An assessment of the occurrence and removal options of microplastics in wastewater treatment processes at the City Ekurhuleni and Midvaal in South Africa

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DECLARATION

I, Tendani Mphaga, hereby declare that this research dissertation submitted by me has never been submitted for review at this or any other institution. From the knowledge that I have, I also confirm that this research dissertation has documented no previously published content written by another person except where due reference is made.

23/03/2023

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Signature **Date**

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ABSTRACT

The main aim of the study was to investigate the occurrence and removal of microplastics in wastewater treatment processes in Ekurhuleni and Midvaal in South Africa. The amount of microplastics and chemical composition discharged into the river pose a threat to aquatic systems and human health. The wastewater samples were collected using 24 h autosamplers between 2021 December to 2022 September. The in-situ measurements for pH and temperature were conducted using an advanced digital HACH HQ40d multi-meter. The analysis for chemical oxygen demand (COD) and suspended solids (SS) was done through colorimetric and gravimetric methods. Analysis for heavy metals was done using inductively coupled plasma mass spectrometry (ICP-MS). The polarized light microscopy (POM) was used to generate high-resolution images and Fourier Transform Infrared Spectroscopy (FTIR) identified polymers and functional groups. The quantification of microplastics was done through image J 1.53 K to determine the microplastics particles (MPs) counts and removal efficiencies. The mass of microplastics was obtained and the loading rate was estimated based on the flow rate. The Pearson correlation coefficient was used to establish the relationships between physicochemical water quality, metals and MPs concentrations and loading. The chemical oxygen demand (COD) and SS showed a small positive correlation with r-value ranging between 0.1-0.3. Temperature and pH showed a negative correlation with MPs concentration. Toxic metals and non-metallic parameters monitored in the influent and effluent of WWTWs also did not correlate significantly, only chloride, Fe, S and SO_4^{2-} showed a smaller correlation at r=0.1-0.3. The MPs vary between 0.01-0.02 mm in the influent and 0.01-0.05 mm in the effluent. The microplastics identified across four seasons were dominated by angular fibres (white), fragments (blue, black and red films (translucent) with concentrations ranging between 145-180 MPs/L in summer, 399-401 MP/L in autumn, 119-798 MPs/L in winter and 152-402 MPs/L in spring. The highest concentrations and loading were observed at WWTW B. The FTIR analysis showed presence of polyethylene (PE), polyethylene terephthalate (PET), polyacrylamide (PAM), polymethyl methacrylate (PPM), polymethyl methacrylate (ABS), polystyrene (PS), polypropylene carbonate (PPC) and thermoplastic vulcanizates (TPV) polymers while the spectra represent functional groups such as saturated aliphatic, alcohol and hydroxyl compounds,

carbonyl and alkenes. The scanning electron microscopy/energy dispersive x-ray (SEM/EDX) analysis resulted in carbon and oxygen ranging between 52-73.46% and 15.9-26% with the composition of MPs additives such as Cr, Ca, Fe, Al, Na, Mg, Zn, Cl, P, S and silicone. Continuous monitoring and regulations of MPs is required to minimize microplastics pollution and adverse impact in the environment.

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LIST OF ABBREVIATIONS

ABS	Acrylonitrile butadiene styrene
AC	Activated carbon
BNR	Biological nutrients removal
BPA	Bisphenol A
COD	Chemical oxygen demand
CSIR	Council for Scientific and Industrial Research
DBPs	Disinfectants by-products
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
DWS	Department of Water and Sanitation
EDCs	Endocrine disrupting contaminants
EDS	Energy dispersive spectroscopy
ERLAB	Ekurhuleni Water Care Laboratory
ERWAT	Ekurhuleni Water Care Company
FTIR	Fourier transform infrared spectroscopy
GC/MS	Gas chromatography/mass spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LWMA	Limpopo Water Management Area
MBR	Membrane bioreactors
MF	Microfiltration

MPs	Microplastics particles
NEMWA	National Environmental Management Waste Act
NWA	National Water Act
PA	Polyamide
PAHs	Polycyclic aromatic hydrocarbons
PAM	Polyacrylamide
PBAs	Phenoxybenzoic acids
PBAT	Polybutylene adipate-co-butylene terephthalate
PBDE	Polybrominated diphenyl ethers
PBDE	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCBs	Polychlorinated biphenyls
PCL	Polycaprolactone
PCPs	Personal care products
PE	Polyethylene
PECA	Poly(ethyl cyanoacrylate)
PES	Polyester
PET	Polyethylene terephthalate
PLA	Polylactide
PMM	Poly(methyl methacrylate)
POCs	Persistent organic compounds

POM	Polarized light microscope
PP	Polypropylene
PPC	Polypropylene carbonate
PPM	Polymethyl methacrylate
PS	Polystyrene
PS	Polystyrene
PST	Primary settling tanks
PU	Polyurethanes
PVC	Polyvinyl chloride
py-GC-MS	Pyrolysis-gas chromatography-mass spectrometry
RO	Reverse osmosis
RQOs	Resource quality objectives
SANS	South African National Standard
SEM	Scanning Electron Microscope
SS	Suspended solids
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TGA-MS	Thermogravimetric analysis-mass spectrometry
TPV	Thermoplastic vulcanizates
UF	Ultra-filtration
UV	Ultraviolet

WHO	World health Organization
WPO	Wet peroxide oxidation
WUL	Water Use Licences
WWTW	Wastewater treatment works

RESEARCH MANUSCRIPTS TO BE PUBLISHED IN 2023

The MSc dissertation will yield 2 publications of which the first one has been submitted to a journal. The title and authors are added below.

1. Mphaga, T., Mhlongo T.N. and Zikalala, S. (proposed publication year is 2023). Identification and quantification of microplastics in wastewater treatment plants by spectroscopic and microscopic techniques in Johannesburg East, South Africa.

CHAPTER 1:

INTRODUCTION

1.1. Background

Water quality is one of the most critical challenges faced globally and amongst other African countries; South Africa is facing similar problems. According to (du Plessis, 2017), 2.4 billion people worldwide live without access to proper sanitation yet poor sanitation is a major contributor to water pollution. Global statistics suggest that one in nine people have access to safe and improved drinking water (William and Loucks, 2015). These global statistics are getting worse every year, putting water security at risk for future generations. The world figures on the scale of water pollution are intriguing. On average, 90% of sewage globally is discharged untreated into rivers, estuaries, and oceans-leaving the environment exposed to different forms of pollutants (Malik *et al*., 2011). Therefore, there is a need to safeguard water for the provision of water security for future use.

Globally, an average of 400 megatons of industrial and domestic waste are discharged into wastewater treatment works annually (Abd-Elhamid et *al.,* 2021). In South Africa alone, 5.12 billion litres of wastewater is collected from industries and domestic activities and 95% of this volume is discharged into rivers while 5% gets disposed of as sludge from Wastewater Treatment Works (WWTW) (du Plessis, 2017; Martin Zhuwakinyu., 2012). The wastewater from the industrial and domestic activities contain different pollutants such as heavy metals, endocrine disrupters, personal care products (PCPs), pharmaceutical product, disinfectants by-products (DBPs) and emerging pollutants (Grassi *et al.,* 2012).

Emerging pollutants are increasingly becoming a global challenge in terms of detection and removal in WWTW (Singh & Gupta, 2017). To date, endocrine disruptors, personal care products (PCPs), pharmaceutical products, disinfectants by-products (DBPs), and microplastics find their way into WWTW where they have shown to accumulate (Tanjung, Hamuna, and Alianto, 2019). This may pose risks to organisms that live or depend on freshwater for a living. Such risk include increased toxicity,

hormonal disorders, deformation of organs as well as increased mortality on aquatic organisms (Rosala., 2010; Archer, 2018).

South Africa has a huge plastic industry with limited complimentary recycling place thus contributing millions of tons of plastic disposed into waste management facilities such as landfills (Adeniran and Shakantu, 2022). Very little is known about microplastics in South African WWTW because of the limited research in the area of microplastics (Verster, Minnaar, and Bouwman, 2017). Studies in microplastics in the South African context are focused on coastal areas while in Europe and Asia they are focused on wastewater and surface water (Verster, Minnaar and Bouwman, 2017; Long *et al.*, 2019, Naidoo *et al.,* 2015). Despite being limited, these studies have confirmed the presence of microplastics in both coastal areas and WWTWs.

According to various studies conducted across the world, WWTWs have been identified as one of the significant sources of microplastics due to the huge quantity of sewage influent received from both domestic and industrial wastewater (Conley et al., 2019a; Kang et al., 2018a; Talvitie *et al.,* 2017a). Among the few studies that have been done in South Africa, is one by Long *et al.* (2019) which has confirmed that most conventional WWTWs are not designed to remove microplastics-leading to the discharge of microplastics-laden effluent into the rivers. Similar findings have been made by several other researchers (Adiloğlu *et al.*, 2012; Maraqa, Meetani, and Alhalabi, 2020; Thakali *et al.*, 2020).

The chemical properties of microplastics vary due to the composition of polymer raw materials and additives (Tarasyuk *et al.,* 1991). Chemical compounds found in microplastics such as bisphenol A (BPA), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), phthalates, and heavy metals have been proven to be endocrine disruptors, carcinogens, liver poisons, mutants of deoxyribonucleic acid (DNA) and reproduction retardants if inhaled or consumed by people (Katyal *et al.,* 2020). Microplastics and toxic chemicals are discharged in wastewater by fragmentation process caused by waves and currents during conveyance to WWTWs. The inherent chemical composition of wastewater is complex and contains toxic chemicals such as heavy metals and persistent organic compounds (POCs) (World Health Organization, 2015). In the presence of microplastics in wastewater toxic

chemicals such as PCBs and heavy metals are adsorbed onto the surface of microplastics. In this way, the microplastics act as contaminant pathways for the pollutants into the environment (Claudia Campanale, Massarelli, *et al*., 2020).

While the research on the availability of microplastics in drinking water is still at its inception, there is already evidence of microplastics occurring in rivers that are the major source of drinking water (Luis *et al.,* 2018). There is also evidence that microplastics affect aquatic species. For example, traces of microplastics have been found in the digestive tracts of fish and sea birds and that this adversely affects their health and increases mortality (Gewert, Plassmann, and Macleod, 2015; Katyal, Kong, and Villanueva, 2020).

Sewage sludge is a major source of microplastics for the soil with additional millions of tons of microplastics being disposed into the environment through the application of agricultural fertilizers (Choy *et al.,* 2020). A study done by Enyoh (2019) demonstrated that microplastics are eventually sequestered from the contaminated soil into fruits and vegetables Therefore, fruits and vegetables are becoming an exposure pathway for human beings to microplastics-with dosages of up to 80 g/kg of body mass. Microplastics can exist in soil for over 100 years and can eventually find their pathway to ground and surface water thus posing a threat to the freshwater ecosystem (Choy *et al.*, 2020, Edo, 2020).

Various sludge applications and treatment options pose different threats to the environment. In other areas sludge is not added into agricultural land, instead, incineration becomes an option. During the incineration of sludge, toxic chemicals that are added during plastic manufacturing and those adsorbed on the surface of the plastic may be released into the atmosphere (Mahon *et al.*, 2017; Simon, Vianello, and Vollertsen, 2019). There is limited information on microplastics globally and in South Africa (Edo, 2020). This study seeks to highlight the presence of microplastics in the influent and final effluent of WWTWs A and B. Recommendations of this study will be shared with the Department of Water and Sanitation, concerned industries and the general public to raise awareness on the significance of microplastics pollution and the role of the sewage conveyance system in transferring microplastics to wastewater and the environment.

1.2. Problem statement

Wastewater treatment works (WWTWs) are supposed to give solutions to reduce pollution by microplastics into the environment. On the contrary, WWTWs are considered sources of microplastics for the aquatic environment because treated water continuously is discharged into the receiving environment in large quantities (Sun *et al.,* 2019). Although WWTWs consists of primary, secondary and even tertiary processes with advanced technologies, microplastics are still not eliminated completely (Hidayaturrahman and Lee, 2019). The WWTWs A and B are conventional activated sludge processes that were not initially designed for the removal of microplastics as it is not mentioned in the design manual (WWTW A manual, 2015; WWTW B manual, 2015). Microplastics removal requires advanced treatment processes such as membrane filtration, ultra-filtration (UF) and reverse osmosis (RO) (Poerio, 2019). The presence of microplastics in wastewater also serves as an indication of pollutants sources. The treatment of wastewater occurs upstream and then the treated water gets discharged into surface water bodies where it is collected downstream for further water treatment (Xu *et al.,* 2020).

Microplastics contain chemicals that are toxic, carcinogenic and act further as carriers of contaminants. Therefore, if not fully removed from drinking water they may pose a threat to human health (Yuan *et al*, 2022). In a country like South Africa there are informal settlements built close to riverbeds downstream of WWTWs (Weimann and Oni, 2019). Communities from such settlements utilise the contaminated wastewater discharged from WWTWs. The detection of microplastics in human stool demonstrate that microplastics are present in human bodies. Danopoulos *et al.* (2020) further stated that treated drinking water is contributing to the introduction of microplastics into the human body. This can be true if WWTWs are not capable of removing microplastics that end up in surface water bodies where abstractions for portable water treatment and irrigations take place.

Microplastics absorb and or adsorb toxic water contaminants onto their surfaces via passive adsorption and then transmit these toxins to animals and human beings, leading to an increased incidence of immune or neurodegenerative diseases (Correia *et al.,* 2020). These effects can strike a single cell, a group of cells, an organ system,

or the entire body (Duncan *et al*., 2017). Among these chemicals, are carcinogens, DNA mutants, endocrine disruptors and reproduction retardants. Most of these chemicals are recalcitrant, therefore tend to bio-accumulate in bodies of living organisms with increasing concentration as the trophic levels increase (Gasperi *et al.,* 2019; Nelms *et al*., 2015). The internal organs that are most commonly affected are the liver, the kidneys, the heart, the nervous system (including the brain) and the reproductive system (Campanale *et al.* 2020).

Many studies have shown that microplastics also enter the food web, leading to human beings being exposed to their potential harm (Davidson *and Dudas, 2016,* (Westphalen and Abdelrasoul, 2018, Duncan *et al.,* 2017). A study reported by Davidson and Dudas (2016), investigated the presence of microplastics in Manila clams in British Columbia. The study found that every clam analysed had at least pieces of microplastics and the concentration in individual clams ranged from 0.07 to 5.5 particles per gram of body mass (Davidson *and Dudas, 2016*). In Brazil, an investigation conducted by Miranda detected significant amounts of microplastics in the stomachs of two important edible species of fish (Westphalen and Abdelrasoul, 2018). Human beings consume microplastics from edible aquatic species and crops capable of adsorbing microplastics (Duncan *et al.,* 2017). The presence of microplastics in the human body causes digestive systems problems and accumulates in the appendix leading to appendicitis (Gasperi *et al.*, 2019).

The impacts of microplastics on aquatic ecosystems include negative effects on microbes, invertebrates, fish and other animals such as birds, turtles and marine mammals (World Health Organization, 2015). Microplastics are also ingested by animals into the stomach, leading to them feeling full and therefore, eating less than the amount of food they need to stay alive. Microplastics also cause internal damage to the digestive tract. In addition, many kinds of microplastics have hydrophobic surfaces that adhere to common pollutants like polychlorinated biphenyls (PCBs) (Lasee *et al.*, 2017). These chemicals would rather be stuck to surfaces like plastic than in the water itself. If these compounds are highly concentrated on plastic, and then ingested by animals, the chemicals can desorb in the low oxygen environment inside the gut (Seidensticker *et al.*, 2018). This could expose the animal to high concentrations of potentially toxic compounds.

1.3. Justification

It is estimated that the global production of plastics surpasses 407 million tonnes annually by 2015, which is a significant increase from 270 million tonnes recorded in 2010 (Geyer *et al.,* 2017). This amount includes plastics used in a variety of processes which including the production of pharmaceutical products, PCPs, household products, industrial products, and other production processes of plastics (Simon *et al.,* 2019). The estimated amount of waste plastic generated and disposed of to the environment in the world is 302 million tonnes, which is 74% of the total plastics produced. In 2010, four to twelve million tonnes of plastics found their pathways into the ocean through littering, wastewater discharge, erosion, storm water, and industrial discharge, this is a global concern (Lebreton *et al.,* 2018). A study done by Jambek *et al.* (2015) showed that more than 630 000 metric tonnes of plastic debris were found in South African coastal areas, making the country to be the 11th most significant plastic pollution contributor in the world.

In South Africa little is known about the number of microplastics entering WWTWs and surface water. More studies focused on the availability of microplastics in coastal areas with some particles greater than 5 mm (Bouwman *et al.*, 2018, Nel and Frontman 2015, Naidoo *et al.*, 2015, Ryan *et al.,* 2014). Substantial amounts of microplastics may reach WWTWs through municipal sewage, industrial discharges, and stormwater drainage. According to Toxopeus (2019), it was reported that South Africa has a total number of 824 wastewater treatment works across 152 municipalities with a collective design capacity of 6.5 billion litres. On estimate, 5.12 billion litres of wastewater are collected by WWTWs with the discharge of over 95% of wastewater received. The amount and quantity of microplastics entering and discharged with wastewater quantities are not well understood in South Africa and the study area.

There is no wastewater treatment process in place that leads to zero discharge of microplastics (Sun *et al.,* 2019). Water from dewatering sludge and water used for cleaning in the plants is recyclable back into the influent yet it carries appreciable amounts of microplastics (Corradini et al., 2019). Much of the microplastics end up in the sludge (Habib *et al.,* 2020). Therefore, contamination of the sludge will determine the fate of the available microplastics in soil and groundwater (Bouwman *et al.,* 2018).

Therefore, the aim of the current study is to determine the removal efficiency of microplastics and recommended solutions to mitigate exposure of soil and downstream waters to highly mobile plastics and their constituent compounds. Particular attention will be on activated sludge processes in the study area.

Studies such as Gies *et al.,* (2018), Murphy *et al*., (2017), Olmos *et al.*, (2016) have confirmed that advanced wastewater treatment such as membrane processes can reduce up to 97-99% of microplastics in water. However, in conventional treatment processes, the removal efficiency varies from plant to plant due to the physical and chemical properties of microplastics (Long *et al.*, 2019). In studies carried out by (Long *et al.*, 2019 and Magni *et al.,* 2019) removal efficiency of 79.33% was reported in one plant and 64% in another plant. The concentration of microplastics discharged into the rivers varies in different wastewater treatment works and there is a need to conduct a study in other locations.

Aquatic animals may ingest microplastics which can affect their respiratory tracts and lead to death (Deocaris *et al.,* 2019). People may indirectly consume microplastics from eating aquatic species such as fish and seafood and this may lead to health-related problems such as cancer or organ failure (Elvis *et al.,* 2019). Further accumulation of microplastics in rivers may affect water reuse such as drinking water from conventional water treatment where toxic chemicals attached to microplastics may be consumed (Sun *et al.,* 2019). Significant amounts of microplastics have been detected in sewage sludge. However, more is yet to be understood on the interaction of microplastics with other compounds during treatment, disposal in the soil, and incineration of sludge (Mahon *et al.*, 2017, Piehl *et al.,* 2018 Rolsky *et al.,* 2020).

Archer 2018 focused on emerging micro-pollutants such as endocrine disruptors, PCPs, and pharmaceutical contaminants in South Africa as well as the study area. The study left a gap in the availability of microplastics as emerging pollutants in WWTW. According to Lee and Kim (2018), it is recommended that future research should focus on the identification and monitoring of microplastics in regions like those in Africa where there is limited data published.

1.4. Research aims and objectives.

1.4.1. Research aim

The study aims to investigate the occurrence and removal efficiencies of microplastics from wastewater treatment processes in Ekurhuleni and Midvaal municipalities in South Africa.

1.4.2. Research objectives

- To collect wastewater samples from influent and effluent of WWTW A and B.
- To perform physicochemical tests such pH, temperature, suspended solids (SS), and chemical oxygen demand (COD) and toxic metals analysis (Aluminium, Cadmium, Chloride, Lead, Iron, Manganese, Fluoride, Sodium, Sulphates and Zinc).
- To analyse samples from selected points in the wastewater to characterise microplastics in terms of morphology, chemical composition, and quantification (concentration & loading rate).
- To determine the removal efficiencies of microplastics by the biological nutrient's removal processes at WWTW A and to compare with WWTW B.
- To determine the correlation between pH, SS, and COD to microplastics concentration and loading rate.
- To determine statistical correlations between the concentration of microplastics and toxic metals (Aluminium, Cadmium, Chloride, Lead, Iron, Manganese, Fluoride, Sodium, and Zinc) and their potential health effects.
- To make output-dependent recommendations about monitoring and the potential health effects of microplastics presence in wastewater effluent.

1.5. Research Questions

1.5.1. Main Research questions

- Do microplastics occur in wastewater treatment plants?

- What is the removal efficiency of microplastics in wastewater treatment plants?

1.5.2. Subsidiary questions

- What tests can be done to evaluate the presence of microplastics quantitatively and qualitatively?
- What are the morphological characteristics, chemical compositions & quantities (concentration & loading rate) of microplastics in wastewater treatment works (WWTW)?
- To what extent does the biological nutrient's removal process in WWTW A and WWTW B remove microplastics?
- To what extent does the physicochemical parameters such as pH, temperature, SS, and COD correlate with the presence of microplastics in wastewater?
- To what extent does the presence of microplastics correlate with toxic metals (Zinc, Chromium, Lead, Copper, Cadmium, Iron, Manganese, and Aluminium) in wastewater?
- What are the potential health impacts associated with the presence of microplastics and toxic metals (Zinc, Chromium, Lead, Copper, Cadmium, Iron, Manganese, and Aluminium) in wastewater?

1.6. Delimitations and limitations

This section provide the delimitations and limitations in the current study.

1.6.1. Delimitation

Although there are scientific methods proposed in various scientific guidelines and papers for the sampling and analysis of microplastics, there are no standards methods for microplastics assessments, therefore researcher can explore various techniques and methods which can also results in development of new scientific body of knowledge.

1.6.2. Limitations

Due to COVID19 Ekurhuleni, the Water Care laboratory was only testing essential samples as a result of staff limitations, the situation extended beyond the research sampling period, and the time frames set for research plans such as sampling and analysis were affected. The microscopic analysis method for microplastics requires precision and experience, performing this analysis without experience and knowledge may lead to errors in results and leading to false conclusive remarks on the subject matter under investigation.

1.7. Ethical consideration

Ethical clearance for the study was obtained from the UNISA College of Agriculture and Environmental Sciences following the college ethics procedures. As part of the ethics clearance requirements, consent was obtained from Ekurhuleni Water Care Company (ERWAT) and their laboratory (ERLAB). The ethical clearance approval letter are presented in Appendix H and G.

CHAPTER 2:

LITERATURE REVIEW

2.1. Introduction

The encroachment of plastics on surface water resources such as ocean, rivers, estuaries, dams and sea has escalated over the years (Govender *et al.,* 2020). Globally, plastics have been responsible for the loss of marine aquatic life species, a phenomenon that results in the disruption of the marine biodiversity (Thushari & Senevirathna, 2020). Due to growing concerns about plastic pollution in the ocean, there has also been a shift of paradigm to focus on the smallest particles referred to as microplastics. Research in Europe and other countries outside the African continent has focused on microplastics in the ocean and inland surface water bodies, including their occurrence in wastewater treatment (Verster, Minnaar and Bouwman, 2017; Long *et al.*, 2019, Naidoo *et al.,* 2015). However, in South Africa research has focused on microplastics occurrence in the ocean due to its noticeable impact on aquatic life, there has been limited research on Wastewater Treatment Works (WWTW) (Bouwman *et al.,* 2018).

Although the occurrence of microplastics in wastewater has not been fully studied in South Africa, it is important to characterize them to determine removal efficiencies and distribution into the environment (Bayo *et al.,* 2021). There are various sources of microplastics in wastewater treatment. Different authors have highlighted the prevalence of microplastics in WWTW as an attribute to discharged industrial effluent, domestic effluent and stormwater drainage into municipal sewage conveyance systems (Conley *et al.*, 2019a; Michalkiewicz, 2019; Tagg *et al.,* 2020). There are different classifications of microplastics as well as their types. These classifications are based on whether the microplastics are degradable or not as well as the composition polymer of the microplastics (Siegfried *et al.,* 2017).

The WWTWs in South Africa are governed by the Water Use Licences (WUL) and Section 21 of the National Water Act (NWA) which does not include microplastics guidelines (Bouwman *et al.*, 2018). Therefore, the monitoring programmes in WWTW do not include microplastics on discharged effluent. The fate and removal of

microplastics in most WWTW in South Africa is not well documented (Verster *et al.*, 2017a).

The prevalence of microplastics in WWTW is linked with disruption of treatment processes such as settling, biological nutrients removal (BNR), anaerobic sludge treatment and disinfection of wastewater treatment (Iyare *et al.*, 2020a; W. Liu *et al.,* 2021a). The inability of WWTW to completely remove microplastics makes WWTW regarded as one of the major sources of surface water and the ocean (Habib, Thiemann and Al Kendi, 2020; Long *et al.*, 2019). Although some microplastics are removed from wastewater through physical processes, the degraded microplastics release contaminants into the water, therefore contributing to water quality degradation (Kjeldsen *et al.,* 2019). The encroachment and penetration of microplastics into the food web also present public health concerns (Rana Zeeshan Habib *et al.,* 2020). Classification and quantification of microplastics in wastewater aid in knowledge and information required in WWTW monitoring programs, process optimization for their removals as well as tracking their pathways into the environment.

The subsequent sections of the literature review surveys the research conducted by different authors on the occurrence and removal options of microplastics WWTW in South Africa and across the world. An overview of existing knowledge relating to microplastics in WWTW is provided with identification of theoretical framework, methodology and gaps in the research previously done, research in progress and research on its inceptions.

2.2. Introduction of microplastics to environment

2.2.1. Origin and classification of microplastics

There are various origins of microplastics particles and these include pharmaceutical products, health care products, clothes and the weathering of large plastics particles, to name a few (Sukrita and Prasongsom, 2020). The use of polymer for different purposes also gives rise to plastic particles of different sizes-some of which fall in the category of microplastics. Natural processes such weathering and biological processes also alter plastic particles into different sizes (Mason *et al.,* 2022; Duan *et al.,* 2021). It is also paramount to understand the different categories used for

microplastics classifications as well as different types of polymers and their composition as stated by Kosovssky (2022).

Since the existence of humankind, plastic has always been manufactured from materials sourced from plants and animals (Guan *et al.,* 2020). With the development in science, the production and processing of natural plastic materials such as rubber, nitrocellulose and collagen involved the addition of chemicals to strengthen them (GESAMP, 2015). The past century has seen a rise in the volumes of synthetic polymers due to the growing demand for plastic use. Over the last 50 years, the manufacturing of synthetic plastics has increased by approximately 8.7% (OECD, 2018). The life cycle of microplastics before the 19th century was short due to use of natural materials that are easily decomposed and weathered by biological and physical processes (Chalmin, 2019). In the early 19th the synthesis of plastics from fossil fuels gained prominence and these synthetic plastics had longer life cycles in the environment due to their resistance to chemical and physical processes (Zhu, 2020). Around the same time, such plastics as Bakelite also took centre stage and added to the environmental load of plastics.

To date, there are growing concerns over the long life cycle of synthetic plastics that has resulted in 8 million metric tons of plastics ending up in the coastal environment and 25% inland surface water bodies (Geyer *et al.,* 2017). These plastics ending in the environment include different polymers which are divided into two groups: naturally occurring and synthetic polymers. Naturally occurring polymers such as cellulose, lignin, chitin, wool and starch can be found in man-made products such as food and clothes (Lusher *et al.,* 2017). (Frias & Nash, 2019). Due to additives such as plasticizers that are included in the production of plastics, synthetic polymers such as thermoplastics and thermoset are complex and diverse (Hahladakis *et al,.* 2018). Polymers such as polyethylene (PE), polyester (PES), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) are the most common thermoplastics used for manufacturing plastic bottles, pipes, textiles, bags, cigarette butts and other insulation materials (Peng *et al.,* 2017). Thermoplastics pellets are not resistant to heat-they melt and reshape when exposed to heat (GESAMP, 2015). Thermoset plastics are synthetic polymers with interconnected chains bonded to ensure any form of resistance from reshaping, remoulding and heat (Lusher, 2015). The most common

thermosets are polyurethanes (PU), epoxy and alkyd, which are used in products such as insulations, adhesives, tires and other products (ter Halle *et al.,* 2017). Polymers occurring in their original manufacturing state are referred to as plastics (Wagner *et al.,* 2014*)*.

Plastics particles that end up in the environment vary in such characteristics as size and shape (Pinto Da Costa *et al.,* 2020). Such differences in particle sizes and shapes are attributed to evolution in synthetic polymers (Khan *et al.,* 2019). Different authors argued that during the new era of synthetic polymers, industrial manufacturing processes started to add plastics to different products such as PCPs, food staff as well as pharmaceutical products, the addition is to providing scrubbing properties in PCPs primarily (Hahladakis *et al.,* 2018; National Research Council, 1994; Pinto Da Costa *et al.,* 2020). The particles such as resin pellets added to products are 5 mm or less and are regarded as primary microplastics (Eerkes-Medrano *et al.,* 2019).

Microplastics are particles less than 5 mm but greater than 0.00001 mm and can be seen through microscopy as shown in Table 2-1 (Murphy et al., 2016). The table also includes the sizes and classification of the plastics ranging from macro plastics, meso plastics and mega plastics.

Table 2-1: Sizes and classification of microplastics

Size	**Nano<0.00001m**	**Micro<5 mm<0.00001m**	**Meso<2.5 cm**	**Macro<1m**	**Mega>1 m**	**Reference**
Source	Primary nano plastics. Fragmentation of microplastics	Primary microplastics. Secondary fragmentation of large plastics	Direct and indirect items abandoned and waste	Direct and indirect items abandoned and waste	Direct and indirect items abandoned and waste	Lusher, Hollman and Mandoza-Hill, (2017**)**
Examples	Primary: Added nanoparticles. Secondary: Fragmented microplastics.	Primary: Resin pellets. Secondary: fragmentation.	Bottle caps and other fragmented plastic.	Plastic bags, packaging or fragments.	Abandoned plastics from different materials, e.g., car tires, rubbers, plastic films, ropes, household appliances, and medical equipment.	Pinto Da Costa *et al. (*2020)

Although microplastics are primarily added to products such as pharmaceutical products, food and health care products; larger particles of plastics with a long life cycle in the environment also generate microplastics if weathered or decomposed by environmental conditions (Reddy *et al.,* 2021). Larger plastic particles such as tires, paint products and normal clothing wear and tear physically which results in small particles of plastics, referred to as secondary microplastics (Andrady, 2017) relate if they link to microplastics development. Other physical processes that result in microplastics are the fragmentation that occurs due to physical promoters such as UV light, mechanical friction or waves. This process allows for large particles of plastics to disintegrate and break into small pieces (Ziajahromi, 2017).

Some plastic materials are biodegradable, meaning they can be broken down into small pieces through microbial processes and mineralization (Gago *et al.,* 2019). Biodegradation of polymers may follow two-step processes, which include hydrolysis where plastic is broken down into fragments which results in monomers and oligomers (Besley *et al.,* 2017). Subsequently, the plastic fragments undergo mineralization by microorganisms to evolve such gases as carbon dioxide and methane (Lusher, 2015). The mineralization process decomposes microplastics and their chemical compounds, more specifically biopolymers into organic materials in which nutrients are also generated (Hahladakis *et al.,* 2018). The oligomers and monomers in the microplastics can also produce toxic chemicals (Lusher *et al.*, 2017a). The most common type of biodegradable plastics is aliphatic polyester and aliphatic-aromatic polyester with common examples such as polybutylene adipate-co-butylene terephthalate (PBAT), semi-crystalline polycaprolactone (PCL), polylactide (PLA), polyglycolide, and polyhydroxyalkanoates (Frias & Nash, 2019). The current study is looking at the presence of microplastics and removal in wastewater.

2.2.2. Fate of microplastics

Wastewater treatment introduce microplastics into the environment through different routes such as discharge of final effluent into the river, application of sludge into the soil as well as irrigation (Liu *et al.,* 2021). Microplastics released in the environment may cause problems due to various process that occur during wastewater treatment and in the natural environment such as fragmentation, biodegradation and weathering

(Kelly *et al.*, 2021). The release of microplastics into the environment is associated with water quality deterioration, death of aquatic species, soil and groundwater contamination as well as human health impacts (Issac & Kandasubramanian, 2021).

Wastewater treatment is characterised by diverse microbial communities such as ciliates, amoeba, flagellates and protozoan which are responsible for the oxidation of contaminants (Babko *et al.,* 2016). The diversity and dynamics of microbial communities render biodegradable plastics to be broken down partially and some completely. The biggest concern is that during the production of some plastics, additives and monomers are added which has toxicological effects (Claudia Campanale, Massarelli, *et al.,* 2020).

Most common plastics additives are phthalates, calcium carbonate, silica, calcium sulphate, flammable chemicals containing bromine as well as stabilizers of high temperatures on plastics (Llorca & Farré, 2021). Some plastic colorants are made up of heavy metals such as Zn, Pb, Cr, Co and Cd. The biodegradation of microplastics during wastewater treatment also introduces the chemicals which were initially added to the microplastics (Iv, 2021). A study done by Gallo *et al.,* (2018) found that the quality of the wastewater effluent contained chemicals such as polybrominated diphenyl ethers, Bisphenol A and organophosphates, which are linked to the processing of microplastics. The release of final wastewater treated effluent is associated with the introduction of chemicals linked to microplastics that may further degrade the environment when released. The most common environmental problems are degraded quality of surface water and introduction of emerging pollutants which are not included in water quality monitoring programmes (Kumar *et al.,* 2020).

2.3. Distribution of microplastics into wastewater treatment.

Wastewater treatment works (WWWs) have been identified as one of the end routes of microplastics into the environment, however, it is not regarded as the primary source of microplastics (Iyare *et al.,* 2020b). The wastewater conveyance systems are connected to industrial discharges, domestic sewage networks as well as stormwater drainages (Mrowiec, 2018). Industrial effluent, domestic effluent and stormwater drainages are regarded as primary sources of microplastics in wastewater (Fältström *et al.,* 2021; Kjeldsen *et al.,* 2019; Wagner & Lambert, 2018).

The production of various product in the industries include the addition of microplastics for different uses such PCPs and pharmaceutical products. (Frias & Nash, 2019). Across the globe, the production of health care products, PCPs, abrasive cleaning products and clothing entails the addition of microplastics (Murphy *et al.,* 2016). During industrial production, the wash water from production processes may contain microplastics emanating from process chains (Stevens, 2018). Industrial processes require high-quality water for processes such as production, cooling systems, fabricating, cleaning and processing of products. The by-products of production processes such as lubricants, clothes, food, rubbers and other plastics are introduced into wastewater stream through wash water. The by-products are composed of plastics particles such as polystyrene (PS), acrylic and PES added during production processes (GESAMP, 2016).

In addition to microplastics pollution, heavy metals are also associated with industrial effluent. A study conducted in South Africa by Iloms *et al.,* (2020), confirms a correlation of five industrial plants with wastewater treatment plant effluent indicating that Zn, Cu and Pb were major contaminants from both systems. The presence of pollutants such as heavy metals in wastewater is a major concern due to microplastics ability to adsorb and transport contaminants into surface water and soil (Torres *et al.,* 2021).

Apart from the industrial processes, a variety of domestic products feed microplastics into the WWTWs (Rana Zeeshan Habib *et al.,* 2020). Such household products include PCPs such as toothpaste, cosmetics as well as health products that after use may end up in WWTWs (Andrady, 2017). In cosmetic products, micro beads are used as scrubbers or to transform products into emulsions while acrylonitrile butadiene styrene (ABS) has been added globally (Eerkes-Medrano *et al.,* 2019). However, these end up in the WWTWs through sewage conveyance from showers or improper disposal into the sewer networks (Iyare *et al.,* 2020b). The use of different pharmaceutical products in the household such as medication that could be eaten or inhaled e.g. nano capsules make the constituent microplastics to find their route to municipal sewage systems (Atugoda *et al.,* 2021). The other domestic source of microplastics is laundry. fibres (Gaylarde *et al.,* 2021). When laundry machines are being used for washing clothes or other related textiles, fibres wear and tear releasing the fibre fragments into

sewer networks (De Falco *et al.,* 2019). A summary of industrial and household activities that serve as end routes of microplastics in municipal wastewater systems is given in Table 2-2.

Table 2-2: Industrial and household use that serves as end route of microplastics into wastewater treatment.

Uses of microplastics	**Products**	**Reference**
Abrasion	Cosmetics, exfoliating creams, toothpaste, detergents, industrial abrasives	Scudo *et al.* (2017)
Emulsifying	Cosmetics, detergents and paints	Eerkes-Medrano *et al.* (2019)
Filler	Construction	Scudo *et al.* (2017)
Ingredients	Medical products, cosmetics, fertilizers, detergents	Atugoda *et al.* (2021)
Surface coating	Papermaking and polishing agents	Scudo *et al.* (2017)
Improve resistance	Coating, paints and plastic cement	Gaylarde *et al.* (2021)
Absorbance	Nappies, water retainer, agriculture	Scudo *et al.* (2017)

Studies done by Sun (2019) and Lee and Kim (2019) showed that a substantial amount of microplastics detected in municipal wastewater influent was attributed to

industrial and domestic sources. The most common microplastics identified in wastewater are pellets, fibre fragments and granules (Pries *et al.*, 2013). Microplastics classification is very complex; they vary due to size, shape and colour. Polymers such as polypropylene (PP), PE, polystyrene (PS), PET, ethylene copolymer and polyamide (PA) are the most kind of classified microplastics in wastewater works (Dychenka *et al.,* 2017). In a study done by Sun *et al.* 2019 it was observed that fibres constitute 52.7% of microplastics observed in wastewater. Although the source of fibres could not be verified, it is believed that 60% of the plant inflow was domestic and might have been a contributing factor to the abundance of this type of microplastics.

There is limited research done on the microplastics contribution by stormwater drainage systems due to the lack of complex collection systems where sampling can be done. However, it is known that the end route is into municipal wastewater systems (Sedlak *et al.,* 2017).

According to a study done by Bonthuys (2018), 66% of microplastics in the ocean are contributed by road runoff which also end up in the storm water drainage. Various plastic items end up on the surfaces such as coatings, fluid absorbance, polishing agents and some plastic materials that wear and tear against the surface (Lassen *et al.,* 2015). These may end up in the storm water drainages which feed into wastewater treatment. In San Francisco Bay, 7 trillion tire particles have been identified and were attributed to surface runoff (Sedlak *et al.,* 2017).

Although the distribution of microplastics into wastewater treatment has been linked with industrial effluent and domestic sewage, there have also been reports of illegal dumping of plastics into municipal sewer networks that end up in wastewater treatment (Chaukura *et al.,* 2021). In the United States of America, one million metric tons of plastics end up in the environment through illegal dumping, which is transported into the sewage systems through storm water (Law *et al.,* 2020). The larger particles give rise to microplastics through fragmentation (Ziajahromi, 2017). In South Africa there is limited recycling and most plastics material end up in landfill sites. However, illegal dumping has been experienced more especially in informal settlements (Rodseth *et al., 2020)*. The improper management of plastics and illegal dumping lead

to larger plastics in sewer systems, through physical processes, microplastics are formed and dispersed into wastewater treatment (Magalhães *et al.,* 2020).

2.4. Factors influencing the occurrence of microplastics in wastewater treatment.

2.4.1. Organic loading

Organic matter is found almost in all water resources ranging from low to high concentration (Nkambule *et al.,* 2012). Organic matter is derived from the decomposition of products of plant material, bacteria, human faeces and other dissolved and soluble materials in wastewater (Kim *et al.,* 2015). The presence of high organic matter promotes the aggregation of microplastics which influences the transport into the WWTWs. These properties of aggregation also promote the transport of particles in the sediments of receiving water bodies (Wang *et al.,* 2021 and Santchi *et al.,* 2021). High organic content in wastewater is associated with high COD and SS. SS loading rate and COD are considered the most factors, which influence the presence of microplastics by shielding microplastics particles. According to a study done by Long *et al.* (2018) the presence of microplastics correlated highly with variation in organic loading such as SS and COD.

In a study by Long *et al.* (2018), further emphasis is given on the directly proportional relationship between the presence of microplastics and organic loading such as SS and COD can be used to measure the abundance of microplastics in wastewater. The study by Long *et al.* (2018) also further highlighted the monitoring and measurements of SS and COD as a simpler procedure, which can save time and cost as compared to microplastics measurements.

2.4.2. Hydraulic loading

Hydraulic loading is defined as the amount of water entering wastewater treatment units. There is seasonal variations on the inflow received in the wastewater treatment attributed to dry and wet weather patterns. During wet seasons, there is high flows entering WWTWs while in dry seasons the flows drop drastically. Microplastics can be estimated based on flow distribution into the WWTWs and discharged effluent (Kwon

et al., 2022). All designated unit processes in wastewater are designed to handle the specific incoming flow. Variation in wastewater incoming flow is associated with the presence of microplastics (Kim *et al.,* 2015). High flows are associated with high microplastics loading rate while lower flows is linked with lower loading rate in WWTWs (Blair *et al.,* 2019). This is because the high flows create turbulence and flow currents that are capable of physically breaking down microplastics. This results into more particle presence in water. When the plant is operating above its hydraulic capacity, unit processes such as primary settling tanks and secondary settling tanks do not perform well-resulting in SS escaping to downstream processes. SS shield a significant amount of microplastics, which can end up being discharged with the final effluent (Ory *et al.,* 2020).

2.5. Wastewater treatment processes and removal efficiencies of microplastics.

Wastewater treatment facilities put in place processes to remove pollutants such as physicochemical and microbial contaminants in wastewater. The sources of wastewater that enters the plant could be industrial, domestic or storm water which finds the pathways to the treatment works through conveyance systems (Magnusson *et al*., 2016). Wastewater treatment can be classified as a conventional or advanced system based on the type of technology (membranes filtrations, sand filtration, activated carbon) and the efficiency to remove contaminants (Gatidou *et al.,* 2019). Several studies have been done to determine the efficiencies of both conventional and advanced processes in the removal of microplastics (Sigh *et al.,* 2021). According to literature by Singh and Gupta (2017), Long *et al.* (2019), and Campanale *et al. (*2020), advanced processes such as RO, membrane bioreactors (MBR), UF and activated carbon (AC) are efficient in the removal of microplastics while conventional processes like activated sludge processes are affected by various operational factors such as hydraulic loading, organic loads, design capacities and level of plant optimization.

2.5.1. Conventional wastewater treatment and the removal of microplastics

Conventional wastewater treatment is the most common and simple way of removing physical, chemical and microbial pollutants in wastewater for safe disposal in the

environment (Murphy *et al.,* 2016). These may include various processes such as physical processes, chemical addition, and the use of microbial activities for the removal of pollutants. Most common pollutants in wastewater involve nutrients, organic and inorganic matter, physical contaminants such as electrical conductivity and pH as well as microbial contaminants (Talvitie *et al.,* 2015). The conventional process involves three-stage treatment composed of preliminary, primary and secondary treatment (Figure 2-1 below) present a typical three stage treatment process.

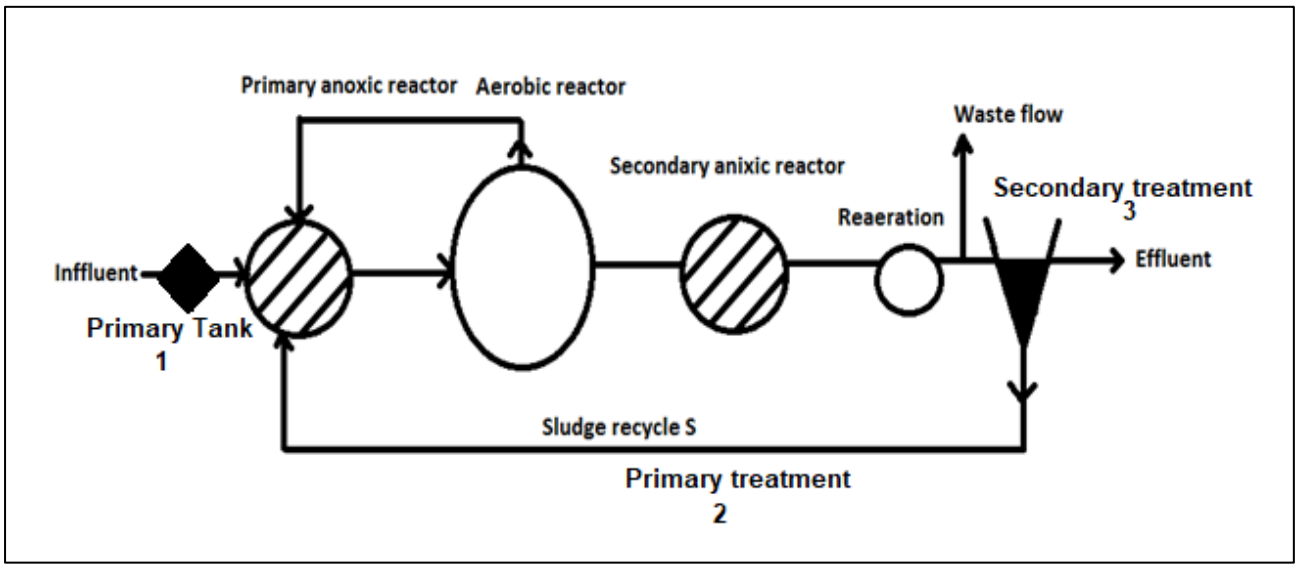

Figure 2-1: Three stage wastewater treatment process (WISA, 2002)

2.5.1.1. Preliminary treatment

Preliminary treatment happens in the inlet works when wastewater enters the treatment plant through conveyance pipes. Wastewater contains large materials such as debris, old scraps, plastics, rags, food particles, sand and gravel which makes up grit when combined with other small particles (Peng *et al.,* 2017). These materials require preliminary treatment to remove them to protect downstream process units and equipment. The first process is the use of screens that block these object from entering the WWTWs and the compaction of screened materials to recover water before disposal into the bins and collection and transportation to landfill site (Lares *et al.,* 2018a). This is followed by the removal of sand and gravel known as grit when combined with small particles. The process of grit removal occurs in the

degritting chambers where the velocity is lowered to enable settling of the grits that is periodically removed by blowing and compressing air in the chambers, this is regarded as the final stage of initial treatment (Mahon *et al.,* 2017). During degritting some of the microplastics do settle and are removed as well while some are transported with the water to next stage. The grit removed are stored in the bins and transported to landfill sites together with removed microplastics. Microplastics often get broken down to releases toxic chemicals such as PCBs and endocrine disrupting contaminants (EDCs) due to biochemical processes in the landfills (Siddiqua *et al.,* 2021).

2.5.1.2. Primary Treatment

Primary treatment is the second stage in conventional wastewater treatment aimed at the reduction of organic materials that bypassed the grit removal such as SS and microplastics (Cristaldi *et al.,* 2020). This stage involves the settling of colloidal solids. This process occurs in primary sedimentation tanks which allow the settling of solids with the reduction of pollutants such as nutrients, metals, and organic loading to BNR (Simon *et al.,* 2019). Conley *et al.,* (2019a) stated that this process was not designed to remove complex pollutants such as persistent organic matter and emerging pollutants like microplastics, but they can be attached to organic matter and other settleable materials and get removed as well to anaerobic processes (Lofty *et al.,* 2022). However, in the primary settling tanks, some microplastics escape due to poor overflow rate and poor settling as well as poor desludging and over capacity (Maraqa *et al.,* 2020). Such factors contribute to a significant amount of microplastics bypassing downstream processes and ultimately escaping from wastewater treatment with discharged effluent into the environment (Alst & Vollertsen, 2018). The settled sludge in the PSTs contain microplastics due to aggregation of solids, which are transferred to the next treatment stage of sludge streams such as drying beds, anaerobic digestion or filter belt pressers (Lvleva *et al.,* 2019).

2.5.1.3. Secondary treatment

The third stage of treatment is BNR which involves the breaking down of organic materials through microorganisms (Long *et al.,* 2019). This involves anaerobic and aerobic bacteria like phosphates-accumulating bacteria, *Acinetobacter*, and nitrifies

like nitrobacteria and *Nitrosomonas* which oxidise nutrients and break down colloidal organic matter (Sun *et al.*, 2019). There are several conventional plant configurations such as the University of Cape Town (UCT), Ludzack Etinger process, Budenpho, 3 stage Pheredox, Johannesburg process, and A_2O process; these are commonly used in South Africa. This processes all uses microbial interaction to oxidise organic compounds in the presence of oxygen (Sizirici and Yildiz, 2020). However, activated sludge processes is able to breakdown biodegradable microplastics, thereby releasing contaminants such as heavy metals from plastics additives (Kelly *et al.,* 2020).

Other conventional processes include tricking filters, which use media and microbes for wastewater treatment (Ziajahromi *et al.,* 2017). The trickling filters provide the filtration mechanisms to remove an estimated 79% of microplastics when used as post treatment for activated sludge effluent (Liu *et al.,* 2020). Microplastics are trapped through the media, however the disadvantages can be the continuous accumulation, resulting into ponding in the treatment unit. Due to limited dissolved oxygen in the trickling filters, the removal of microplastics through microbial processes is difficult, oxidation is influenced by aeration.

It is difficult to biodegrade microplastics through trickling filters due to poor microbial communities, some bacteria's that degrade microplastics are not in abundance in trickling filters processes (Estahbanati & Fahrenfeld, 2016). Biopolymers are biodegradable by tricking filters, through hydrolysis and mineralization, this results in toxic chemicals released in the effluent increasing contaminants concentration in the receiving environment (Sedlak *et al.,* 2017). Some microplastics like thermoplastics and thermosets are not easily degraded, thus a large amount of these plastics are transferred from trickling filters to the clarification stage, where they can escape into the environment (Dyachenko *et al.,* 2017). The availability of non-biodegradable microplastics also influences the transport of contaminants such as heavy metals, nutrients and pathogens through their biofilm (Ziajahromi *et al.,* 2016).

2.5.1.4. Disinfection

The wastewater effluent is disinfected using various chlorine products such as chlorine gas, calcium hypochlorite, sodium hypochlorite and advance processes such as UV light and Ozone (Gelete *et al.,* 2020). The biggest challenge is the reaction of chlorine

with various compounds including microplastics to form DBPs (Miao *et al.,* 2022). Chlorination also has the ability to degrade low density polymers such PS. However, high density polymers such as PE are resistant to chlorination (Nandakumar *et al.,* 2022).

2.5.1.5. Sludge treatment

The sludge withdrawn from the primary settling tank contains high concentrations of microplastics (Lofty *et al.,* 2022). From the settling tank, the sludge is then sent to anaerobic digestion for treatment. The anaerobic sludge treatment involves heating, mixing and methanogenisis for degradation of organic compounds and production of methane and carbon dioxide (Meegoda *et al.,* 2018). Microplastics present in sludge and biological treatment sections may not be completely degraded by microorganisms and thermal processes (Lv *et al.,* 2019). This is because most plastics are partially biodegradable and resistant to thermal hydrolysis due to additives such as flame retardants that gives strength to microplastics (GESAMP, 2015).

Settled sludge in secondary clarifiers is treated through various dewatering techniques such as filter belt presses and gravity thickeners (Talvitie *et al.,* 2015). The settling and drying processes also does not remove microplastics from sludge-therefore, large amounts of microplastics are present. The discharged final effluent in wastewater has become the main pathway for microplastics into rivers, streams, estuaries and the ocean. Microplastics from this pathway account for 25% of microplastics present in surface water globally (Verster *et al.,* 2017). Sludge from WWTWs is occasionally disposed in landfills or incinerated and some used as fertilisers while the water may be used for irrigation (Mokonyama *et al.,* 2017). Application of sewage sludge and irrigation of final effluent in the agricultural field has been documented to introduce microplastics in the soil, which ultimately encroach on water resources through surface groundwater interaction and erosion (Milojevic *et al.,* 2021).

Table 2-3: Reference studies on microplastics occurrence and removal of conventional processes.

Conventional treatment	**Microplastics in particles/L**	**MPs Classification**	**Removal efficiencies in (%)**	**Reference**
Primary & Secondary	1.05	Fibres	99	Lares *et al.,* 2018
Primary & Secondary	91	Fibres	72	Leslie *et al.,* 2017
Primary & Secondary	3.7, 17.6 & 17.2	Fibres	97.6, 85.2 & 85.5	Conley *et al.,* 2019
Primary & Secondary	1.73	Fibres, pellets, granules & fragments	97.8	Long *et al.,* 2019
Primary & Secondary	50	Fibres	83	Dries *et al.,* 2015
Secondary	33	Fibres, fragments	73	Gundogdu *et al.,* 2018
Primary & Secondary	0.4	Fibres, fragments, pellets	84	Magni *et al,* 2019
primary	159	Fibres	66	Ziajahromi *et al.* 2017

Removal of microplastics from wastewater treatment plants is carried out via physical processes or through biodegradation (Rana Zeeshan Habib *et al.,* 2020). The physical processes do not completely remove microplastics, therefore, leading to distribution into the surface water with final effluent (Park and Park, 2021). The failures of complete removal of microplastics from conventional processes is due to the initial designs which did not consider the removal of micro particles in wastewater (Kwon *et al.,* 2022.

2.5.2. Advanced wastewater treatment and the removal of microplastics

Advanced wastewater entails tertiary treatment and integrated wastewater treatment which may include physical, chemical and biological processes in a single treatment unit (Al-Rekabi *et al.,* 2007). Following the conventional wastewater treatment, additional process units may be included to enhance further removal of pollutants (Lares *et al.,* 2018b). Such additional process units are considered tertiary processes. The most common post-treatment processes in wastewater in South Africa are sand filtration and AC adsorption (Guyer, 2011). The purpose of sand filtration and AC adsorption is to remove the remaining pollutants to meet specific regulatory requirements such as WUL, resource quality objectives (RQOs) and SANS241. The use of AC can reduce persistent organic materials and emerging pollutants such as endocrine-disrupting chemicals which are not removed through a normal conventional treatment process (Talvitie *et al.,* 2017a).

Advanced treatment processes (tertiary) such as membrane filtrations, MBR, AC, ozone and other treatment unit such as RO are common in industrial wastewater treatment and water reclamation plants which may combine processes such as UF, RO and bioreactors (Talvitie *et al.,* 2017a). Table 2-4 below summarises studies done on testing the removal efficiencies of advance treatment processes.

Table 2-4: Reference studies on microplastics occurrence and removal using membrane processes

Advanced treatment	**Microplastics in particles/L**	**MPs Classification**	**Removal efficiencies in (%)**	**Reference**
Tertiary	90	Fragments & fibres	99	Carr *et al.,* 2016
Tertiary	0.3	Beads, fragments & fibres	99	Talvitie *et al.,* 2017
Tertiary	70	Fibres	95.6	Talvitie & Henonein 2014
Tertiary	13.5	Fibres & fragments	97.6	Talvitie *et al.,* 2015
Tertiary	10.1	Synthetic fibres	97	Minting *et al.,* 2017
Tertiary	59	Fibres, fragments &	99	Michaelson *et al.,* 2016
Tertiary (MBR)	13	Fibres & fragments	99.5	Lv *et al.,* 2019

2.5.2.1. Membrane processes

Membrane processes are pressure-driven processes which have been used in industrial processes and are now been preferred in the water and wastewater sector due to the diversity of emerging pollutants (Yoon, 2015). Membranes can be classified as synthetic and biological depending on their origin, but the most commonly used membranes are synthetic membranes (Ganiyu *et al.,* 2015). Although membranes serve the same purpose, which is to remove organic and inorganic contaminants, they are made up of different materials, which distinguish them from each other (Moreira *et al.,* 2017). Membrane materials are susceptible to destruction if exposed to chlorine, acidic pH and high alkalinity water, thus water may require some form of pre-treatment before being exposed to membrane processes (Ory *et al.,* 2020). Conventional treatment processes such as screening, clarification, disinfection, dechlorination, adjustment of pH and addition of scale inhibitors may be required to protect membranes and increase their life spans (Talvitie *et al.,* 2015). The subsequent subsections describe the most common membrane method used in water and wastewater and their history in microplastics removal:

Microfiltration (MF)

Microfiltration is a separation process with membrane sizes which ranges from 0.05 to 10 µm. They normally operate under pressure ranging between 140-200 Kpa for the removal of viruses, larger colloidal particles, precipitates and coagulants. Microfiltration has been widely used as pre-treatment for RO feed because of its ability to remove problematic fine particles, which causes RO membranes fouling (See Figure 2-2, typical fouled membrane). According to a study done by Talviet *et al.* (2017), Magnusson, and Wahlberg (2014) microfiltration was successfully used to remove 98% of microplastics in wastewater.

Figure 2-2: Fouled membrane

Ultrafiltration (UF)

UF is a separation membrane process with pore sizes ranging at 10 to 1000 nm. The membranes operate under pressure ranging between 200 to 300 Kpa. The membrane is likely to remove colour, proteins and dissolved organic material in water. UF has a molecular weight cut off range of 1000 to 100 000 kDa with typical transmembrane pressure of 100 to 700 Kpa. Concerning the study done by Ma *et al.* (2018) UF was used in conjunction with the aid of a coagulant. The coagulant removed 15% of microplastics while UF removed 90.9% of microplastics. UF has not been largely used in microplastics removal; thus, it has been used dominantly as RO pre-treatment. Although UF is utilised as a pre-treatment, it is capable of removing 96.97% of microplastics (Tadsuwan and Babel, 2022)

Membrane Bioreactor (MBR)

MBR is a common advanced wastewater treatment processes where biological treatment is combined with membrane filtrations. Membranes are configured to replace the clarification of effluent to produce high-quality effluent. MBR has been seen to benefit wastewater treatment across the world by reducing the need for more equipment while removing substantial amounts of suspended and colloidal solids. The significant benefit of MBR is that wastewater treatment capacity is improved without the requirements of more reactors. MBR can operate in the range 10000 to 12 000 mix liquor SS providing effective retention of SS. According to a study by Lvleva *et al.* (2018) in China, MBR removed 99.5% of microplastics in a feed influent of

13 particles/L. Further emphasis was also given to MBR in a study done by Lares *et al.* (2018) where the MBR was operated at a pilot scale with feed water containing 1.05 particles/L combined with activating sludge process. The average removal efficiencies were 99%-therefore, demonstrating the significance of advanced processes in microplastics removal.

2.6. The impact of microplastics in the environment.

The current section discusses the impact posed by microplastics on different environmental components and human health. Due to end route of discharged WWTW effluent containing microplastics into rivers, various environmental components are linked which ultimately affect human health. The impact of microplastics on surface water, aquatic systems, drinking water, soil, ground water and human health are discussed in this section.

2.6.1. Impact of microplastics on surface water quality

In recent reports, polychlorinated phenols (CPs) have been detected in sediments, surface water in Western Europe and Asia, more specifically in the USA originating from microplastics pollution (Jing *et al.,* 2018; Lloyd-smith, 2018). In Southern Africa, the presence of CPs in the surface water occurs in low concentrations as compared to Europe and Asia (Kampire & Rubidge, 2017; Moodley *et al.,* 2016). Although microplastics are known for containing chemicals such as PCBs, they are also known to have the ability to adsorb toxic substances such as metals (Aluminium, Cadmium, Chloride, Lead, Iron, Manganese, Fluoride, Sodium, Sulphates and Zinc) and pathogens (virus, protozoa and bacteria) on their surface which is influenced by pH variation and organic loading in surface water bodies (Desforges *et al.,* 2014, Wu et al. 2017, Seidensticker, 2018). In Japan, resin pellets were collected from the surface water for analysis of contaminants and results showed that microplastics surfaces also contained traces of endocrine disrupting chemicals such as PCBs, phenoxybenzoic acids (PBAs), phthalates and polybrominated diphenyl ethers (PBDE) (Wirnkor *et al.,* 2019).

The presence of microplastics in rivers changes the water quality regime and contribute to increasing toxicity. A study conducted by Ziajahromi (2018) indicated high

mortality rate of *C. dubia* when exposed to PET and PE fibres. Table 2-5 below present chemical compounds and toxicity description in water.

Table 2-5: Summary of toxicological impact of chemical composition of microplastics

Chemicals	Toxicity	Impact	Reference
PCBs	-Carcinogenicity	Cancer	Kumar *et al.,* 2020 and Choi *et al.,* 2004)
PBAs	-Immunotoxicity	Diseases	
PBDE	-Neurotoxicity	Brain damage	
Heavy metals	-Mutagenicity	Genetic mutation	

Microplastics also increases the pathogenic organisms in rivers by acting as carriers from effluent discharged (Walker *et al.,* 2022). The presence of pathogenic organisms such as *E.coli,* faecal coliforms and total coliforms indicate that water has microbial contaminants (Khan, 2020).

2.6.2. Impact of microplastics on aquatic species

Although microplastics are associated with water quality deterioration, their presence in surface water also causes problems in the aquatic biota (Issac & Kandasubramanian, 2021). The microplastics that accumulate in surface water end up in the aquatic food chain at both lower and higher trophic levels cite. Aquatic species ingest microplastics for their day-to-day food sources due to their shapes and sizes (Anderson *et al.,* 2016). Yu *et al.* (2020) studied several nematode species and their microplastics ingestion, focusing on the PS, the organisms showed injuries and damage within their stomach leading to poor metabolism and shock. It is also proven that injuries associated with ingestion of microplastics are also caused by polymers such as PES, PA, PP, PE, ethylene terephthalate, nurdles, pellets, and PVC ingested by aquatic species such as fish (Lei *et al.,* 2018).

Microplastics does not only cause intestinal damage in aquatic life, but they are also responsible for liver and kidney problems as well as reduced food intake and starvation in species such as turtle, Mollusca, fish and nematode (Hossain *et al.,* 2019; Banaee

et al., 2020 and Lo and Chan 2018). In Europe and Asia, there is evidence that edible aquatic species have traces of microplastics in their bodies, which affect their health (Nelmas *et al.,* 2016, Santillo 2017).

2.6.3. Impact of microplastics on soil and groundwater

Thermo-tolerant microplastics are resistant to treatment such as thermal hydrolysis and methanogenisis, therefore, are disposed of with the sludge used for fertilizers on arable land (Buta *et al.,* 2021). Biodegradable microplastics such as Polyhydroxyalkanoates (PHAs) and non-heat resistance microplastics generated from polymers such as PVC release chemicals during methanogenisis and thermal hydrolysis (Quecholac-Piña *et al.,* 2020). Chemical contaminants such as bisphenol A, phthalates, flame retardants and heavy metals released also end up in the soil when sludge is used as fertilizers (K. Kumar *et al.,* 2017). Through soil sorption and infiltration, contaminants reach groundwater in fractured zones. In South Africa, the presence of microplastics identified in groundwater systems of four boreholes in Potchefstroom is associated with sludge application in the agricultural field (Bouwman *et al.,* 2018).

Mineralization also occurs in the soil through microbial activities such as fungi and other organisms capable of decomposing organic and inorganic compounds, this leads to the release of contaminants into the soil and sorption by the plant (Kumar *et al.,* 2017). According to literature such as Pathan et al. (2020) and Wang *et al.* (2021), there are various biodegradable microplastics which release contaminants into the soil and groundwater. According to Su *et al.* (2021), microplastics were detected in soil and groundwater at Jiaodong in Peninsula. The study by Su *et al.* (2021) showed a positive correlation between the microplastics detected in the soil as well as groundwater with PE being dominant polymer.

2.6.4. Impact of microplastics on drinking water quality.

The source water for drinking water is rivers which already contain chemical compounds linked to microplastics such as endocrine disrupters and heavy metals (Singh *et al.,* 2022). The water treatment processes cannot fully remove contaminants such as PCBs, BPAs and heavy metals presence in source water, therefore, final

treated water may contain traces of microplastics and their contaminants. Microplastics are known to carry microorganisms, therefore, accumulations of pathogens may result in the formation of biofilms in distribution networks and reservoirs (Cholewinska *et al.,* 2022 and Chen et al., 2022).

Microplastics have been identified in drinking water treatment plants in metropolitan municipalities such as (Ekurhuleni, Mbombela, Tshwane, and Tlokwe) in South Africa with an average of 4 to 15 particles per litre (Bonthuys, 2018). The origin and sources of microplastics in these regions are still unknown.

2.6.5. Impact of microplastics on human health

Since microplastics and their associated chemicals are reported in surface water, groundwater, seafood and drinking water, their impact on human health remains a major concern (Claudia Campanale, Massarelli, *et al.,* 2020). The direct effects of microplastics on human health are still debatable due to limited data. However, research has shown that when microplastics are consumed with water and foodstuffs, they result in toxicity and affect the functioning of the immune system (Hirt & Body-Malapel, 2020). According to Issac & Kandasubramanian (2021), microplastics are associated with human health impacts such as infertility, hormone disorder, cancer, respiratory failures, stomach damage as well as skin irritation. The human health impact is linked to chemical toxicity such as additives (plasticizers) as well as toxic substances adsorbed in microplastics surfaces (Heavy metals).

Particles of microplastics ingested can also accumulate microbial pollutants in humans and animals (Hwang *et al.,* 2020). The accumulation of microplastics fibres in veins of the pulmonary artery has been reported in the human body, this can be linked with lung and heart conditions (Kannan & Vimalkumar, 2021). Leslie et al. (2022) also studied the presence of different polymers in 22 volunteers and the results showed that an average of 1.6 µg/ml of plastic particles were present in human blood. The particles may travel through veins and cause inflammations and heart disorders. The evidence of human stool containing microplastics particles is also an indication that they accumulate in the human body and cause inflammatory diseases (Mohamed Nor *et al.,* 2021).

Microplastics chemicals such as PVC and heavy metals such as lead are of high alert in water and food products (Wirnkor *et al.,* 2019). Chemical contaminants linked with microplastics cause health effects such as endocrine disruption, hormonal disorders, cancers, obesity, cardiovascular diseases and coronary heart diseases in humans (Gasperi *et al.,* 2018, Campnale 2019).

Aquatic species such as fish forms a larger part of the food chain in Asian countries and coastal areas, therefore microplastics can be transferred from seafood to humans. Ingestion of food and drinking water contaminated with microplastics may render contaminants to the human body, which can be detrimental to people living with underlying conditions such as cardiovascular conditions and Human Immune Virus (HIV) (Lehner *et al.,* 2018, Wright *et al.,* 2017, Toussaint *et al.* 2019, Cox *et al.,* 2019). South Africa has 14% of people living with HIV in 2017, People living with HIV are immunocompromised (Zuma *et al*., 2017). Consuming water, crops and aquatic animals contaminated by microplastics, and adsorbed chemicals may further exacerbate their conditions. (Naidoo *et al*., 2020).

2.7. Methods used for sampling and characterization of microplastics.

Microplastics sampling and characterization are comprised of different methods. In this section, different techniques used for collecting microplastics samples and characterization are discussed. The microplastics samplings entail systematic procedures followed in collecting water samples such as grab samples, net or plankton and filtration pumps. Although there are no uniform methods for the analysis of microplastics, researchers are still urged to follow methods in published research work to avoid errors and inconclusive results. It is, therefore, important to ensure that there are no organic materials that interfere with the analysis of microplastics samples. To eliminate organic materials and other particles, pre-treatment processes such as filtration, digestion and density separations are used before identification and analysis of microplastics (Xiang *et al.,* 2022). Characterization of microplastics can be done through identification and analytical methods. The identification of microplastics is done to determine the physical characteristics while analytical methods are used to determine the chemical characteristics of microplastics (Woo *et al.,* 2021). The

subsequent subsections give detailed description of sampling and characterization methods:

2.7.1. Microplastics samples collection

To decide on the most suitable sampling technique for the collection of water samples in WWTW, it is important to consider the objectives of the research and other characteristics such as designated sampling points. There are no harmonized sampling techniques. However, depending on the objectives of the study, researchers have opted to use methods such as composite sampling, grab sampling, plankton (Net) and filtration sampling (Claudia Campanale, Savino, *et al*., 2020; Hung *et al.,* 2021). Most researchers in WWTW are concerned with the occurrence of microplastics during low flows and high flows. This has influenced the systematic procedures that need to be followed when sampling microplastics in WWTW.

The most common methods for the collection of samples in wastewater are grab samples and auto samplers. The grab samples are usually collected from a point in WWTW at a specific period representing a single sample (Barrows *et al.,* 2017). The limitation of grab samples is that they are not representative of the actual conditions over 24-h period as the sample is collected once off for a period not exceeding 15 min (See Table 2-6). Grab samples are collected by lowering a bucket connected with a chain into the wastewater inflow, outflow or any other sampling points in the WWTW (Shan *et al*., 2022). When water samples are collected, further treatment is done for the removal of organic materials to enable the analysis of microplastics. The grab sampling technique has been used widely by many researchers. However, to determine the microplastics quantities such as loading rate, a 24 h representative sample is more accurate and reliable (Branch *et al.,* 2022). Auto samplers have been used in the collection of microplastics samples because of their ability to provide representative samples. The sampling of microplastics in wastewater is done through auto samplers through a pipe submerged into the water between 0.5m to 1m in depth connected to a smaller vacuum pump which creates suction and allows water to be transferred to sampling bottles at different intervals over 24 h. Studies such as Hung *et al.,* (2021) and Talvitie *et al*. (2017) have successfully used grab sampling and auto sampling methods in surface water and wastewater samples. Although grab

samples can be used in the collection of wastewater samples, in the current study auto samplers were used to collect samples to ensure that the estimation of loading rate is based on the incoming flow over 24 h.

Apart from grab and auto sampling, pump filtration and net (trawl) sampling are also utilised in the collection of wastewater samples. The use of manta trawl nets is common in the collection of microplastics samples in surface water such as rivers and oceans (Claudia Campanale, Savino, *et al.,* 2020). Hung *et al.,* (2021) used trawl nets to collect surface water samples. The manta trawl nets are made up of an aperture shaped in a box form with two wings tightened on the inside of the frame to lift the net frames to allow for efficient collection of samples on the surface water column (Karlsson *et al.,* 2020). The trawl sampling was also not considered because of its inability to collect a representative sample.

Filtration pumps are also common methods for microplastics sampling. As stated by Murphy *et al,* 2016 and Talvitie et al, 2016 using pump filtration can improve the number of microplastics collected from wastewater. However, due to its suitability to collect microplastics samples in larger surface water systems, the sampling method was not considered. The pump filtration method collects samples using a peristaltic pump which collects the water samples from surface water samples between 0-60 cm depth and passes them through various stainless steel sieves (Martin *et al.,* 2018). This technique enables the collection of samples at different flow rates.

Table 2-6: Summary of sampling methods for microplastics

Sampling methods	Advantages	Limitations	References
Manta trawl nets	-It is easy to collect microplastics samples using trawl nets -Larger particles of microplastics can be collected in a short space of time. -Trawl nets can collect microplastics to the smallest sizes up to 0.1 mm.	-Samplers require a boat to move across the sampling points in surface water sources, this is regarded as expensive. -The nets are susceptible to clogging.	(Claudia Campanale, Savino, *et al.,* 2020; Haris *et al.,* 2020; Hung *et al.,* 2021; Sedlak *et al.,* 2017; Shan *et al.,* 2022).
Filtration pumps	-Larger quantities of microplastics were collected. -Minimizes the sampling handling procedure. -Eliminate possible contamination.	-May require to be fitted in the boats for collection of large quantities of microplastics in oceans and rivers. -Samples may not be representative.	(Haris *et al.,* 2020; Hung *et al.,* 2021; Talvitie *et al.,* 2017b)

	-Good in alternating sampling locations in a flexible manner.	-Logistics are required for the transportation of samples.	
Composite auto samplers	-Samples collected are representative. -Eliminate contamination. -sampling handling is easy. -Volume of samples collected is well defined.	-Auto samplers are expensive equipment. -Installations require professionalism. -Maintenance is extensive and requires an instrument technician -The operations require an energy source.	(Claudia Campanale, Savino, *et al.,* 2020; Talvitie *et al.,* 2017b)
Grab samples	-Easy to sample and fast to collect samples. -Cheap sampling methods, requires less technical expertise.	- Samples are not representative. - Possible cross contamination of samples. - Not suitable for conditions where contaminants fluctuate.	(Barrows *et al*., 2017; Hung *et al.,* 2021; Shan *et al.,* 2022)

2.7.2. Microplastics pre-treatment

Wastewater contains different contaminants which may interfere with the identification and analysis of microplastics (Martinho *et al.,* 2022). The most common contaminants in wastewater are organic materials such as SS which may shield microplastics and create opaque conditions which make it difficult to characterize microplastics using techniques such as microscopy (Woo *et al*., 2021). Therefore, it is very essential to do pre-treatment of samples to isolate microplastics particles. According to Masura *et al.* (2015), microplastics sample pre-treatment is done through filtering, sieving, digestion and separation of particles using methods such as density separator (Park & Park, 2021).

Filtration or sieving are primary methods used for microplastics pre-treatment. The filtering is done to ensure that the targeted particles <5 mm are captured to distinguish them as micro-particles (Leslie *et al.,* 2022). Various filters can be used to filter microplastics, the most common ones used are the Whatman fibre class filters, polycarbonate filters and stainless steel sieves (Halfar *et al.,* 2021). To ensure that there are fewer interferences with sample analysis, most researchers are promoting the use of polymer-free techniques such as the use of stainless steel sieves or drying the sample after density separation or isolation of microplastics (Lenz *et al.,* 2021). Wastewater samples are high in contaminants; therefore, it is necessary to remove impurities that may affect the identification and quantification of microplastics particles.

Before the microplastics are identified and analysed, digestion of organic materials is done through methods such as enzymes, alkaline and acid digestion (Xiang *et al.,* 2022). The most common method used for wastewater samples is wet peroxide oxidation (WPO) which is followed by density separation to isolate microplastics from contaminants (Masura *et al.,* 2015a). The WPO is done by the addition of 30% of hydrogen peroxide (H_2O_2) in the sample and acid/iron sulphate to speed up the oxidation reaction, the process will digest organic material (Zobkov *et al.,* 2020). Some researchers opted to use potassium hydroxide; however, it caused degradation of microplastics, therefore limiting the concentrations of microplastics in the samples (Schrank *et al.,* 2022). The use of enzymes is limited because of the stringent conditions of pH and temperature which need to be

maintained. The process is also viewed as time-consuming due to experimental procedures to keep the conditions required. Therefore, the WPO was considered in the current research. The WPO is followed by density separation which allows microplastics to float on the surface of samples and can be easily collected for drying and further analysis (Nguyen *et al.,* 2022).

2.7.3. Methods used for characterization of microplastics

Microplastics morphology is complex, although some particles can be seen through the naked eye their shapes and sizes are different (Boyle & Örmeci, 2020). Some microplastics particles require the application of various techniques to describe their features and distinguished nature (Zhang *et al.,* 2018). To identify and classify microplastics, their characterization is essential. Microplastics have been identified in surface water, drinking water, groundwater, wastewater, sludge and other environmental parameters such as biota (Toussaint *et al.,* 2019; Ubomba-Jaswa & Kalebaila, 2020). The sizes and shapes of microplastics identified are very distinctive, therefore, this makes their characterization even more complex (Miller *et al.,* 2021*)*. A detailed discussion of microplastics characterization methods.

Various techniques have been applied in the characterization of microplastics which involves identifying the polymer class as well as the type (ALusher *et al.,* 2017). Microplastics classes include fibres, biofilms, micro-beads and pellets (McCormick *et al.,* 2016). The classes of microplastics are made up of different types of polymers which also requires rigorous analysis for their quantification in water samples (Rana Zeeshan Habib *et al.,* 2020). Microplastics identification methods and analytical methods are different and unique (Rana Zeeshan Habib *et al.,* 2020). Over the years, the identification of microplastics has been done through the use of microscopy while analytical methods such as spectroscopy and thermal analysis were used minimal due to cost.

The dissecting microscopy is used for microplastics analysis due to its ability to perform analysis on the three dimensions, allowing the observation of microplastics in two different ranges and angles providing a competitive advantage to obtain more than one image (Marine & Environmental Research Institute, 2015). At very low magnification, microplastics can be observed using light reflection. Most researchers

have detailed dissection microscopy as a fast and easy technique to use which allows the identification of the shape, sizes and colours of microplastics (Hamilton *et al.,* 2021). The dissecting microscopy, however, does not provide the polymer composition and the microplastics morphology (Wightman, 2020). Yoganandhan *et al.* (2019) successfully used dissecting microscopy to analyse and measure particle sizes in the water samples coupled with motic image plus analysis application. In the study by Yoganandhan *et al.* (2019), visual images were generated and dominantly fibres of different sizes were identified on the raw water samples and the final effluent samples. Other studies which used dissecting microscopy for the identification of microplastics include (Hamilton *et al*., 2021; Stolte, 2014; Wightman, 2020).

Another common technique used is scanning electron microscopy (SEM) which is capable of giving data on the formation and shapes of microplastics generating visual images with clear resolution of the microplastics surfaces (*Mariano et al.,* 2021). The SEM uses the image pixel, map resolution and magnification. This technique is used to provide the chemical composition of microplastics, sometimes equipped with energy dispersive spectroscopy (EDS) with primary and secondary electrons (Ricciardi *et al., 2021)*. The elements detection in the samples is the key in the image generation that provide the morphology of microplastics. in a recent study done in the united kingdom, microscopy and elemental characterization were performed using the SEM (Blair *et al.,* 2019). Although Blair *et al. (*2019) successfully identified different microplastics such as fibres and fragments, micro-pellets were deemed non-plastics due to the abundance of silica. Microscopy is deemed a common method for the identification of microplastics, some techniques are more suitable for investigating the toxicological effects on aquatic health (Dąbrowska *et al.,* 2021). The use of transmission electron microscopy (TEM) is a perfect example of a technique which has been used to assess growth inhibition, chemical content as well as elemental efficiencies and reaction generation with nitrogen and oxygen (Mariano *et al.,* 2021).

Contrary to microscopy, analytical methods such as Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy are used for polymer quantification (Veerasingam *et al.,* 2021). FTIR spectroscopy is an analytical method which has been used in majority of times in the microplastics characterization through chemical bonds

(Jung *et al*., 2018). The application of FTIR to particle characterization involves four procedures, namely: transmission, reflectance, adsorption as well as attenuated total Reflection (ATR) (Tagg *et al.,* 2015). The FTIR is non-destructive and enables the detection of chemicals that microplastics contain accurately (Cowger *et al.,* 2020). Another analytical method is Raman spectroscopy which uses the radiation and material which interact with each other producing a spectrum. Similar to FTIR, Raman spectroscopy is also a non-destructive technique with the capability of visualizing microplastics (Asamoah *et al.,* 2021). The setbacks of Raman microscopy are interferences and the cost of instrument maintenance coupled with the lengthy procedure which requires more time (Eberhardt *et al.,* 2015).

Researchers have also resorted to the use of thermal techniques due to the complexity of microplastic particles and thermal tolerance (Ivleva, 2021b). The main reason behind thermal methods is to enable the investigation of the changes in microplastics' physical and chemical characteristics which distinguish polymers from each other (Frigione *et al.,* 2021). The degradability of particles allows the identification of polymers. The most common thermal processes include differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) (Teh *et al.,* 2021).

DSC is the most common method applicable for thermal analysis studies. It has been used for the chemical characterization of microplastics, more concerned with the physical properties of microplastics (Fernández *et al.,* 2022). The changes in crystal and fluctuations in temperatures as well as enthalpy and entropy play a crucial role in determining the morphological characteristics of microplastics (Woo et al., 2021). The limitations of DSC are detailed in Table 2-7 which were considered when opting for FTIR in the current study.

The thermographic analysis is essential because it can qualitatively and quantitatively analyse samples by observing the changes in the mass of samples when exposed to higher temperatures through heating under specific atmospheric pressures (Mansa & Zou, 2021). Due to its limitation on polymer detection, TGA was not considered in the current study. Wastewater is composed of diverse types of polymers due to different sources such as industrial and domestic activities, therefore employing

a technique that is limited to specific polymers was not applicable. Although TGA alone has limitations, it can be coupled with various methods such as TGA-FTIR, TGA-DSC, TGA-MS and a combination of TGA-FTIR-GC/MS, this can enable the identification of various polymers (Nel *et al.,* 2021). There is a complex combinations required to analyse microplastics using TGA, therefore FTIR was considered.

The Py-GC-MS which analyses gases pyrolysed to determine the presence of polymers in the samples (Woo *et al*., 2021). The analysis of pyrolysed gases is contrasted to the standard polymers to confirm the presence of microplastics in the samples (Mariano *et al.,* 2021). The method is limited to the identification of PS, therefore could not be considered in the current study. A detailed summary of identification, analytical and thermal methods are given below in Table 2-7:

Table 2-7: Summary of microplastics characterization methods

Methods		**Advantages**	**Disadvantages**	**References**
Identification methods	Dissecting microscopy	-It is simple and produces results fast. -Efficient in morphological characterization.	-It enables the provision of chemical or elementary composition analysis of microplastics. -The plastic particles require further confirmation.	(Mariano *et al.,* 2021)
	SEM	-It provides high-quality pictures which can be used for microplastic quantification. -High-resolution pictures can be used for the morphology of microplastics. -Elemental composition of microplastics is achieved if used with EDX.	-Very costly method. -Can not provide detailed information on polymers. -Systematic procedures for analysis are lengthy.	(Mariano *et al.,* 2021; Woo *et al.*, 2021)
	TEM	-Provide resolution that allows for the detection of nanoparticles.	-Identification and analysis procedure is lengthy.	(Mariano *et al.,* 2021; Woo *et al.,* 2021)

		-It can provide the chemical composition of polymers if coupled with EDS.	-Extensive sample preparation is required on nanoparticles. -Expensive method and costly to maintain. -Can not be used for polymer identification, an additional method is required.	
	POM	-Unlike another microscopy, POM has been successfully used for polymer analysis.	-POM can not be used for samples that are dark or contain contaminants. -Polarized light intensity can be affected by the structure of polymers and thickness.	(Woo *et al.,* 2021).
Analytical methods	FTIR	-Simple to operate and high level of accuracy -The polymer library is diverse enabling instruments with high detection limits.	- FTIR analysis requires more time. -Can not detect particles >0.02 mm. -Not suitable for the detection of aged particles.	(Mariano *et al.,* 2021; Woo *et al.,* 2021; Xiang *et al.,* 2022)

		-Spectrums can be used to identify functional groups linked to the presence of microplastics.	-Not suitable for inhomogeneous material. -Expensive methods.	
	Raman spectroscopy	-Not affected by interference from water. -Spatial resolution is very high.	-Contaminants affect analytical efficiency. -Samples pretreatment is highly recommended	(Mariano *et al.,* 2021; Woo *et al.,* 2021; Xiang *et al.*, 2022)
Thermal methods	DSC	-DSC can be coupled with TGA for microplastic identification. -Enthalpy and entropy are distinguishing features that enable confirmation of physical properties of polymers, highly recommended over other methods.	-Limited to microplastics such as PE and PP. -Polymers with similar melting temperatures result in overlap in DSC.	(Fernández *et al.,* 2022; Mariano *et al.,* 2021; Woo *et al.,* 2021)
	TGA	-Can be used for qualitative and quantitative analysis.	-Phase transitions may overlap, therefore limiting the identification of polymers.	(Mansa & Zou, 2021; Nel *et al.,* 2021; Woo *et al.,*

				2021; Xiang *et al.,* 2022)
	py-GC-MS	-Sample volume can be as small as possible. -Can be used for both qualitative and quantitative analysis. -No addition of reagent is required, therefore reducing chemical costs.	-The experimental conditions are strict. -Limited to the identification of polystyrene (PS) derivatives. -Limited microplastics can be detected.	(Mariano *et al.,* 2021; Woo *et al.,* 2021; Xiang *et al.,* 2022)
Visual observation		-Cheap and easy method for microplastic identification.	-Only particles >1 mm can be visualised. -Organic contaminants may interferences. -Validity and reliability are questionable.	(Xiang *et al.,* 2022)

2.8. Legislation and guideline managing plastics

The responsibilities of municipalities is to enforce municipal bylaws which include the discharge of industrial effluent into municipal sewer systems (Hansen, 2015). The municipal bylaws, however, are more focused on physicochemical water quality parameters-the monitoring and standard for microplastics are not included. This results in large amount of microplastics find their way into municipal WWTWs (Booth *et al.,* 2020). The WWTWs are managed and regulated through WUL which stipulate the requirements for treated effluent discharged into the rivers (NEMA, 1998). The WUL standards as guided by NWA does not include the monitoring and standards for microplastics. Similarly, there is no microplastics standards for water intended for irrigation use in South Africa (Bouwman *et al.,* 2018).

Globally, there are no standards regulating the discharge of wastewater in the environment. However, through section 116376 of health and safety in the California Safe Drinking Water Act the state water boards starting from 2020 are compelled to analyse microplastics in drinking water (Health and Safety Code: 116376, 2018). The results on microplastics must be made available to the public for awareness.

CHAPTER 3:

RESEARCH METHODOLOGY

3.1. Preamble

The current study aims to assess the occurrence and removal options of microplastics in wastewater treatment processes. During the assessments of microplastics, sampling locations, sampling techniques and analysis methods play a pivotal role in the quality and reliability of the results.

In the current section, the study location which includes the sampling sites and their characteristics such as present economic activities, demographics and catchment conditions are described. Information on economic activities such as industrialization and urbanization is important in identifying the possible sources of microplastics pollution. Qualitative and quantitative research approaches have been widely used as the most common methods for data collection and analysis in this study. Data collection methods and sampling techniques are also described, including the justification for choosing specific procedures over the other ones.

After data is collected, it is significant for data to be analysed using scientific instruments, mathematical formulas, and statistical approaches. A brief description of microscopic analysis for microplastics characterization including a method for physicochemical analysis of water samples are described in the current chapter. In light of quantitative analysis, the statistical approach plays an important role in signifying the potential correlation and relationships across research variables, therefore it was also necessary to describe the statistical approach used.

Ethical clearance for the study was obtained from the UNISA College of Agriculture and Environmental Sciences (CAES) following the college ethics procedures. As part of the ethics clearance requirements, consent was obtained from Ekurhuleni Water Care Company (ERWAT) and their laboratory (ERLAB).

Due to COVID19 and the resultant staff limitations, Ekurhuleni Water Care Laboratory (ERLAB) was only testing essential samples. UNISA laboratories were operating under strict regulations for a period that extended beyond the research sampling

period. As such, the time frames set for research plans such as sampling and analysis were affected. The Council for Scientific and Industrial Research (CSIR) laboratories were utilised during this period.

3.2. Description of the study area

In the current section, a description of the study area is explained. This includes the geographic location of two WWTWs i.e., WWTW A and WWTW B. In addition to the location, the general description of the study sites such as sampling points, treatment technology, design capacity, operational capacity, water quality, catchments and potential sources of pollution such as industries connected to WWTW is explained.

3.2.1. Study site location

3.2.1.1. WWTW A

The WWTW A is Located at portion 4 of the farm 17IR in the East of Kempton Park at East Rand R25 (Bapsfontein Road). The community represented by the study area is Norkem Park which falls within the DD3 drainage district. The main catchment is the Limpopo Water Management Area (LWMA) in which the upper part of quaternary catchment number A21A is located (WWTW A Biomonitoring Report, 2022). The geographic coordinates of the area are (S26º 01’ 25.8 / E28º 17’ 10.0). See Figure 3-1 for a detailed map of the location (Arc GIS, 2010).

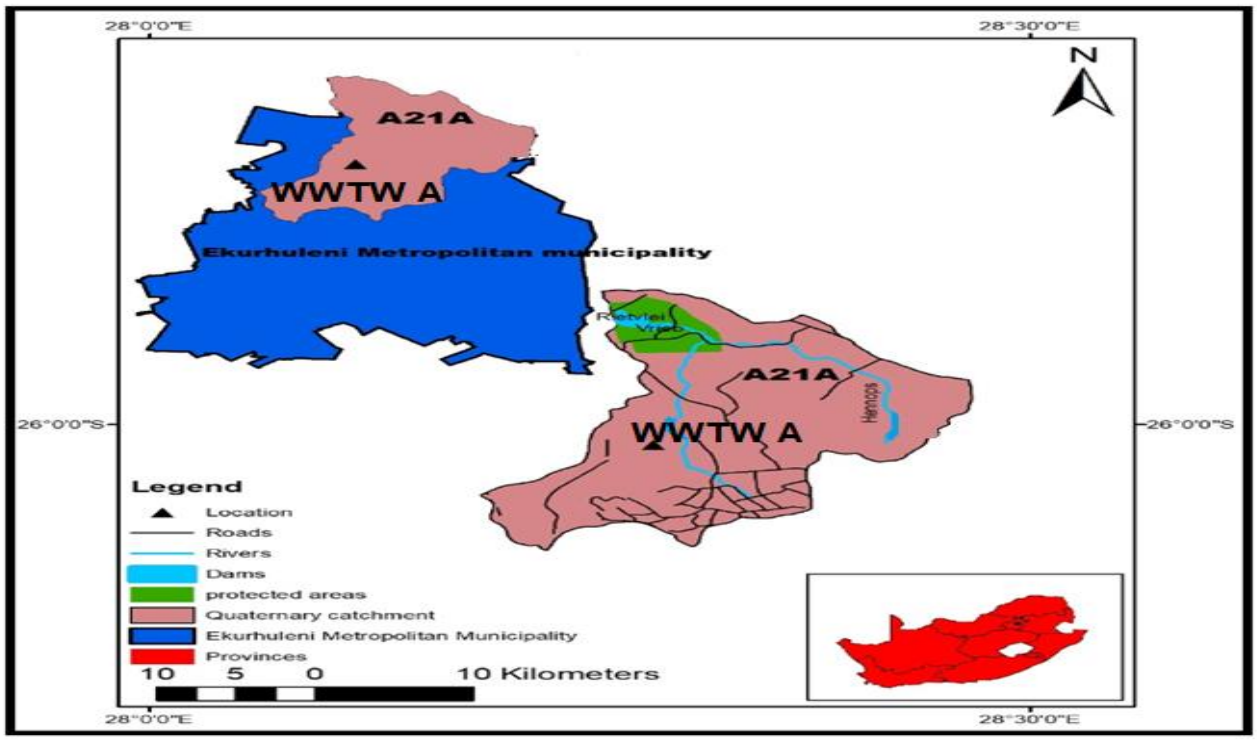

Figure 3-1: Map of the study area for WWTW A (Arc GIS, 2010).

3.2.1.2. WWTW B

The WWTW B is Located in portion 50 of the farm 150 IR in the South of Johannesburg, on the Midvaal R59 Road. DD6 drainage district of Vaal River Catchment (WMA) covers the study location. Klip River is the mainstream that drains into the Vaal River at Vereeniging, which is a tributary of the Orange River (WWTW B Water Quality Report, 2021). The geographic coordinates of the area are (S26º 01' 25.8 / E28º 17' 10.0). See figure 3-2 for a detailed map of the location (Arch GIS, 2010).

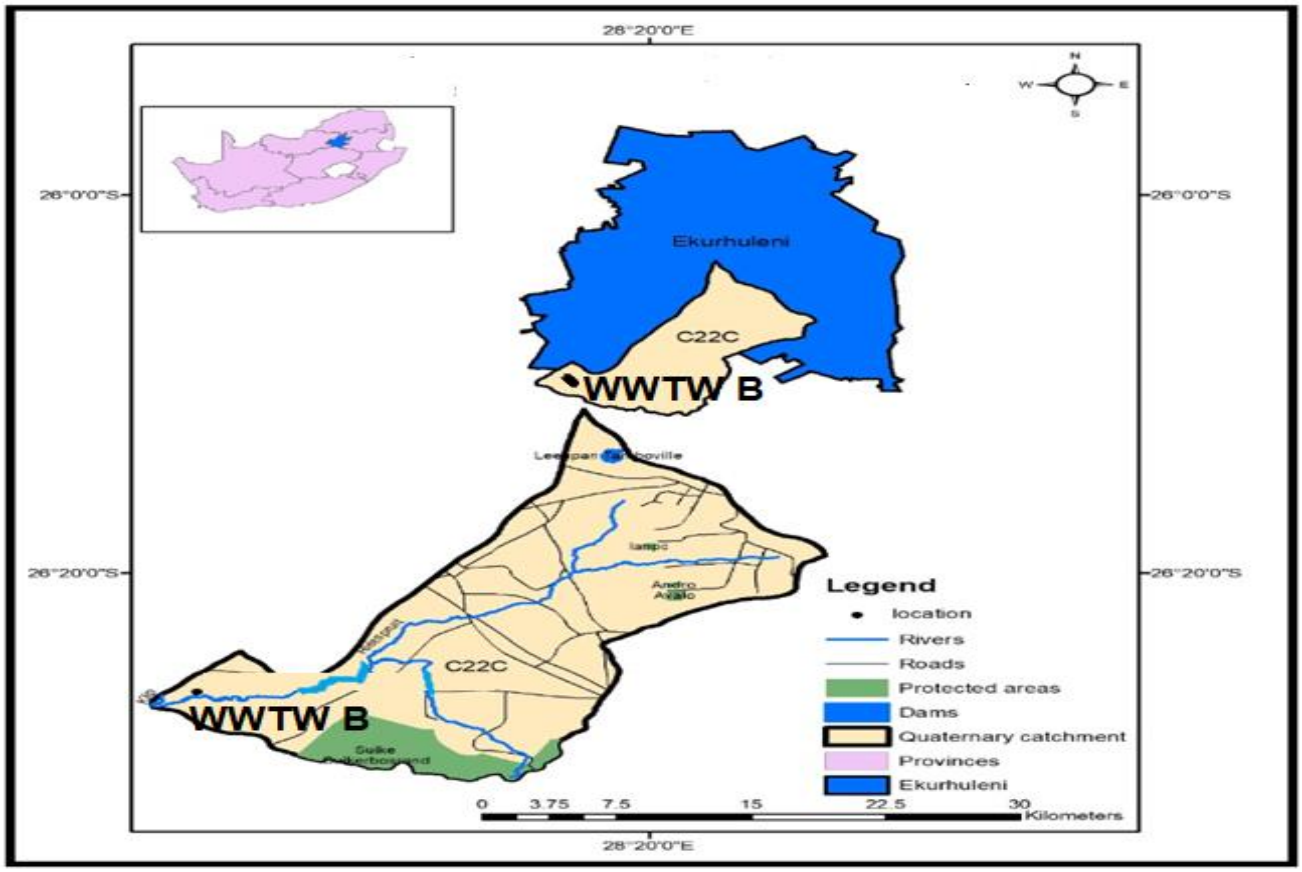

Figure 3-2: Map of the study area for WWTW B (Arch GIS, 2010)

3.2.2. General description of the study area and sample locations

3.2.2.1. General description of WWTW A

The WWTW A was designed to treat 63 ML/d, during the last financial year of 2021/2022, the works operated at 63 ML/d which is 100% of its capacity utilised (WWTW A Water Quality Report, 2021). Although the work operated at its design capacity, it is at risk of exceeding its design capacity. The treatment works receive its influent from 51 industries scattered around Kempton Park and 53 777 households with a population of 171 575 in Kempton Park. The influent is received in a proportion of 60% domestic and 40% industrial wastewater (WWTW A Manual, 2015). There is a potential source of microplastics from industrial and domestic influent which is received in the WWTW.

The WWTW A utilises a conventional treatment technology. It is comprised of inlet works equipped with three mechanical fine screens and degritting chambers for the

screen, sand and grit removals as a preliminary treatment process (WWTW A Manual. 2015). Preliminary treatment is followed by four primary settling tanks (PST) aimed at solid and COD reduction to reduce the loading into the activated sludge processes (Archer, 2018). The supernatant from PST overflows into activated sludge processes which are configured as University of Cape Town (UCT configuration) in treatment Section 1-3 and Modified Ludzac Etinger processes in treatment section 4 (WWTW A Manual, 2015). Although treatment configurations are different in the sections, the secondary treatment of the BNR process remains the same across the treatment units. In the secondary treatment, microorganisms are used to oxidise nutrients and organic matter. Overflow from the BNR is subjected to tertiary treatment using final settling tanks (FSTs). This is the final polishing step for the removal of SS before the effluent is disinfected using chlorine gas (WWTW A Water Quality Report, 2021). The wastewater residue such as waste activated sludge (WAS) and primary sludge from PSTs is treated in cold open digesters equipped with rotor mixers. However, the anaerobic digestion process is not heated (Eustina Musvoto, Nomvuselelo Mgwenya, Hazel Mangashena, 2018).

The final effluent and sludge treated at WWTW A are expected to comply with the Water Use License (WUL) granted in terms of the National Water Act (Act 36 of 1998) under Section 21. Although there is WUL in place, microplastics are not regulated. The final effluent treated at WWTW A is discharged to Rietspruit River which is a tributary of Hennops River draining to Rietvlei dam (WWTW A Biomonitoring report, 2022). Downstream of the Rietvlei dam, there is the Rietvlei water treatment which supplies drinking water to the City of Tshwane. The Hennops River and Rietvlei dam is experiencing deteriorating water quality with eutrophic conditions being the most prevalent (DWS, 2021). Microplastics are not regulated, but according to several authors, their presence in freshwater presents many problems in drinking water (Koelmans *et al*., 2019; WHO, 2019). Similarly, sludge at the works is irrigated on land, it is known that a high percentage of microplastics are present in sludge (Lusher *et al.,* 2017). This has the potential to contaminate groundwater and surface water through interactions (Re, 2019). The WWTW A was selected as one of the study sites because it is connected to 51 industries and 53 777 households, which are potential sources of microplastics in wastewater.

3.2.2.2. WWTW A sampling points location

The WWTW has two sampling points located at the inlet works (See section 1 of Figure 3-3) and a second sampling point located at the final effluent after the chlorine contact tank (See section 25 in Figure 3-3). The geographic coordinates for the sampling points are presented in Table 3-1 below.

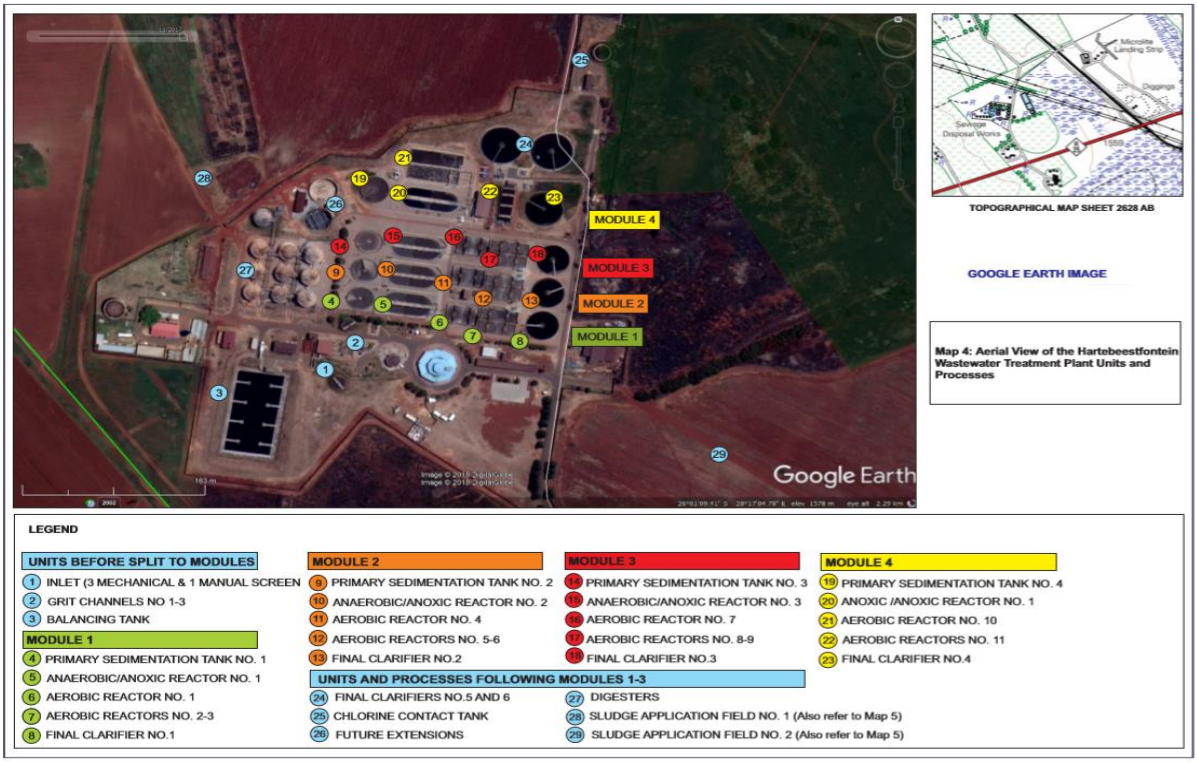

Figure 3-3: Aerial photo of the study area (Water Quality Report, 2021)

Table 3-1: Coordinates for sampling points.

Site name	Position		Location
	Latitude (S)	Longitude (E)	
Influent	26°01'.160''	28°16.977''	WWTW A
Final effluent	$26^0$0'58.6872",	$28^0$17'10.3488"	WWTW A

3.2.2.3. General description of WWTW B

WWTW B is designed to treat a maximum of 155 ML/d from primarily domestic households with an average population of 111 612 and 38046 households (WWTW B Water Quality Report, 2021). Only 5.41 ML/d (4%) of wastewater is contributed by industries. The wastewater treated is from domestic households in Everton and surrounding communities. The plant is comprised of four treatment modules with three each treating 35 ML/d and one treating 50 ML/d. The treatment process employed is conventional treatment with primary, secondary, and tertiary treatment mainly as the final stage (WWTW B Water Quality Report, 2021). Primary treatment consists of six mechanical screens and six pre-screen grit for screen and debris removal. It is also equipped with six grit chambers for sand and grit removal (WWTW B Process Audit, 2019). Four modules comprised of four primary settling tanks for further removal of SS and sand which escaped from grit chambers. Solids settle as raw sludge and are further treated in an anaerobic hydrolysis process, filter belt pressers' and paddies. The activated sludge process is used for secondary treatment for oxidation of nutrients in the presence of microorganisms (Eustina *et al.,* 2018). It also entails secondary clarification for further removal of SS and polishing of final effluent. The last treatment process is disinfection for the removal of microorganisms using chlorine gas.

The WWTW B is also regulated by the WUL. The final effluent discharged into Klip River must comply with the conditions of WUL (WWTW B WUL audit report, 2021). Klip River is a tributary of Vaal River Catchment. In the Vaal River, the water quality has deteriorated and the aesthetic impacts can be seen visible by unaided human eye. The water is brownish, showing evidence of pollution from partially treated sewage (Kruger-Frank, 2019). The study location was chosen because it discharges into a sensitive catchment prone to pollution, therefore making it important to safeguard the catchment from further deterioration.

3.2.2.4. WWTW B sampling points location

The WWTW B has two sampling points located at the inlet works (See section A1 of Figure 3-4) and a second sampling point located at the final effluent after the chlorine

contact tank (See sections 1 and 2 in Figure 3-4). The geographic coordinates for the sampling points are presented in Table 3-2 below.

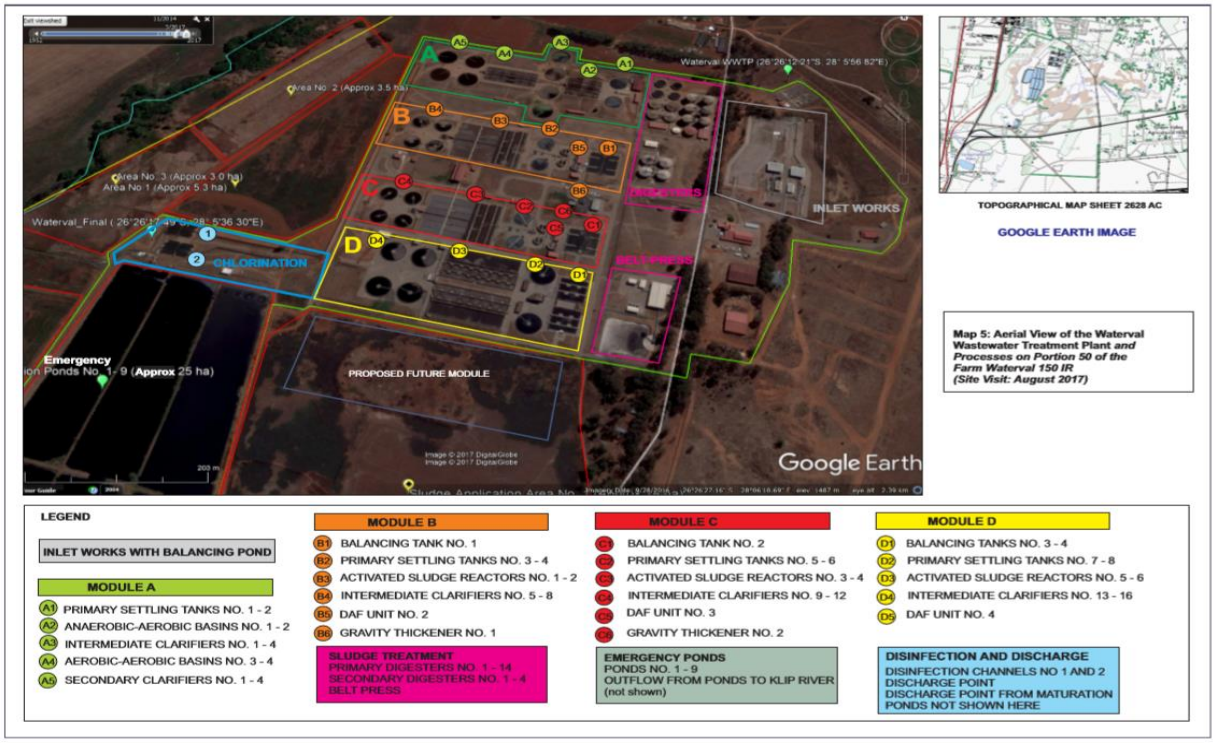

Figure 3-4: Aerial photo of the study area (Water Quality Report, 2021).

Table 3-2: Coordinates for sampling points.

Site name	Position		Location
	Latitude (S)	Longitude (E)	
Influent	26°26.212'	28°06.124'	WWTW B
Final effluent	$26^0$0’58.6872”,	$28^0$17’10.3488”	WWTW B

3.3. Research method

In the current research, both qualitative and quantitative methods were used for data collection and analysis, this is often referred to as a mixed method. The mixed method was used because it uses systematic procedures that enables data collection and

analysis using both qualitative and quantitative methods in one study to solve research problems as stated by Timans *et al.* (2019). To use mixed methods effectively, it was important to understand both qualitative and quantitative research methods.

The purpose of using quantitative experiments was for the generation of numerical water quality data and microplastics quantities which were analysed statistically and using mathematical formulas and software such as Pearson correlation component of Statistical Package for Social Sciences (SPSS). Quantitative analysis was also used to determine the chemical composition of microplastics particles (MPs) to confirm the presence of polymers and elements linked to the presence of microplastics in wastewater. To perform a quantitative analysis through a statistical approach, data need to be assessed for whether the data is distributed normally or not as stated by Franzese and Iuliano (2018). Data may be described as normal or not normally distributed. In the current research, Pearson correlation was considered for correlation analysis for the data that is normally distributed. In the case of data that is not normally distributed, a non-parametric statistical method can be used to determine if the significance level of p is within the range of <0.05 as shown by Sedlak *et al.* (2017).

A qualitative method was also utilized to identify and classify microplastics according to sizes, shapes and colours in the water samples. This was done through microscopic analysis at the selected laboratory. MPs were evaluated by observations under the microscope following the procedure stated by Masura *et al.* (2015). Relationships between microplastics identified and water quality were established.

3.4. Research design

A research design can be described as a formulation of research questions in order to ensure that the results obtained adequately address the research problems (Lewis, 2015). The most common research designs used are causal-comparative design, explanatory design, correlational design, descriptive design, exploratory and the convergent parallel designs which are described in details by authors such as (Suter, 2014; Creswell, 2018 and Berman, 2017).

Although there are various research designs methods, the convergent mixed parallel design was used for the analysis of data. The convergent mixed parallel design

enables the researcher to collect and analyse both quantitative and qualitative data simultaneously to solve research problems and answer the research question as stated by Dawadi *et al.* (2021). The convergent parallel design was used because both the qualitative and quantitative methods in the current research had equal value in understanding and solving the research problem. Both the qualitative and quantitative data were collected and analysed followed by linking the results and interpretation to establish the relationships between research variables. Figure 3-5 is a diagram showing the sequence of convergent mixed parallel design:

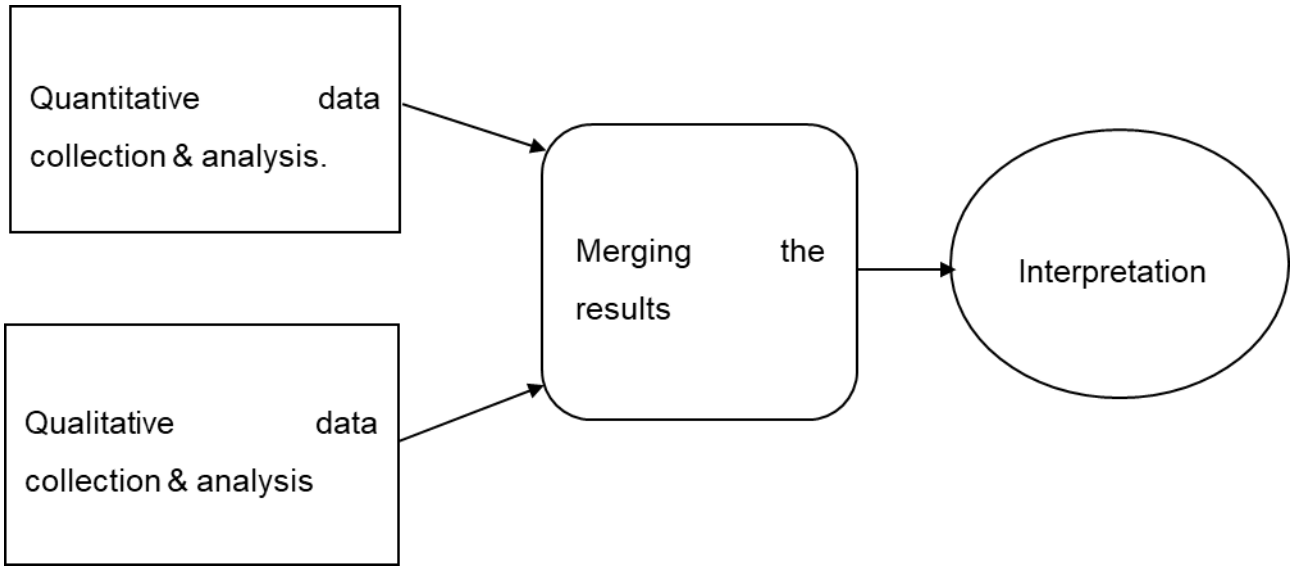

Figure 3-5: Systematic procedure for convergent parallel design (Schoonenboom & Johnson, 2017).

3.5. Sample collection

To sample water quality and microplastics, a standard guide for scientists investigating the occurrence of microplastics across matrices was used as described in Brander *et al. (*2020). Across the world, it is common to use auto samplers for wastewater sample collection in WWTWs as a standard method that guarantees homogeneity. Subsections 3.5.1 and 3.5.2 give a detailed description of the target parameters and sample collection method.

3.5.1. Microplastics Sampling at WWTW

Samples were collected from December 2021 to September 2022 to give representative samples over four seasons of the year i.e., summer, autumn, winter

and spring. A total of 32 samples were collected across WWTWs. Each season, 4 samples were collected in each WWTW A and B influent and final effluent, making a total of 8 samples per season and 32 samples over 4 seasons.

The microplastics sampling was done using the MS 10 composite auto sampler which is equipped with a 3-litre sampling bottle. Auto samplers were located at the inlet and outlet of two WWTW to collect samples from influent and final effluent. The MS 10 composite auto sampler was equipped with a vacuum pump which allowed the suction of water at 10-20 min intervals. The sampler timer was set to collect an equivalent of 2.5 litres of the representative sample over 24 h. A sample volume equivalent to 1 L was collected by opening the auto sampler dispenser pipe to fill up the glass bottle to its maximum capacity. The samples were preserved in a cooler box and transported to the CSIR laboratories for further analysis.

3.5.2. Physicochemical sampling at WWTW

A volume of 100 mL for analysis of each physicochemical sample was collected from the initial 1 L samples of microplastics to test for physicochemical parameters. The sample was collected from the initial samples to ensure uniformity and homogeneity in the sample representation. Precaution was taken was to ensure that the samples were stored in a cooler box maintained at 4˚°C between sample collection and analysis and that analysis at ERLAB was done within 6 h from the time of collection. Samples were collected from December 2021 to September 2022 to give representative samples over four seasons of the year i.e., summer, autumn, winter and spring.

3.6. Data collection

In this section a step-by-step systematic procedure followed for analysis of preliminary test, physicochemical and microplastics analysis is discussed.

3.6.1. Temperature and potential hydrogen (pH) analysis

Temperature and pH were measured onsite/in-situ using a digital HACH HQ40d multi-meter following the instrument instructions. The measurements of temperature were taken by switching on the instrument and putting it in temperature mode. The probe was submerged in the water sample and the values of temperature were recorded in

degree Celsius once the readings were stable on the meter display screen. The instrument mode was switched to pH to take measurements to continue with the analysis of water samples. To measure pH, the values on the instrument display screen were allowed to be stable while the probe is submerged in the water sample. The values on the instruments display screen were recorded allowing in-situ measurements of pH.

3.6.2. Physicochemical samples analysis

The physicochemical analysis parameters that were measured included SS, COD, inorganic ions and metallic ions concentration (Al, Cd, Cl^-, Pb, Fe, Mn, F^-, Na, and Zn). A detailed description of the COD, SS and metals procedure followed for wastewater sample analysis is discussed in the subsections below:

3.6.2.1. Total Suspended Solids analysis

SS analysis was done using the standard method 2540 D for water and wastewater 23rd edition by Rice *et al. (*2017). To perform SS analysis 100 mg/L standard solution was prepared using distilled water with conductivity <0.5 mS/m. The standard solution was used to do a quality control test for SS. The 1 L glass bottle was shaken to mix the sample. A volume of 250 mL of sample was transferred from a 1 L bottle to a 500 mL glass tube. The Rundfilter MN 85/90 filter papers with a diameter of 110mm and pore opening of 0.5µm purchased from Merck group were prepared and placed on the filtration unit with the wrinkled side placed side up. The filter was washed with 20 mL of deionised water three times using a vacuum in the filtration unit. The volume of 250 mL of sample was shaken and poured in through the filter while the vacuum is sucking the water entering the filtration unit. The filter was taken from the filtration unit using forceps and placed in the oven for 1 hour at 105 °C. The dried filter was allowed to cool down for 30 min inside the desiccator. The dried filter and the filter without dried particles were placed on an analytical balance and weighted to the nearest 0.001g. The SS was calculated using equation 3-1:

$$SS = \frac{(m1 - mb) \times 1000000}{V} \qquad \textit{Equation 3-1}$$

Where:

mb= mass of filter paper in g;

m1= mass of filter paper plus dry residue in g;

V= volume of sample used in mL.

The SS results were reported as mg/L (at 105 °C).

3.6.2.2. Chemical Oxygen Demand (COD) analysis

A colorimetric method was used for the analysis of COD following the standard guide for water and wastewater by Rice *et al.,* (2017). A 100 mL Sample from a 1 L glass bottle was transferred to a 100 mL tube. The solution of potassium acid phthalate (KPH) was prepared by adding 85 g of previously dried chemical at 120° °C in 1 L of distilled water. The solution was allowed to dissolve. This was followed by the addition of 2 mL of KPH solution into a 100 mL tube containing the sample. A volume of 3 mL of COD_2 reagent was added. The sample was heated for 2 h and allowed to cool to room temperature. Dichromate ions ($Cr_2O_7^{2-}$) were reduced to green chromic ions (Cr^{3+}). The sample tube was placed in the adapter of the colorimeter and COD measurements were taken.

3.6.2.3. Toxic metals analysis

To confirm the presence of heavy metals in wastewater, ICP-MS was used as a tool for data analysis. This spectrometry tool used the ionization capacity to analyse the water samples (Wilschefski & Baxter, 2019). It created an atomic ion that allowed the detection of non-metals and metals on the samples. This tool was used due to its capacity to precisely detect metals without alteration from non-metals.

Toxic metals analysis (Al, Cd, Cl^-, Pb, Fe, Mn, F^-, Na, and Zn) were done using the standard method 2540 D for water and wastewater 23rd edition by Rice *et al.* 2017. The stock solution for Al, Cd, Cl, Pb, Fe, Mn, F, Na and Zn was prepared by pipetting 50 mL of the stock solution into a 500 mL A-grade volumetric flask. A volume of 5 mL of HNO_3 was added to the A-grade volumetric flask containing the stock solution. The deionised water was added to make up the volume of the A-grade volumetric flask to full. The solution was used to calibrate the Inductively Coupled Plasma Mass

Spectrometry (ICP-MS). A volume of 50 mL of sample was transferred from a 1 L bottle to an Erlenmeyer flask. A volume of 10 mL of concentrated HNO_3 was added to a 50 mL sample inside the Erlenmeyer flask. The sample was digested on a hotplate to a volume ranging at 10-20 mL before precipitation happened. The concentrated HNO_3 was added while heating the sample continuously until the solution was light and clear. The clear solution was removed from the hotplate and allowed to cool down. A volume of 50 mL of the solution was transferred to the volumetric flask. The ICP-MS was warmed up for 20 min prior to loading the sample. The sample tubing was placed from the peristaltic pump into the rinse solution, the pump started up automatically after clicking the plasma button on the ICP-MS. The ICP-MS analysed each sample by ionizing the sample, which creates atomic ions that were detected to process metal analysis. The results of each metal analysis were reported in mg/L.

3.6.3. Analysis for microplastics

In this section a step-by-step systematic procedure followed for analysis of microplastics in wastewater is discussed in detail below and presented in Figure 3-6.

3.6.3.1. Microplastics samples pre-treatment

The steps for microplastics pre-treatment are given below:

I. Step 1: Filtering the sample in the laboratory

Samples collected from an auto sampler in 1 L glass bottles were filtered into the glass beaker using the Millipore xx2004708 stainless steel sieve with 5 mm pores purchased at Merck group. Materials retained from a 5 mm stainless steel sieve were discarded. The filtering of samples was done to remove particles greater than 5 mm which are not considered microplastics. Stainless steel sieves were used because they do not contain plastic materials that may contaminate the samples.

II. Step 2: Wet peroxide oxidation (WPO)

The WPO procedure for removal of organic contaminants to isolate microplastics in wastewater samples was done as stated by Masura *et. al* (2015) and Saipolbahri *et al. (2020)*. The removal of organic materials was done to reduce interferences which

may cause errors during microplastics identification. To remove organic contaminants, 100 mL of wastewater sample was taken from initial filtered water in step 1 of pre-treatment. The 100 mL volume of wastewater samples was transferred into a 250 mL glass beaker. To catalyse the reaction, 20 mL of iron sulphate at (0.05 M of Fe (II)) was added to a 250 mL beaker containing a 100 mL water sample. Then 20 mL of H_2O_2 at 30% concentration was added. The mixture was allowed to stand for 5 min at room temperature prior to heating at 75° °C on a hotplate. The beaker was removed from the hotplate as soon as gas bubbles were observed, and the sample was allowed to stand for 30 min. The organic compounds were degraded and only microplastics remained due to their resistance to peroxide oxidation. The H_2O_2 was continuously added whenever the organic material remains visible on the sample, more especially the influent wastewater. For safety precautions mask and gloves were used when handling the H_2O_2 reactive mixture.

III. Step 3: Separation and recovery of microplastics

After the removal of organic contaminants, the solution of wet H_2O_2 was transferred into a density separator and allowed to settle over 24 h. The settled organics were discarded, and the clear sample was transferred into the 250 mL glass beaker for drying.

IV. Step 4: Drying the microplastics samples

The 250 mL glass beaker containing microplastics sample free from organic material was placed in the Memmert vacuum oven-PM400 at 90°C for 72 h to ensure proper drying. The MPs remained on the surface of the glass beaker.

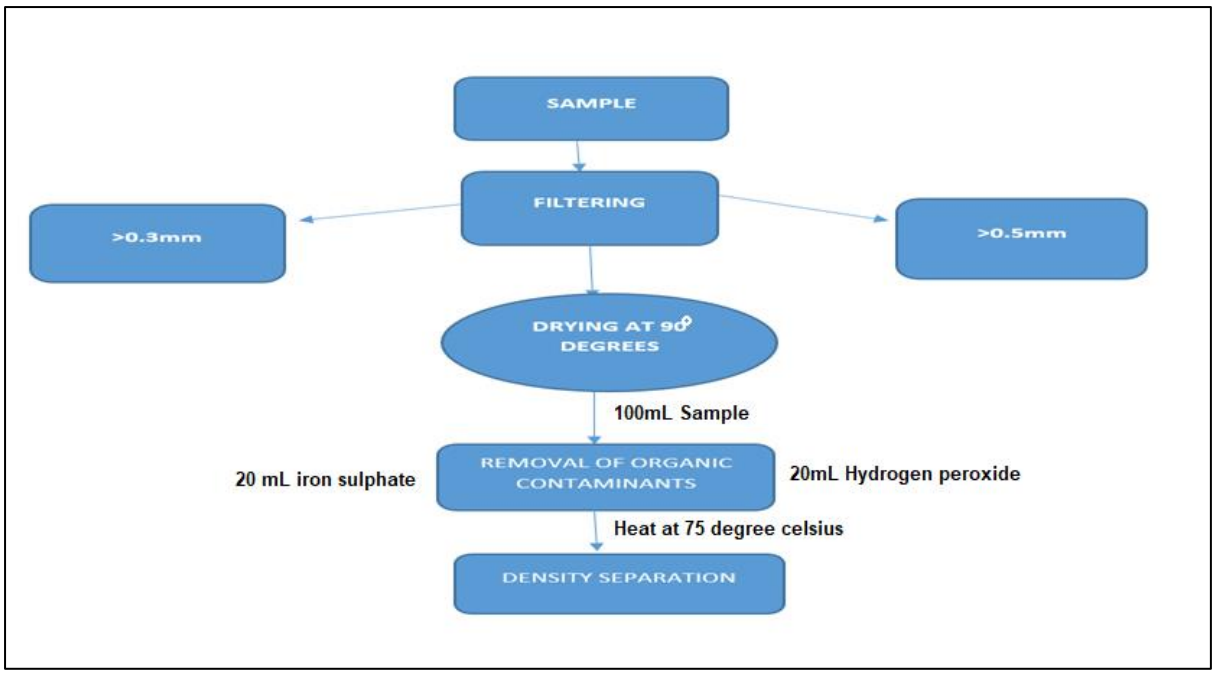

Figure 3-6: Microplastics pre-treatment and WPO (Masura *et al.,* 2015)

3.6.3.2. Characterization of microplastics

The characterization of microplastics included the morphological and chemical properties of microplastics as well as their quantities in wastewater samples. Morphological characterization was done using the SEM and the polarized light microscope (POM). To do an elementary analysis of microplastics, FTIR was used to identify polymers and the SEM coupled with EDX was used to perform an elementary analysis of MPs.

I. Morphological Characterization

To identify and classify microplastics under POM and SEM microscope, the procedures by Carolina & Crime, (2015) and Frandsen, (2016) were used as detailed below.

(a) Morphological characterization using POM.

POM was used for the identification of microplastics based on the shapes and colour of the observed particles. POM can rapidly identify microplastics in wastewater (Nguyen *et al.,* 2021). Due to its optical capacity which allows less magnification of

samples analysis, the POM allowed light reflection on microplastics surfaces for better identification. The high level of credibility of the results was vested in the instrument due to its ability which provided several viewing angles on both sides of observation eyepieces. Microplastics were observed and identified on a three-dimensional view to allow more accurate and precise observations. *The step by step working procedure for POM is described below:*

Step 1: Magnification of the POM microscope was adjusted to 40X using the top light.

Step 2: The MPs were placed on the POM microscope stage micrometre with a rectangular size of 25 mm × 75 mm.

Step 3: Two ocular lenses of the microscope were adjusted through the bottom object lens until the MPs on the micrometre were seen.

Step 3: The objective lens was used to observe the specimen (micrometre with dried MPs), this is often referred to as rough focus which was used to compensate for any variance in strength by alternating left and right eyes to obtain the best image focus.

Step 4: The dried MPs on the micrometre were observed and images were taken to categorise microplastics based on the colour and shapes of fragments, fibre, film, micro-beads, and foam.

(b) Morphological characterization using Scanning Electron Microscope (SEM/EDX).

For physical identification of shapes and sizes of microplastics, scanning electron microscope was used. The first procedure was to open the specimen chamber on the scanning electron microscope to allow the specimen stage to be visible. Microplastics particles were transferred from petri dish into the specimen stage of scanning electron microscope coated with carbon at 50 nm to avoid conflicting with peaks of other compounds. The chamber on the scanning electron microscope containing specimen was closed and the pressure equalization was maintained by switching on the vacuum pumps for 5-10 seconds at 10^{-3} Torr. The scanning electron microscope coupled with energy dispersive X-ray was operated at 15 kV voltage. To produce primary electron, the magnification was set between 400-6400× and the sample was scanned with a

beam at high energy with map resolution of 512 by 384. The scanning electron microscope coupled with energy dispersive X-ray was operated at backscattered imaging mode where the primary electron produced was allowed to interact with the samples to produce secondary electrons, backscattered electrons and X-rays. Scanning electron microscope and X-ray detectors were allowed to collect signals from electrons and X-rays to create images at pixel size of 0.06 µm and maximum magnification of 5200 which were displayed on the computer monitor. Below is a step by step systematic procedure for SEM/EDX analysis:

Step 1: The specimen chamber on the SEM/EDX was opened to allow the stage to be visible.

Step 2: The MPs were placed on the specimen stage and the chamber was closed.

Step 3: Vacuum pumps were switched on for 5-10 s to perform pressure equalization inside the specimen chamber and MPs were focused on the electromagnetic lens inside the column for electron beam generation.

Step 3: The magnification was set between 400-6400× and the sample was scanned with a beam at high energy to produce primary electron.

Stage 4: The primary electron was allowed to interact with the samples to produce secondary electrons, backscattered electrons and X-rays.

Step 5: The SEM detector and X-ray detectors were allowed to collect signals from electrons and X-rays to create images which were displayed on the computer monitor.

II. Chemical characterization

A detailed discussion of the step-by-step systematic procedure for chemical characterization is discussed below:

(a) Polymer identification

To characterise the spectra of samples, Perkin Elmer Spectrum 100 spectrometer was used. It was also important to achieve the required signal-to-noise ratio, therefore the FTIR spectra of all microplastics samples were measured in the range of

550-4000 cm^{-1} and 32 scans were made per spectra which were co-added and averaged. In all the samples, the spectra resolution was maintained at 16 cm^{-1}. The FTIR was composed of various reference spectra of polymer which included PE low density, PVC, polycarbonate carboxylic acid amine salt film and other spectra not identified on the samples. Below is a step-by-step systematic procedure used for polymer identification using FTIR as stated by Kovač Viršek *et al.* (2016):

Step 1: To avoid contamination, the detection unit was cleaned with alcohol and a clean cloth free from fibre to avoid contamination.

Step 2: The MPs were placed on the glass micrometre with a rectangular size of 25 mm×75 mm.

Step 3. The control joystick was used to position the specimen and MPs were scanned on the sample table.

Step 4: The optical photo was taken and the location where the sample was characterised was marked.

Step 5: The upper spectrum representing the measured particle against the lower spectrum representing the searched substance was read on the database. Then the polymer types identified were recorded.

(b) Functional group identification using the FTIR spectra

To identify the functional groups for each polymer identified in the wastewater samples, the FTIR wavelength peaks between 1500-4000 cm^{-1} were identified and linked to IR correlation tables. The FTIR functional group region ranges between 1500-4000 cm^{-1} which was used to identify alcohol, hydroxy compounds, acids, saturated aliphatic (alkyne) and olefinic (alkene) mostly composed of single, double and triple bonded compounds.

(c) Elemental particle analysis

The elemental analysis was done using the SEM/EDX analysis. This was done to identify elements such as heavy metals that are linked to the presence of microplastics in water. The presence of elements such as Chloride, Aluminium, Manganese, Zinc,

Iron, Silicon and heavy metals on microplastics present the potential of microplastics to distribute contaminants. The step-by-step systematic procedure for SEM/EDX has been explained above on morphological characterisation. The identification of elements was based on the reaction of primary electrons which interacted with samples to create secondary electrons, scattered electrons and X-rays. The X-ray detector was used to detect elements as stated by Hamm *et al*. (2018).

3.6.3.3. Quantification of microplastics.

The gravimetric method adapted from Masura *et al.* (2015) was used to determine microplastics loading while the ImageJ 1.53K adapted from Houck (2019) was used to quantify microplastics concentration. Below is a step-by-step systematic procedure followed for the quantification of microplastics using the gravimetric method and ImageJ 1.53K.

I. Gravimetric method

The gravimetric analysis was done by determining the mass of microplastics which was used to calculate the Loading rates.

II. Mass of microplastics

The mass difference between the beaker and the dried microplastics particles was calculated to eliminate the weight of the beaker. Firstly, the beaker without MPs was weighted on the analytical balance and the beaker with microplastics particles was also weighted. The mass of microplastics was reported in mg/L. Equation 3-2 from Masura *et al.* (2015) was used to calculate the total mass of microplastics:

$$Total\ mass\ \left(Microplastics\ in \frac{mg}{l}\right) = \frac{(A-B)\times 1000}{Sample\ Volume,mL} \qquad \textit{Equation 3-2}$$

Where:

A= Mass of the beaker with dried solids

B= Mass of the tarred beaker

III. Microplastics Loading

Equation 3-3 below was used to determine the microplastics loading. The mass of microplastics to average flow received per day and discharged by the WWTW was calculated to determine the loading to the WWTWs and receiving water bodies.

$$Microplastics_{loading\ in\ (\frac{kg}{/d})} = M_{microplastics} \times A \qquad \textit{Equation 3-3}$$

Where:

M= Mass of microplastics in mg/L

AF= Average flow in ML/d

IV. ImageJ 1.53K particle count

The ImageJ 1.53K java-based application by the National Institute of Health (NIH) of the USA was used to perform particle counts to determine the microplastics concentration in the influent and final effluent of WWTW. Below is a step-by-step systematic procedure followed to count MPs on SEM and POM images:

Step 1: Import the image from the computer.

The ImageJ 1.53K software installed on the computer was opened from the desktop taskbar. Once the software is running, the file tab was used to navigate to import images. The sequence images were imported from the saved file in the computer and uploaded to ImageJ 1.53K. The image was displayed below the software taskbar for easy editing.

Step 2: Editing the image.

The image was edited to ensure that the MPs on the image are visible enough for processing and particle counts. The image was edited by setting the threshold colour to white and saturation ranges of 120 to 255. The image size was interpolated as bilinear at a pixel width of 481 and height of 332 with a constrained aspect ratio and average when downsizing. The image contrast and colour balance were adjusted to 50%.

Step 3: Processing and analysing particles.

The particles were processed by setting the image to smooth and sharpened mode. The edges on the image particles were determined automatically on the ImageJ application. The particles were analysed by setting the pixel from 0 to infinity and circularity ranging between 0.001 to 1. The application analysed the particles automatically by pressing an ok button and the results summary table was displayed with particle counts of microplastics in MP/L.

Step 5: Exporting the edited image from the application.

The edited image was exported and saved on the computer file to compare with the original image from POM and SEM microscope.

3.6.4. Removal efficiencies of microplastics

To determine the removal efficiencies of WWTW for microplastics, the concentration and loading of microplastics quantified at the influent and effluent were used. Equation 3-4 was used to determine the removal efficiency of microplastics based on the microplastics concentration, adapted from Conley *et al.,* (2019) while Equation 3-5 was used for the calculation of removal efficiencies based on the microplastics loading rate.

$$RE = \left[\frac{C_{MP,influent} - C_{MP,effluent}}{C_{MP,influent}}\right] \times 100\% \quad \textit{. Equation 3-4}$$

Where:

RE= Removal efficiency.

$C_{MP,\ influent}$= Concentration of microplastics in the inflow into the plant in MPs/L and mg/L.

$C_{MP,\ effluent}$ =Concentration of microplastics in the final effluent discharged into the environment in MPs/L and mg/L.

$$RE = \left[\frac{L_{MP,influent} - L_{MP,effluent}}{L_{MP,influent}}\right] \times 100\% \quad \textit{.Equation 3-5}$$

Where:

RE= Removal efficiency.

$L_{MP,\ influent}$= Loading of microplastics in the inflow into the plant in kg/d.

$L_{MP,\ effluent}$ = Loading of microplastics in the final effluent discharged into the environment in kg/d.

3.6.5. Statistical analysis of microplastics and correlation between organic loading and physicochemical parameters

The statistical analysis was performed on physicochemical water quality and metal concentrations to establish the existing correlations from samples collected in the influent and effluent of WWTW A and B. The Origin pro 2022 with correlation plot software v1.31 was used to determine the correlation coefficient and its P-values. The Pearson correlation coefficient value (r) was used to determine the strength and direction of the relationship between data i.e., microplastics and physicochemical water quality. The strength and direction of relationship was determined by comparing the actual Pearson correlation coefficient (r) calculated values to the standard (r) values ranging from smaller (0.1-0.3), medium (0.3-0.5) and high (>0.5). The Pearson correlation coefficient was also used to determine the significance between correlated data i.e., physicochemical water quality, metals to microplastics concentrations. For all the r values showing positive correlation, the significance P-values was considered to be above 0.05.

3.7. Validity and reliability

Validity and reliability of results were done by ensuring that the laboratory instruments used are calibrated and testing is done at an approved laboratory (ERLAB and CSIRLAB). This was achieved by doing instrument calibration using standard samples. The guidelines for sampling were followed and samples were preserved to ensure no deviation from the time of sampling to the analysis at laboratory. The contamination of samples was avoided by wearing the required sample handling clothing such as latent gloves. A Series of blank samples were also conducted during sample analysis at the laboratory. The procedure for microplastics blanks as stated by De Witte *et al*. (2014) was followed for microplastics. An air filter to avoid air-borne

contamination was used to vacuum the sample analysis environment. The observation of microplastics and their presence were confirmed using polymer standards loaded in the FTIR library. Reliability was also done through the Statistical Package for Social Sciences (SPSS) using the component of the ANOVA F test.

CHAPTER 4:

RESULTS AND DISCUSSIONS

4.1. Introduction

This chapter reports on the results of primary data collected from wastewater samples at two selected WWTW (i.e., WWTW A and B) at the influent and final effluent. The preliminary physicochemical tests results conducted during sample collection such as temperature, pH, COD and SS are first to be presented and discussed in this chapter followed by the results interpretation and discussions for heavy metals. Thereafter, microplastics characterization in terms of morphology, chemical properties and quantification is discussed. After the quantification of microplastics, the removal efficiencies of two activated sludge processes for WWTW A and B are determined, results and discussions are presented in the current section. Finally, the statistical analysis results are also discussed in this chapter to determine the influence of preliminary test as well metals on microplastics concentrations and loadings. The empirical findings of the current research are also compared with the available literature of studies done in South Africa and across the world. Most importantly, literature pertaining the occurrence and removal of microplastics in wastewater and the impact therefore to the receiving environment as well as implications to human health.

4.2. Preliminary physicochemical tests for wastewater

The preliminary physicochemical tests done include temperature, COD, SS and pH on the influent and final effluent of wastewater samples on selected WWTW.

4.2.1. pH test results

The results are presented in Figure 4-1 using bar graph to show seasonal concentrations i.e., summer, autumn, winter and spring. Figure 4-1 shows the seasonal variations of pH for WWTW A and WWTW B. During summer season, pH at WWTW A ranged between 7-7.1 in the influent and 7.6-7.7 in the final effluent while at WWTW B ranged were between 6.8-7.1 in the influent and 7.7-7.8 in the final

effluent. There was a slight variation of pH in the influent and effluent between the two WWTWs.

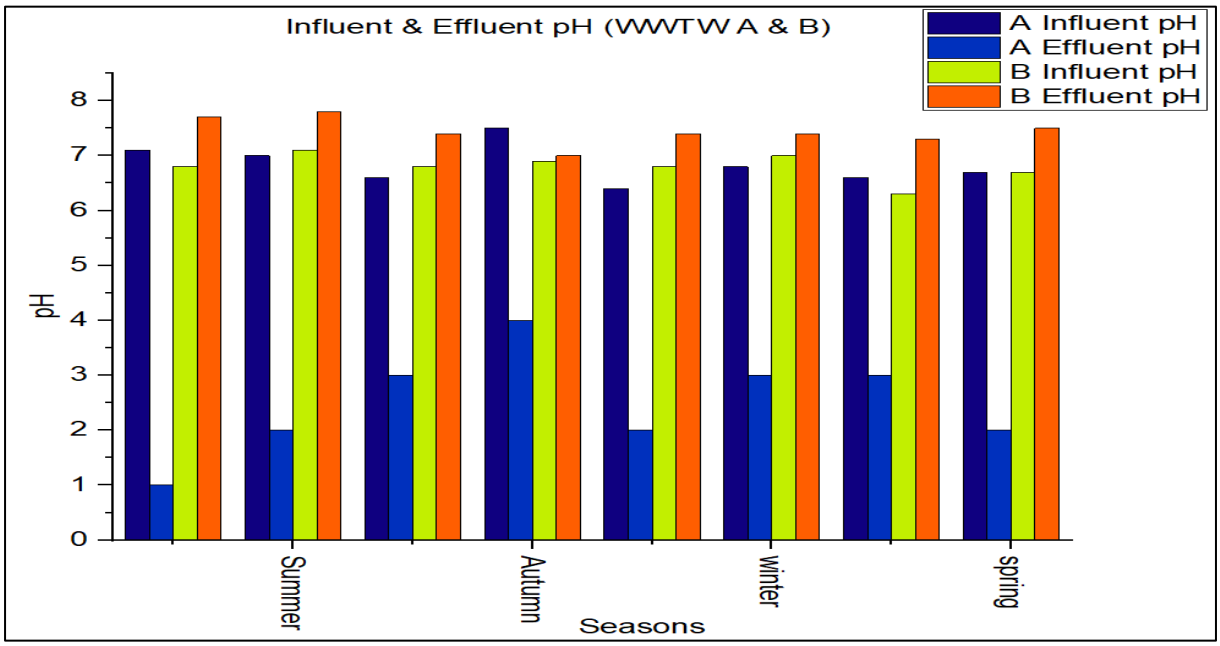

Figure 4-1: Seasonal pH for WWTW A and B influent and effluent for the period December 2021 to September 2022.

During autumn season there was a slight decrease in the influent pH at WWTW A ranging between 6.6-7.5, however the effluent ranged between 7.1-7.5 also showing slight decrease as compared to summer. The influent pH for WWTW B in autumn ranged between 6.8 and 6.9 while for the final effluent was 7-7.4, there was a decrease in the effluent pH in autumn as compared to summer.

The WWWT A showed a similar pattern of pH in winter and spring, with more acidic pH in the influent ranging between 6.6-6.7 and 6.8 respectively, the final effluent was still comparable to summer and autumn with ranges between 7.5-7.6. For WWTW B there was variation in pH over winter and spring seasons. The influent pH was in the range of 6.8.-7 in winter and 6.3-6.7 in spring, however the final effluent for both seasons ranged between 7.4 –7.3-7.5. Although pH seems to vary in the influent and effluent of wastewater, it is noticeable that the influent is more acidic except at WWTW B during the summer season.

According to Seidensticker *et al.* (2018), microplastics have the ability to adsorb contaminants onto their surfaces and the pH ranging 6-7 is has been identified as the

optimal for the ma transfer of the pollutants from the with liquid to the surface of the microplastics. Therefore, it is important to monitor the seasonal variations of pH in order to determine the possible hydrophobic conditions in wastewater samples. Across summer, autumn, winter and spring, pH ranged between 6.6-7.1 in the influent and 7.1-7.8 in the final effluent. The influent pH is within the ranges stated by Seidensticker *et al* (2018), therefore likely to create sorption conditions for contaminants.

4.2.2. Temperature test results

Figure 4-2 shows that the temperature of influent and final effluent ranges between 18-19 °C except at WWTW B influent were a maximum of 20 °C was noticeable in winter season. There is no huge variation in temperature of influent and effluent at WWTW A and B, all seasons showed similar patterns.

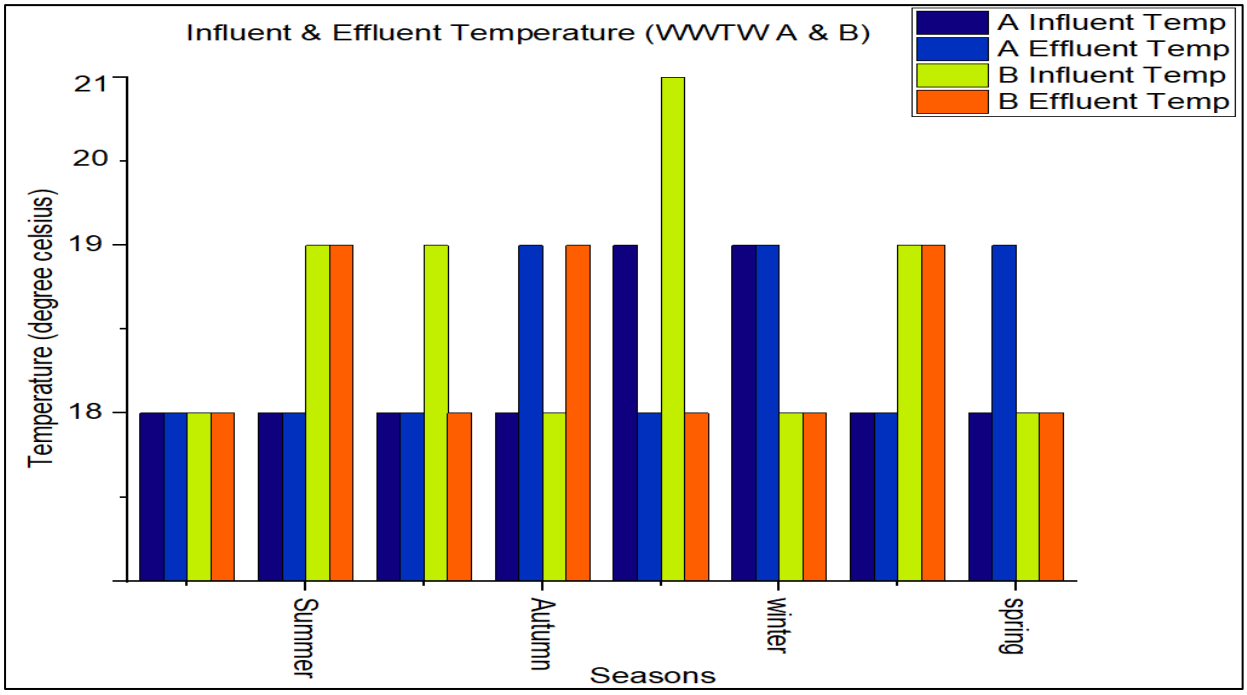

Figure 4-2: Seasonal Temperature for WWTW A and B influent and effluent for the period December 2021 to September 2022.

Due to variation of MPs concentrations during hot and cold seasons, it is important to monitor temperature in wastewater (Dalu *et al.,* 2021). The variations in temperatures of wastewater influent and discharged effluent also influences transport and uptake of microplastics (Williams, 2020).

4.2.3. Chemical oxygen demand (COD) test results

Figure 4-3 shows COD seasonal pattern. The WWTW A showed high COD concentrations in the influent across four seasons with ranges of 185-1139 mg/L (Summer), 669-1009 mg/L (autumn), 579-669 mg/L (winter) and 640-778 mg/L (spring) in the influent. However, the effluent concentrations are lower than influent which is attributed to treatment processes such as primary settling and secondary settling tanks that removes large amount of organic material contributing to high COD. In summer, the effluent COD ranged between 10-25 mg/L as opposed to autumn, winter and spring where ranges were between 35-45 mg/L, 45-98 mg/L and 45-59 mg/L respectively. Although there is a peak of 98 mg/L of COD, the final effluent is lower than influent COD for WWTW A seasonal samples.

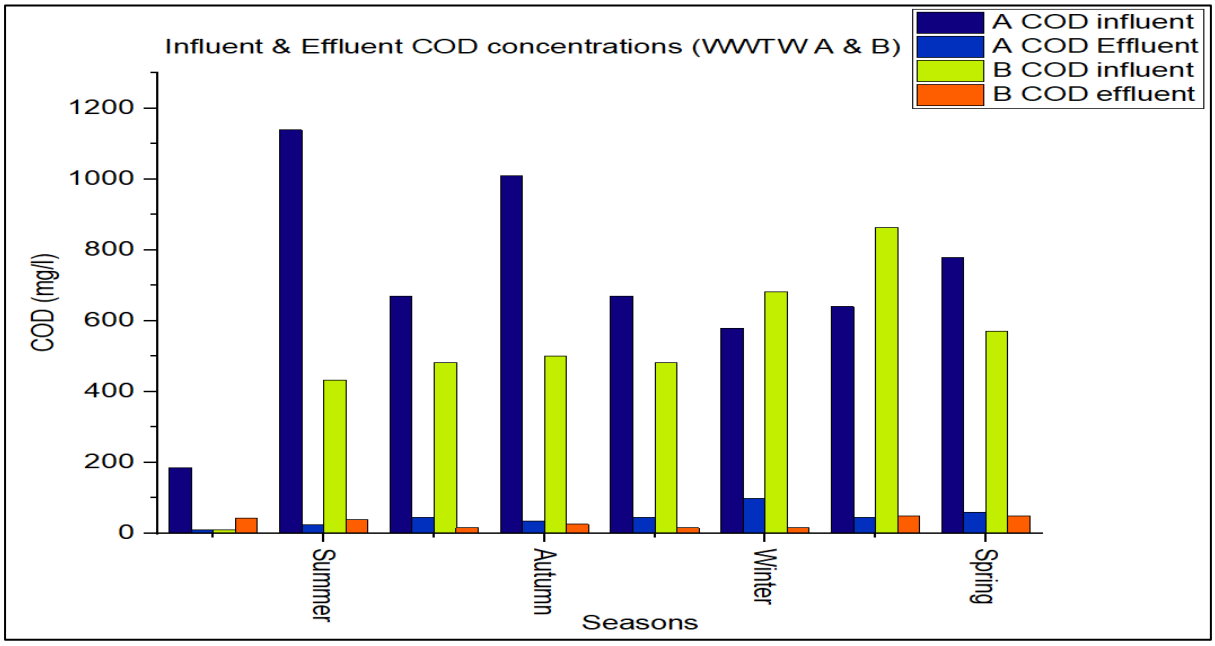

Figure 4-3: Seasonal COD for WWTW A and B influent and effluent for the period December 2021 to September 2022.

The WWTW B has low influent COD as compared to WWTW A, this was attributed to the fact that WWTW B is not connected to industries, while WWTW A is connected to large industrial grid with total of 51 industries. The COD for WWTW B INFLUENT ranged between 10-432 mg/L (summer), 482-500 mg/L (autumn), 482-682 mg/L (winter) and 570-863 mg/L (spring) most likely higher than final effluent. The final effluent COD for WWTW B on most occasions across autumn and winter is lower than the concentrations at WWTW A except in the summer and spring seasons where it

ranged between 38-43 mg/L and 48 mg/L. It is noticeable that during spring, the final effluent COD at WWTW B was influenced by high COD concentrations in the influent. The WWTW A receives high COD during summer, autumn, and winter WWTW B receives high COD during spring. The variation of COD concentrations in the influent shows a direct impact in the final effluent COD, there is direct proportional relationships between influent and final effluent concentrations.

According to Kwon *et al* (2022), COD is regarded as an indicator of organic content that is directly linked to various microbes and microplastics. Kwon *et al* (2022) further highlighted that organic elements associated with high COD are removed in activated sludge processes, microplastics are also reduced, and this is likely due to particles hydrophobic characteristics. Nikolopoulou *et al* (2023) stated that microplastics present various bacteria's when discharged to the environment, this is mainly due to degradation in processes that takes place in the bioreactors or such as filtration processes.

4.2.4. Suspended solids (SS) test results

Figure 4-4 present the seasonal variations in the SS concentrations for influent and effluent at WWTW A and B. The WWTW A has high concentration of SS across all the seasons in the influent with ranges between 105-257mg/L (summer), 189-215 mg/L (autumn), 166-215 mg/L (winter) and 117-146 mg/L (spring), contrary to high SS at influent WWTW A can reduce SS in the final effluent with a range recorded between 10-15 mg/L across all seasons.

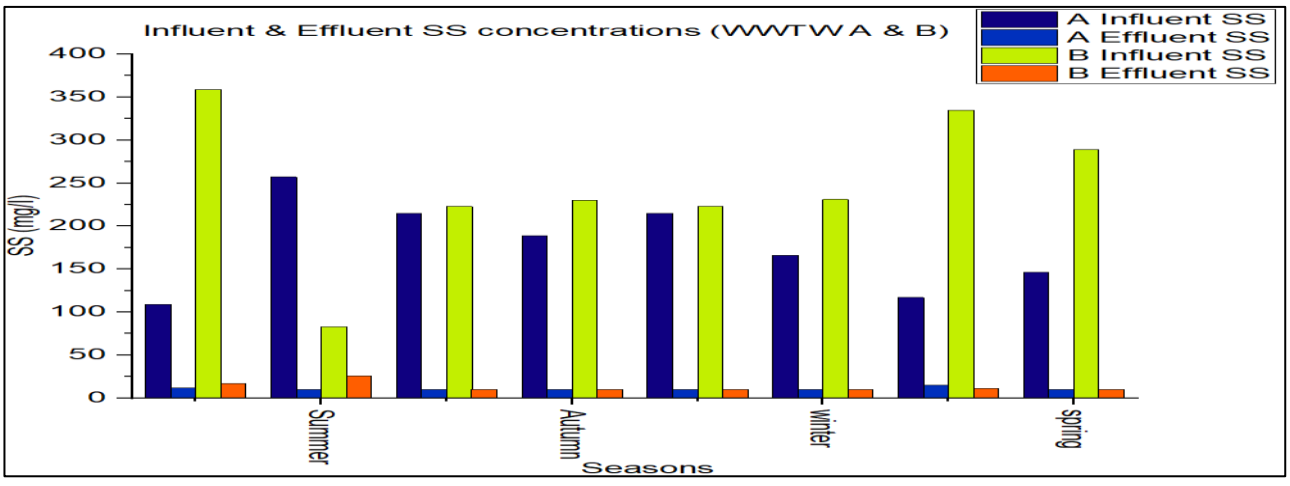

Figure 4-4: Seasonal SS for WWTW A and B influent and effluent for the period December 2021 to September 2022.

The WWTW B influent SS ranges between 83-359 mg/L (summer), 223-230 (autumn) 223-230 mg/L (winter) and 289-335 mg/L (spring). Influent SS concentrations at WWTW B is higher than WWTW A across all seasons. The final effluent SS concentration is low in WWTW B ranging between 17-26 mg/L (summer), 11-10 mg/L (autumn, winter and spring). The final effluent SS concentrations at WWTW B is higher than WWTW A during summer, this attributed to high flows which the plant receives with operational capacity reaching maximum of 389 ML/d and above during wet seasons, resulting into high weir overflow rate in the final settling tanks which lead to solid carry overs.

SS is also an important parameters that indicate the organic concentrations and loading. According to Kwon *et al* (2022) SS in wastewater is known to contain large amounts of microplastics and other non-plastics particles, therefore a positive correlation suggest that the discharge of effluent with high SS is an indication of microplastics contamination.

4.2.5. Toxic inorganic parameter analysis for wastewater

A total of six metallic species (Al, Cd, Pb, Fe, Mn and Zn) and 2 non-metallic species (Cl^- andSO_4^{2-}) were analysed at WWTW A. While at WWTW B, a total of one metal and three non-metallic species were monitored and analysed, amongst them include Cl^-, S, SO_4^{2-} and F^-. Figure 4-5, 4-6 and 4-7 below present the seasonal results obtained for analysis of non-metallic parameters and heavy metals at influent and effluent of WWTW A and B.

4.2.5.1. Non-metallic parameters for WWTW A

Figure 4-5 presents the seasonal concentrations for Cl and SO_4^{2-} at WWTW A influent and effluent. The concentrations of Cl^- and SO_4^{2-} are high as compared to Al and Cd and this is attributed to 40% influent emanating from domestic households, with a potential to contain high levels of chlorides and sulphates. The concentrations of Cl^- in the influent ranged between 51-52 mg/L (summer), 53 mg/L (autumn), 49-50 mg/L (winter and spring). The effluent concentrations for Cl^- ranged between 48-51 mg/l (summer), 54 mg/L (autumn), 45-48 mg/L (winter) and 47-53 mg/L (spring). There is

a slight decrease of Cl^- concentrations from influent to final effluent at WWTW A. According to Figure 4-5, WWTW A has high concentrations of SO_4^{2-} in the influent with a slight decrease in the final effluent. The influent concentrations of SO_4^{2-} ranged between 75-146 mg/L (summer), 72 mg/L (autumn), 70-114 mg/L (winter) and 72-92 mg/L (spring) while the effluent concentrations ranged between 34-56 mg/L (summer), 63 mg/L (autumn), 50-55 mg/L (winter) and 50-63 mg/L (spring).

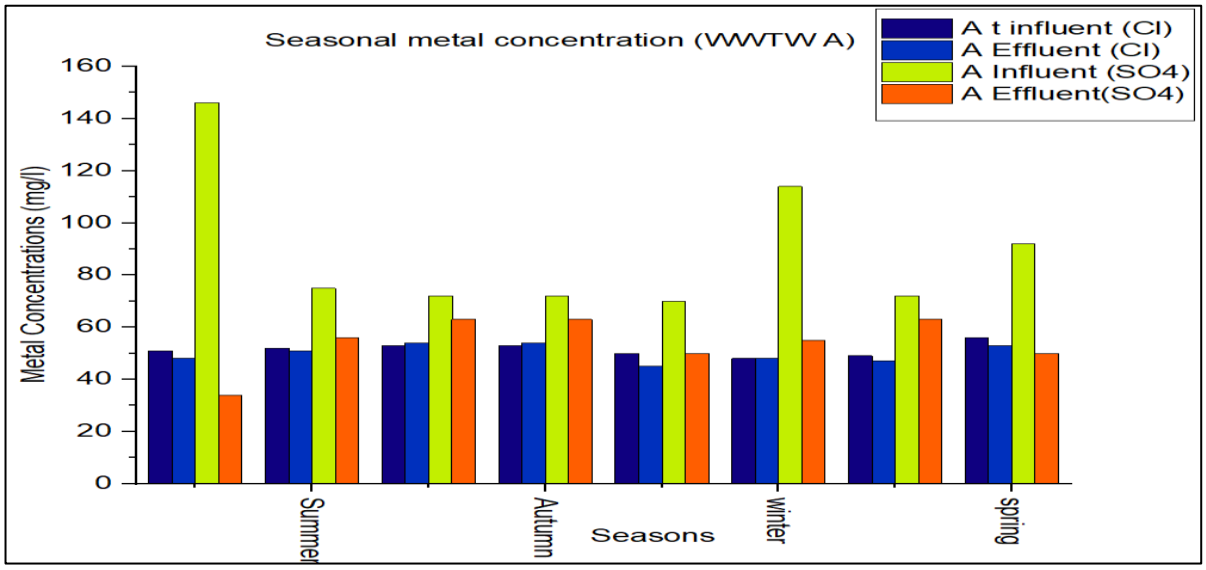

Figure 4-5: Seasonal non-metal concentrations for WWTW A influent and effluent for the period December 2021 to September 2022.

4.2.5.2. Metallic analysis for WWTW A

Figure 4-6 presents the results for Al and Cd for both influent and effluent. The concentration of Al in the influent ranged between 0.2-0.34 mg/L (summer) and 0.2-0.4 mg/L (autumn, winter, and spring). The effluent Al concentration at WWTW A was 0.1 mg/L across all seasons. The concentration of Cd in the influent and effluent was 0.04 mg/L across all seasons at WWTW A.

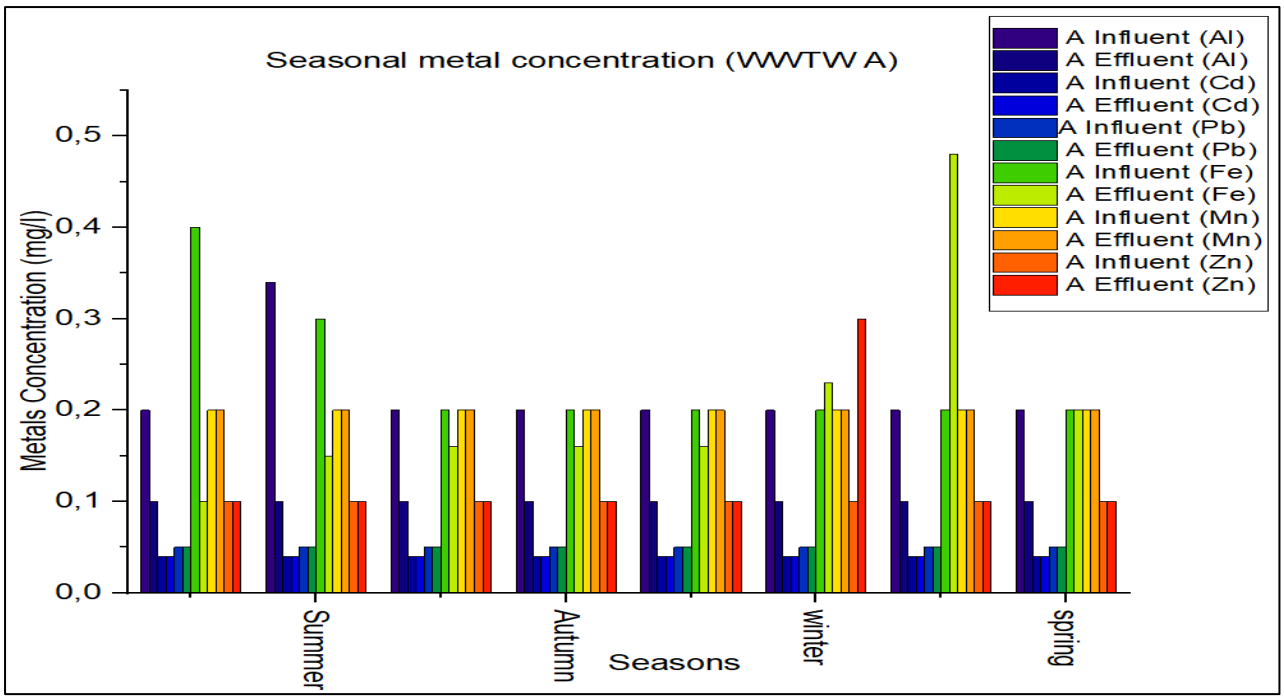

Figure 4-6: Seasonal metal concentrations for WWTW A influent and effluent for the period December 2021 to September 2022.

The influent and effluent Pb concentrations was recorded to be 0.05 mg/L at WWTW A across four seasons. Fe concentrations were slightly variable across four seasons in the influent with concentrations ranging between 0.3-0.4 mg/mg/L (summer), 0.2 mg/mg/L (autumn), 0.2-0.23 mg/L (winter) and 0.2 mg/L (spring). The Fe concentrations in the effluent was slightly lower during summer and autumn ranging between 0.15-0.16 mg/L. There was an increase in the effluent concentrations for Fe in winter and spring to 0.23 mg/L and 0.48 mg/L respectively at WWTW A. The concentrations of Mn and Zn in the influent was ranging between 0.1-0.2 mg/L across all four seasons, there was only single peak of 0.3 mg/L recorded in the final effluent of WWTW A. All monitored heavy metals shows higher concentrations in the influent and a slight decrease in the effluent concentrations. There is not much variation between influent and effluent concentrations, therefore the WWTW biological processes do not fully remove heavy metals.

4.2.5.3 Non-metallic analysis for WWTW B

Figure 4-7 presents the results for WWTW B non-metallic concentrations over summer, autumn, winter and spring. According to Figure 4-7, chloride and sulphur are highly variable in the influent and effluent of WWTW B. The concentration of Cl^- in the

influent ranged between 91-103 mg/L (summer), 82-101 mg/L (autumn), 82-84 mg/L (winter) and 88 mg/L (spring) while the effluent concentration ranged between 57-97 mg/L (summer), 54-95 mg/L (autumn), 45-82 mg/L (winter) and 94-103 mg/L (spring). There is slight drop in chloride concentrations in the effluent accept in the spring where effluent is higher than influent at WWTW B. The concentration of sulphur varies across the seasons in the influent with concentrations ranging between 102-124 mg/L (summer), 106-109 (autumn), 78-87 mg/L (winter) and 89-105 mg/L (spring). The effluent concentrations of sulphur ranged between 102-103 mg/L (summer), 67-80.4 mg/L (autumn), 89-95 mg/L (winter) and 80-124 mg/L (spring). The concentrations of sulphur on the effluent are slightly higher than influent during winter and spring at WWTW B.

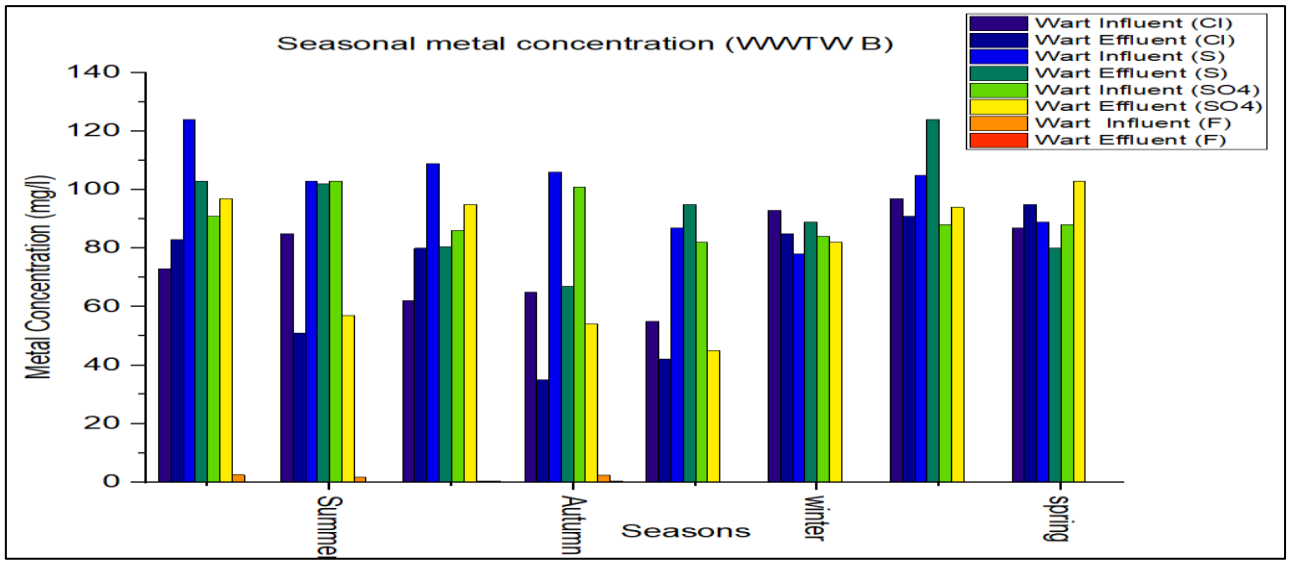

Figure 4-7: Seasonal non-metal concentrations for WWTW B influent and effluent for the period December 2021 to September 2022

The concentrations of sulphates ranged between 91-103 mg/ mg/L (summer), 86-101 mg/ mg/L (autumn), 82-84 mg/ mg/L (winter) and 88 mg/ mg/L (spring). During summer, autumn and spring, the effluent concentrations of SO_4^{2-} is slightly higher than the influent. The concentrations ranged between 57-97 mg/L (summer), 54-95 mg/L (autumn), 45-82 mg/L (winter) and 103 mg/ mg/L (spring). Seasonal concentrations of fluoride ranged between 2.5-1.8 mg/ mg/L (summer), 0.3-2.4 mg/L (autumn), 0.1-0.13 mg/L (winter) and 0.13-0.18 mg/L (spring). The hghest influent concentrations are recorded in summer and autumn seasons while winter and spring shows lower concentrations. The effluent concentrations of fluoride are lower than the

influent, the concentrations are ranging between 0.21-0.24 mg/L (summer), 0.31-0.44 (autumn), 0.14-0.18 mg/L (winter) and 0.2-0.21 mg/L (spring). Overall, there is higher concentrations of metals in the influent and recorded lower concentrations in the effluent of WWTW B. Some concentrations of non-metals are slighly higher on the effluent as compared to influent, more specifically sulphur and chloride.

Microplastics can be carrier of heavy metals due to hydrophobic characteristics, the wastewater discharged may posed threat to receiving waters ecological regime and through interaction in the environment, the wellbeing of humans can be compromised (Liu *et al.,* 2021). At WWTW A and B, heavy metals were detectable in the influent and effluent of wastewater, the likelihood of metal distribution by microplastics was high due to their occurrence even after the wastewater has been treated. A study conducted by Liu *et al.,* (2022) tested the ability of different polymers such as PP, PS and PVC to adsorb metals such as Pb, Cu, Cr and Cd. The results indicated that microplastics can adsorb contaminants and promote microbial inhibition. Ultimately, the microplastics end up in the environmental waters, soil and the food chain through consumption of food and drinking water (Amobonye *et al.,* 2021).

Some concentrations of heavy metals such as Al, Cd, Mn, Zn, and Fe show the same trend in the influent and effluent of WWTW A. Only SO_4^{2-} and Cl showed some fluctuations across four seasons which are non-metallic parameters. The WWTW B showed a difference in the heavy metals' concentrations across summer, autumn, winter and spring with fluctuations in fluoride and non-metallic species such as chloride, sulphur and sulphates. The minimal change in heavy metals and non-metallic parameters concentrations from influent to final effluent may indicate two factors: (1) inability of activated sludge to remove contaminants, and (2) the distribution of contaminants by microplastics. A study done by Nkosi *et al* (2022) indicated high concentrations of metals such as Zn, Cu and arsenic analysed from samples containing microplastics were observed.

4.3. Microplastics concentrations and quantification

This section presents the results and discussions on the microplastics concentrations and quantification on the influent and final effluent of WWTW A and B.

4.3.1. Seasonal microplastics concentrations

The concentrations of microplastics were obtained through adjusting and processing of POM images to determine the particle counts on image J 1.53 K. The seasonal concentrations of microplastics for influent and effluent at WWTW A and B are presented in Figure 4-8. Detailed descriptions of microplastics shapes and their concentrations are also presented in Table 4-1 and 4-2 for both WWTWs.

The concentrations of microplastics vary seasonally across the sampling points. According to Figure 4-8 and Table 4-1, during summer season the influent concentrations at WWTW A contained 145 MPs/L fibres and 148 MPs/L fragments while the effluent contained 54 MPs/L fibres and 138 MPs/L fragments. The composition of WWTW B did not vary in the influent during summer; the influent concentrations ranged between 79-180 MPs/L and a total of 180 MPs/L were fibres (See Table 4-1). The effluent at WWTW B during summer contained 78 MPs/L fragments and 21 MPs/L fibres. During summer both WWTWs influent and effluent were composed of fragments and fibres. The concentrations of microplastics are generally higher in the influent and lower in the effluent across all sampling points with WWTW A receiving higher concentrations of MPs during summer season.

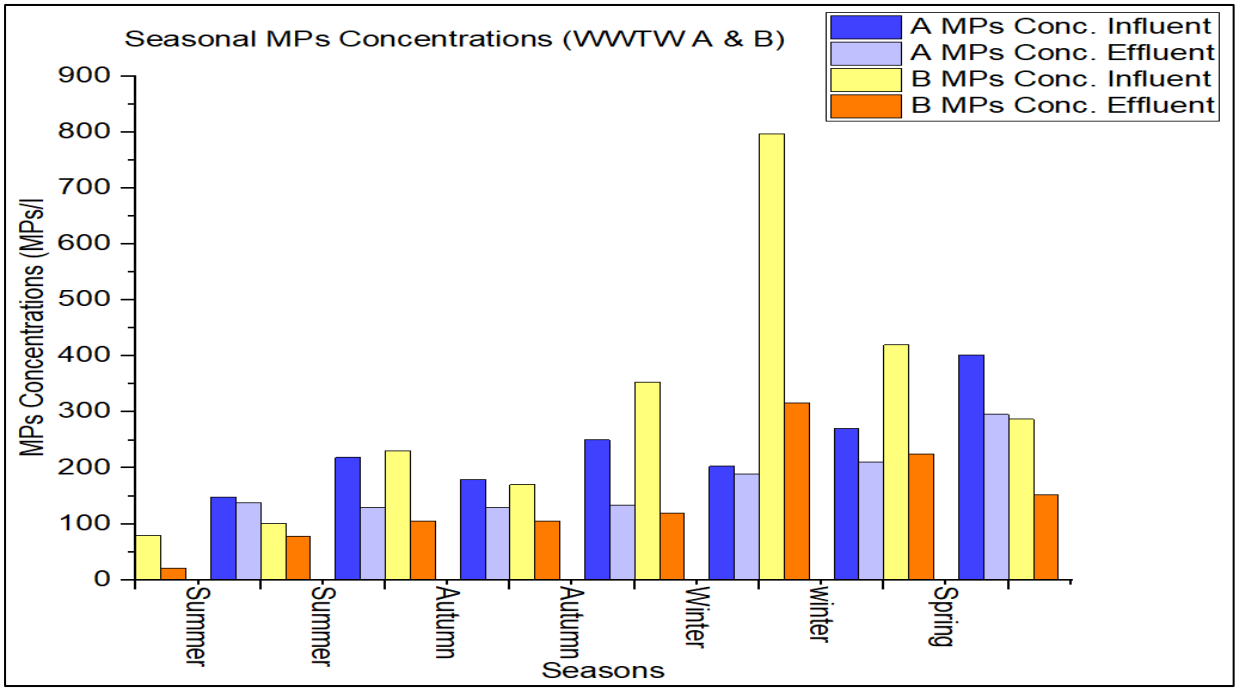

Figure 4-8: Seasonal microplastics concentration for influent and effluent at WWTW A and WWTW B.

The concentrations of microplastics during autumn slightly increased in the influent and effluent of both WWTW A and B. With reference to Figure 4-8 and Tables 4-1 and 4-2, the influent concentrations of microplastics at WWTW A during autumn season ranged between 219-180 MPs/L, in which a total of 399 MPs/L composed of fragments. The effluent concentration ranged between 129-138 MPs/L and a total of 298 MPs/L were observed as fragments. The WWTW B influent microplastics concentrations ranged between 170-231 MPs/L, with a total of 401 MPs/L composed of fragments. The effluent concentration also consisted of 210 MPs/L predominantly fragments. During autumn, the concentrations of MPs were only composed of fragments in both WWTWs. The influent microplastics concentrations for WWTW B was higher than WWTW A.

Table 4-1: Seasonal MPs concentrations based on shapes for WWTW A influent and effluent.

Sample id	**Seasons**	**Shapes**			
		Angular	**Fragments**	**fibres**	**Film**
Influent	Summer	0	148	145	0
	Autumn	0	399	0	0
	Winter	250	0	203	0
	Spring	402	271	0	0
Effluent	Summer	0	138	54	0
	Autumn	0	258	0	0
	Winter	134	0	189	0
	Spring	296	0	0	211

During winter, the concentrations of microplastics continued to increase on the influent and effluent of WWTW A and B, with the highest peaks being 250 MPs/L and 798 MPs/L in the influent and 189 MPs/L and 316 MPs/L in the effluent. A morphology analysis showed that he influent at WWTW A contained 250 MPs/L angular and 203 MPs/L fibres shaped particles. Although the effluent concentrations dropped, the angular and fibre shaped particles were identified with concentrations of 134 MPs/L and 189 MPs/L respectively. The WWTW B influent microplastics concentrations was 354 MPs/L angular and 798 MPs/L fibrous particles. Of these concentrations, the effluent microplastics were also composed of angular and fibre shaped particles with concentrations of 119 MPs/L and 316 MPs/L respectively. During winter season, WWTW B received the highest influent concentrations of microplastics resulting in high effluent concentrations. This is likely due to activated sludge processes unable to handle higher MPs loading.

The spring season was accompanied by slight drop in the influent concentrations. However, the effluent concentrations remained high in both WWTWs. The influent microplastics concentrations at WWTW A comprises of 271 MPs/L fragments and 402 MPs/L angular shaped particles while the final effluent comprises of 211 MPs/L films and 296 MPs/L angular particles. The WWTW B influent in spring comprises of 287 MPs/L angular and 420 MPs/L fragmented particles. The concentrations dropped in the effluent with 152 MPs/L angular and 225 MPs/L fragmented particles. Both WWTWs load of microplastics were consisting of angular and fibres at the influent and effluent. Across the four seasons, films were only noticeable during spring season at WWTW A. The concentrations of microplastics at WWTW A influent was higher than WWTW B. However, WWTW A recorded highest concentrations in the final effluent indicating insufficient treatment of microplastics.

Table 4-2: Seasonal MPs concentrations based on shapes for WWTW B influent and effluent.

Sample id	Seasons	Shapes		
		Angular	**Fragments**	**Fibres**
Influent	Summer	0	0	180
	Autumn	0	401	0
	Winter	354	0	798
	Spring	287	420	0
Effluent	Summer	0	78	21
	Autumn	0	210	0
	Winter	119	316	0
	Spring	152	225	0

The microplastics concentrations were quantified based on the particle count of different shapes such as angular, fragments, fibres and film. According to literature, various shapes of microplastics concentrations has be detected in the influent and effluent of WWTWs (Conley *et al.* 2019; Kang *et al.,* 2018). Although various studies used similar techniques, the concentrations of microplastics observed were different. In a study done by Conely *et al.* (2019) the MPs concentrations in the influent had a difference factor 2.5 count/L while the effluent factor was 4.8 count/L. There was no seasonal variations observed. The findings in the current study show variation in the MPs influent and effluent concentrations. However, lower concentrations were observed in summer and autumn while high concentrations were observed in winter and spring. Conely *et al.* (2019) did not quantify microplastics based on their shapes while the current study quantified the number of angular, fragments, fibres and films identified seasonal as shown in Tables 4-3 and 4-4.

Long *et al.* (2019) reported the MPs concentrations ranging from 1.57-13.69 items/L and 0.2-1.73 items/L in the influent and final effluent respectively. Long *et al* (2018) further quantified the concentrations based on shapes such as pellets, fragments, fibres and granules. The study done by Long *et al.* (2019) is comparable to the findings of the current study in that the concentration of microplastics were high in the influent and lower in the final effluent with different shapes identified. Although the two studies are comparable, the microplastics samples in the study done by Long *et al.* (2019) were collected over 2 days in September- therefore seasonal differences in the concentrations could not be quantified. The reported high concentrations in the influent and low concentrations in the effluent suggest that MPs are reduced in other treatment units of activated sludge process.

Table 4-3: Summary of sizes, shapes and colours of MPs identified at WWTW A.

Sample id	Seasons	Particle size (mm)	Shape				Colour			
			Angular	Fragments	Fibres	Film	White	Blue	Black	Red
Influent	Summer	0.01	×	✓	✓	×	✓	×	×	✓
	Autumn	0.01	×	✓	×	×	×	✓	×	×
	Winter	0.05	✓	×	✓	×	✓	✓	×	×
	Spring	0.01	×	✓	×	×	×	×	✓	×
		0.002	✓	×	×	×	✓	×	×	×
Effluent	Summer	0.01	×	✓	✓	×	✓	✓	×	×
	Autumn	0.01	×	✓	×	×	×	✓	×	×
	Winter	0.05	✓	×	✓	×	✓	✓	×	×
	Spring	0.002	✓	×	×	✓	✓	×	×	×

×=particle or colour not identified, ✓=particle or colour identified

Table 4-4: Summary of sizes, shapes and colours of MPs identified at WWTW B.

Sample id	Seasons	Particle size (mm)	Shape			Colour			
			Angular	Fragments	Fibres	White	Black	Brown	Red
Influent	Summer	0.01	×	×	✓	✓	×	×	×
	Autumn	0.01	×	✓	×	×	×	✓	×
	Winter	0.01	✓	×	✓	×	✓	×	×
	Spring	0.01	×	✓	×	×	✓	×	×
		0.002	✓	×	×	✓	×	×	×
Effluent	Summer	0.01	×	×	✓	✓	×	×	×
		0.02	×	×	✓	×	×	×	✓
	Autumn	0.01	×	✓	×	×	✓	×	×
	Winter	0.01	✓	✓	×	×	✓	×	×
	Spring	0.002	✓	×	×	✓	✓	×	×
		0.005	×	✓	×	×	✓	×	×

×=particle or colour not identified, ✓=particle or colour identified

According to Tables 4-3 and 4-4, the size of MPs ranged between 0.01 mm to 0.02 mm in the influent and 0.01 mm to 0.005 mm in the effluent. Angular, fragments and fibres were dominant across all four seasons. Rosal (2021) stated that fragments and fibres are amongst most reported shapes of MPs in most studies. Most angular and fibrous shaped MPs occurred in white colour with some showing fouling in the recent study. Some of the fragmented materials appeared blue and reddish in colour similar to the current study. Angular and fibrous shaped particles were observed across all four seasons in the influent and effluent of both WWTWs, except for film shaped particles which were only observed in spring. The results of the current study were comparable with the findings of Rosal (2021) where dominant MPs identified were fragments and fibres with various colours ranging from white, blue and black, however the differences were observed in the sizes of MPs which ranged between 0.0016-5 mm.

4.3.2. Seasonal microplastics loading

Figure 4-9 below presents the results for seasonal microplastics loading rate for WWTW A and B. The microplastics loading varied across the two samplings sites with higher loading rate observed on the influent and effluent of WWTW B. During period of summer, WWTW A microplastics loading rate in the influent ranged between 154-175 kg/d while effluent was between 78-136 kg/d. There were higher observed

loadings at WWTW B with influent ranging between 1031-1452 kg/d and effluent between 671-868 kg/d.

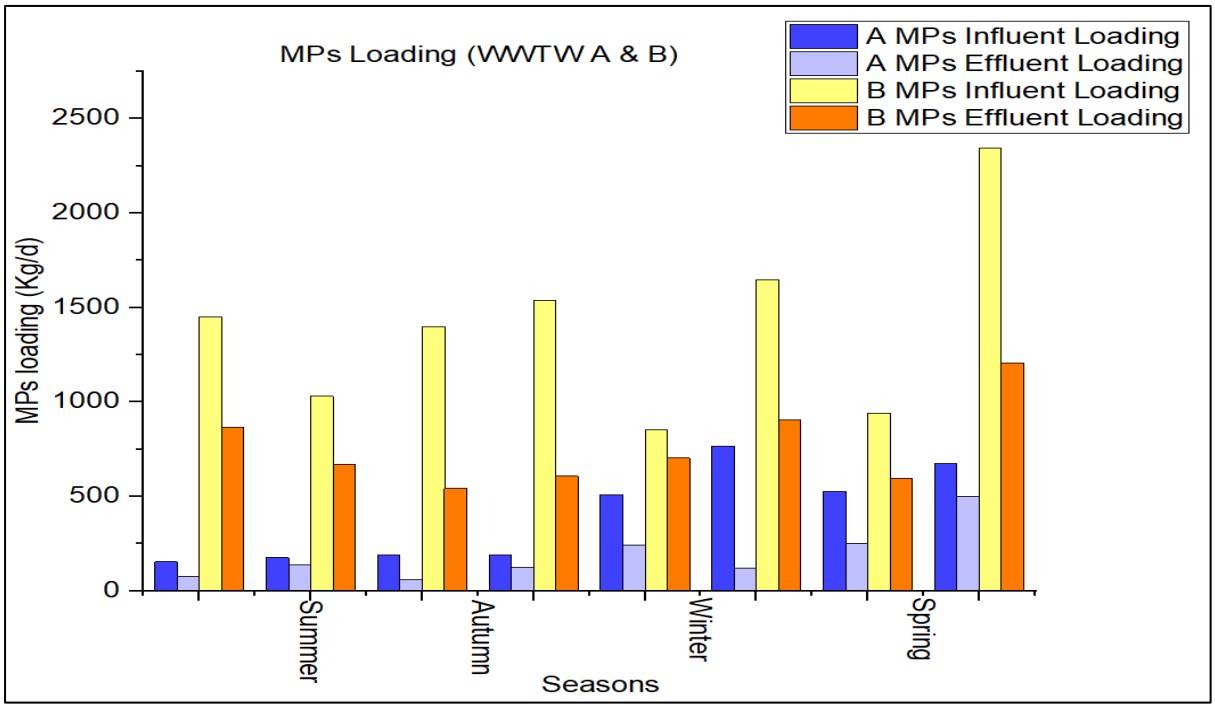

Figure 4-9: Seasonal microplastics loading for influent and effluent at WWTW A and B for the period from December 2021 to September 2022.

During autumn season, the influent loading rate at WWTW A observed ranged between 188-510 kg/d while the effluent was between 125-242 kg/d. The WWTW B influent recorded microplastics loading rate ranging between 1398-1539 Kg/d while effluent was 607-542 kg/d. The WWTW B recorded the highest loading rate in the autumn season.

During winter seasons there was an increase in the influent and effluent loading rate of microplastics at WWTW A and B. The microplastics loading at WWTW A influent in winter ranged between 510-766 kg/d and 121-242 kg/d in the effluent. The influent loading rate at WWTW B ranged between 852-1648 kg/d and 703-904 kg/d in the final effluent. Although there was a slight drop in the loading rate during summer season, highest peak of 2343 kg/d was observed at WWTW B influent. The loading rate during spring at influent and effluent of WWTW A ranged between 528-674 kg/d and 251-502 kg/d respectively. The influent at WWTW B was ranging between 942-2343 kg/d and 594-1206 kg/d in the effluent. The highest peak loading of 2343 kg/d was recorded at WWTW B influent in spring season. The microplastics loading rate at WWTW B

remained higher across summer, winter, autumn and spring, this is due to high hydraulic loading received.

The microplastics loading was quantified in kg/d based on the inflow and outflow measured in ML/d. The microplastics loading rate were estimated in order to determine the possible mass entering and leaving the WWTWs. The loading rate patterns indicated that WWTW B is distributing more MPs into the environment across four seasons i.e., summer, autumn, winter and spring. The high loading rate at WWTW B was attributed to the average operational flow capacity at 389 ML/d as opposed to 63 ML/d at WWTW A WWTW.

Conley *et al*. (2019) confirmed the high microplastics loading using measured inflows and outflows in the WWTWs and microplastics concentrations which were measured in counts/L. The study was able to estimate a maximum of 0.68 g of microplastics annual per capita discharged from WWTWs accounting for 500-1000 MPs/d. Schmidt *et al.* (2020) then reported 7×10^{12} MPs/year in the wastewater in Germany. The two studies confirms that large quantities of MPs are distributed into the environment. These studies agree with the findings of the current study. In addition, Troost (2021) put more emphasis on monitoring microplastics in single location over time in order to determine the seasonal loading trends-which is in support with the computed seasonal loading trends in the current study. The continuous loading of microplastics into the environment may cause physical damage and death of aquatic species through ingestion. Chemical additives such as PCBs may cause toxicity while the adsorbed contaminants such as Zn, Pb and Cd on microplastics surfaces increase toxicity of the river systems (H. Zhao *et al.,* 2022).

4.4. Polymer identification and their functional groups

Figure 4-10 and 4-11 below present the FTIR spectra for all polymers and their functional groups identified in summer seasons. According to figure 4-10 (a) and (c) and Figure 4-11 (a) and (c) the influent at WWTW A contained microplastics consisting of PE and ABS while WWTW B influent contained PE and PET. The functional groups identified linked to polymers identified include alcohol, hydroxy compound, methylene ($>CH_2$), methyl ($-CH_3$), carbonyl compound, olefinic (alkene), silicon-oxy compound,

aromatic ring (Aryl), nitrogen-oxy compounds, inorganic ion and ether and oxy compound.

The polymers identified in the effluent during summer period for WWTW A and B are presented in Figure 4-10. (b) and (d) and Figure 4-11 (b) and (d). The FTIR library indicates that at WWTW A effluent, PAM and ABS were identified in summer while PE and polycarbonate were identified at WWTW B effluent. Due to the polymers identified in the influent being similar to those in the effluent, the FTIR peaks identified are similar and are composed of compounds linked to polymers such as normal polymeric –OH stretches, esters, carboxylate, silicone and vinyl

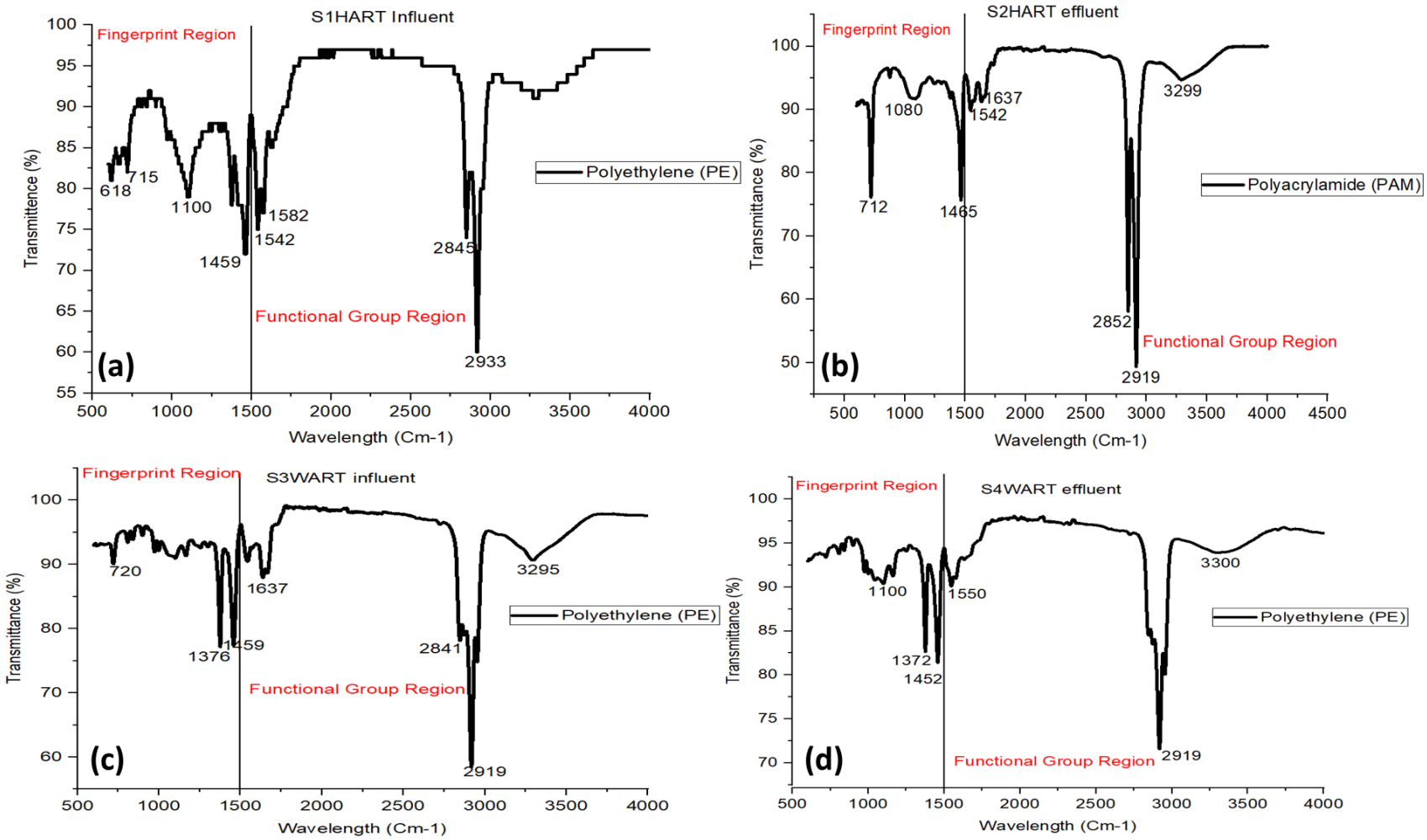

Figure 4-10: FTIR analysis Influent and effluent of WWTW A & B, (a) S1HART-A influent, (b) S1HART-A effluent and (c) S3 WART-B influent, (d) S4 WART-B effluent (summer).

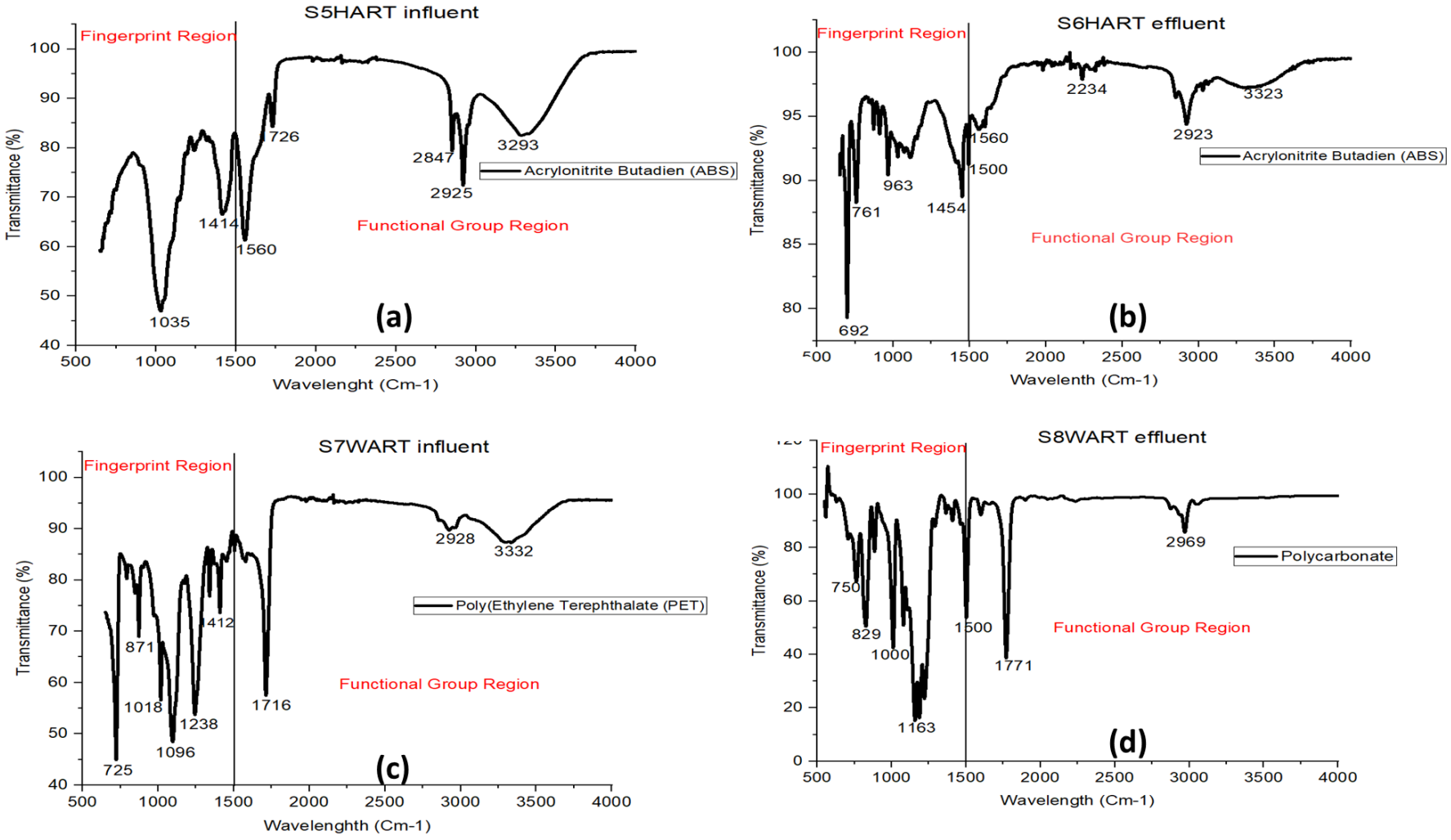

Figure 4-11: FTIR analysis, (a) S5HART-A influent, (b) S6HART-A effluent, (c) S7WART-B influent and S8WART-B effluent (summer).

The polymers and functional groups identified in autumn are presented in the FTIR spectra in Figure 4-12 and 4-13 for both WWTW A and B. Figure 4-12 (a) and (c) and Figure 4-13 (a) and (c) shows the polymer identified and functional groups in the influent during autumn. The WWTW A influent was composed of ABS and PAM while WWTW B was composed of poly(ethyl cyanoacrylate) (PECA) and poly(methyl methacrylate) (poly(methyl methacrylate) (PPM). The functional groups associated with polymers identified in the influent during autumn include alcohol and hydroxy compound, methylene ($>CH_2$) methyl ($-CH_3$), carbonyl compound, olefinic (alkene) and aromatic ring (Aryl).

Figure 4-12 (b) and (d) and Figure 4-13 (b) and (d) presented below show the polymers identified in the final effluent during autumn season. According to the FTIR library and spectra on the graphs, the effluent at WWTW A was composed of only ABS, while the composition at WWTW B was calcium pantothenate and PMM. Similar functional groups identified in the effluent were detected in the final effluent for both WWTW A and B. Mainly common compounds identified include polymeric –OH stretch, aromatic ring stretch, esters, amine, carboxylic acid salts and rest of functional groups identified in the influent as described above for autumn season.

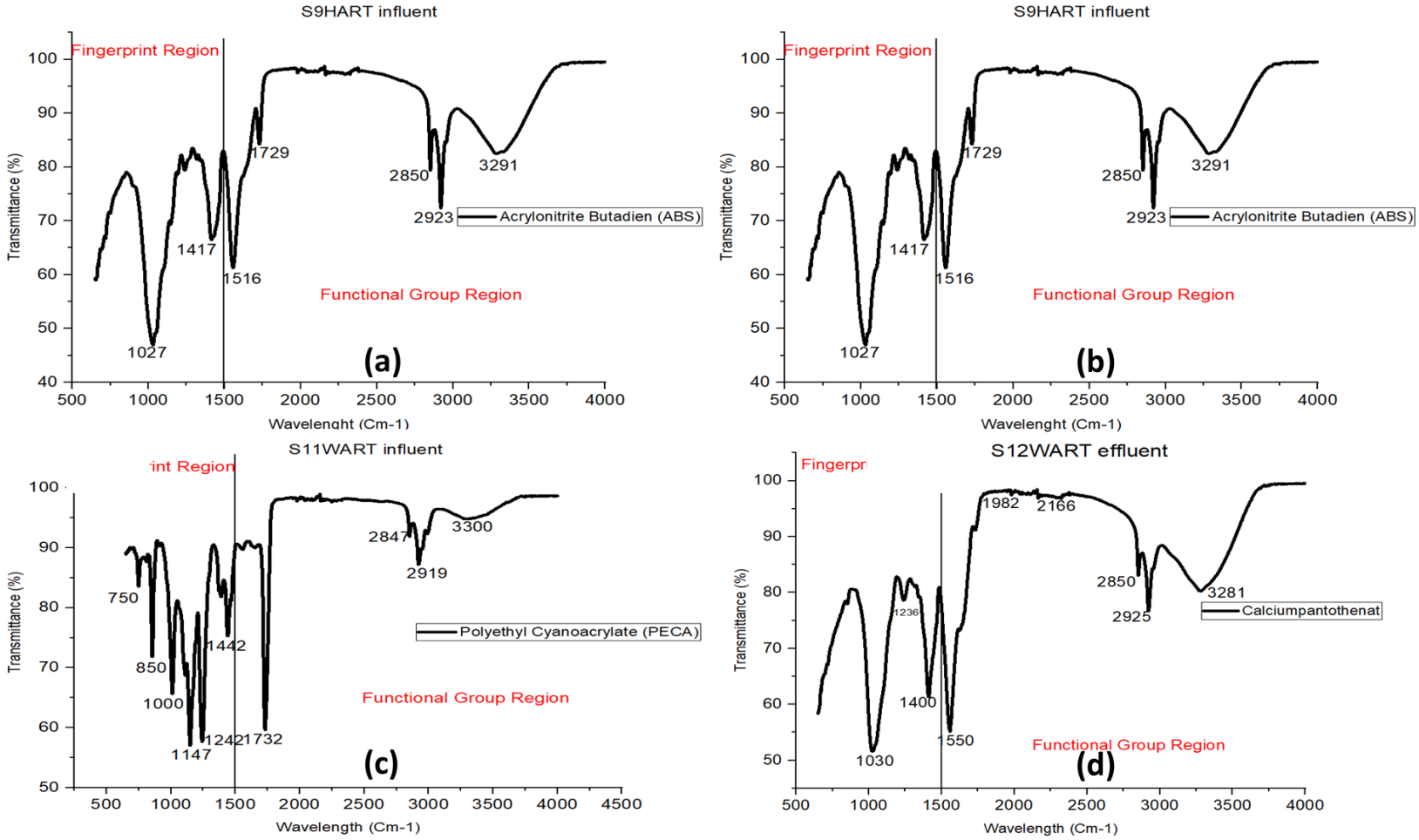

Figure 4-12: FTIR analysis, (a) S9HART-A influent, (b) S10HART-A effluent, (c) S11WART-B influent and S12WART-B effluent (autumn).

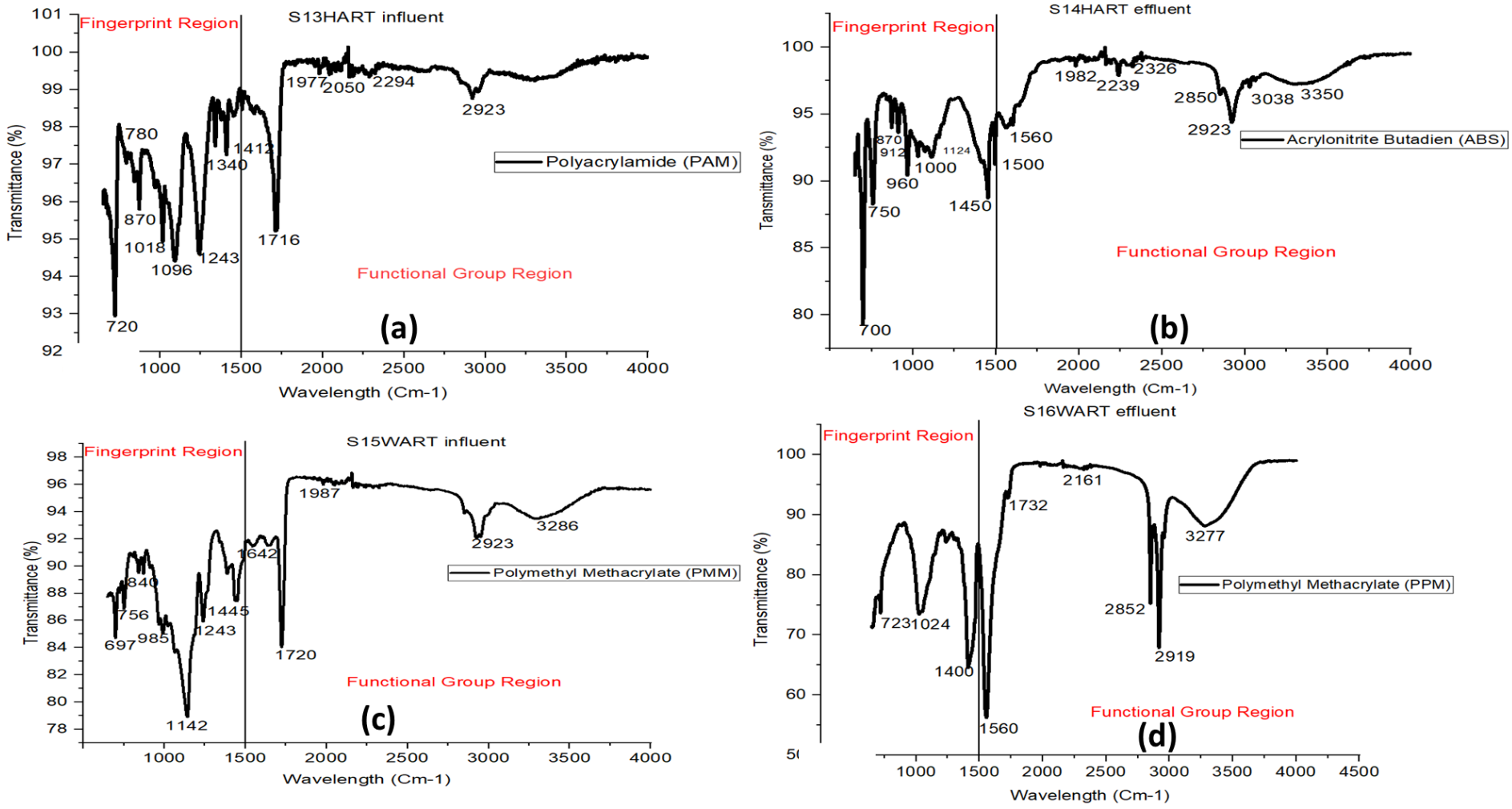

Figure 4-13: FTIR analysis, (a) S13HART-A influent, (b) S14HART-A effluent, (c) S15WART-B influent and S16WART-B effluent (autumn).

The winter season was composed of very unique and complex polymers as compared to summer and autumn; all polymers identified during the winter season were not present in the summer and autumn seasons. The polymers and functional groups identified during summer seasons for influent and effluent are presented in Figure 4-14 and 4-15. Figure 4-14 (a) and (c) and Figure 4-15 (a) and (c) present the influent FTIR spectra and functional groups for WWTW A and B. The WWTW A influent in winter was characterized by acrylic, PPC, PET, thermoplastic rubber (TPV), PE, and PS while WWTW B was only composed of PS. There is a distinct composition of polymers between the two WWTW in the influent in winter, this could be attributed to WWTW A receiving 40% industrial effluent, which is dominant during dry seasons. The main functional groups identified in the influent include Methylene ($>CH_2$), methyl ($-CH_3$), methyne (>CH–) carbonyl compound, olefinic (alkene), normal polymeric –OH stretches. Figure 4-14 (b) and (d) and Figure 4-15 (b) and (d) present the effluent results for FTIR spectra and identified polymers. During winter season, effluent at WWTW A was composed of PP and PPC while WWTW B was composed of PP glass fibre and hydropropyl cellulose (HPC). The functional groups identified were mainly methylene ($>CH_2$), methyl ($-CH_3$), methyne (>CH–) carbonyl compound, olefinic (alkene), normal polymeric –OH stretches which are similar to the influent.

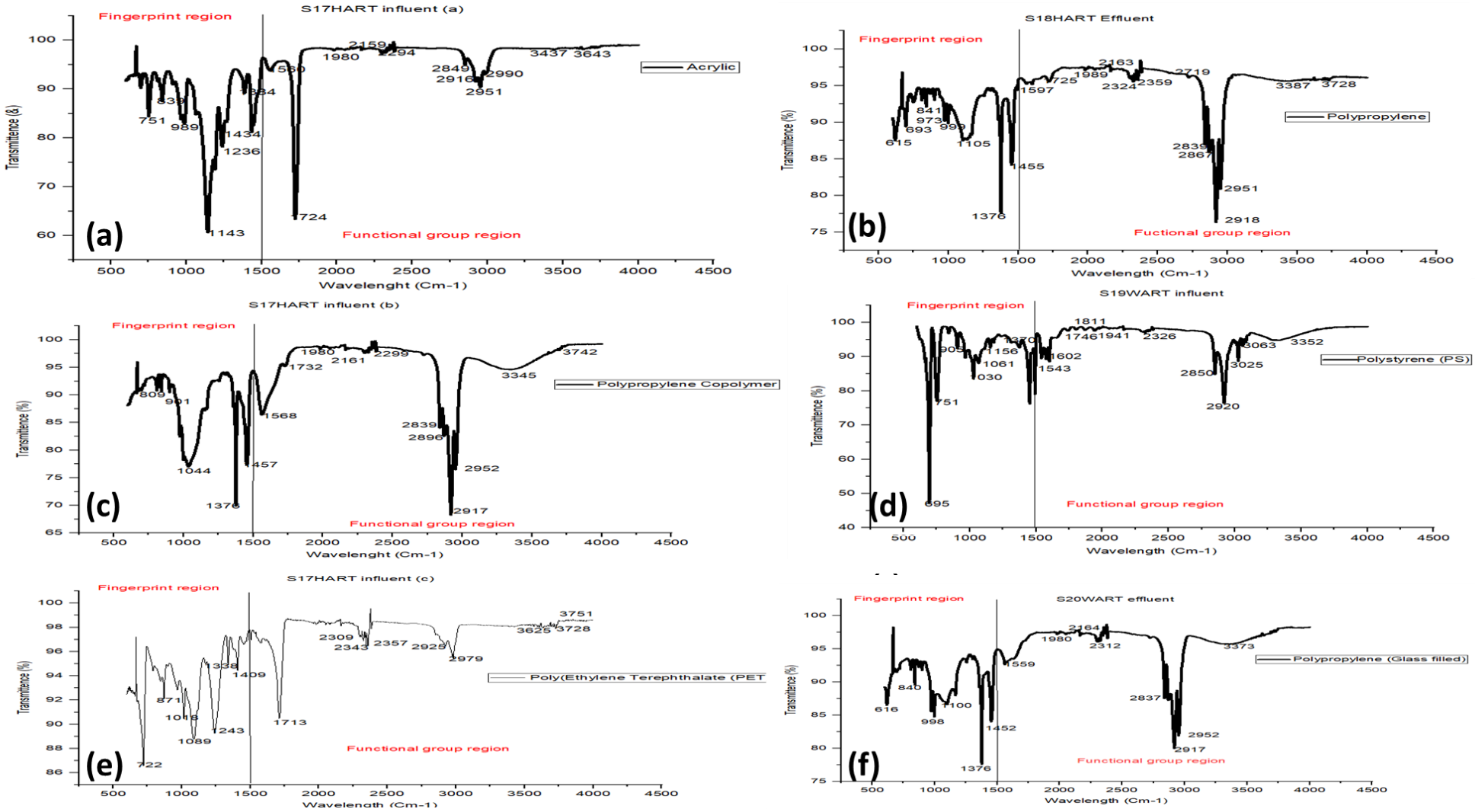

Figure 4-14: FTIR analysis, (a) S17HART-A influent, (b) S18HART-A effluent (c) S19WART-B influent, (d) S20WART-B effluent

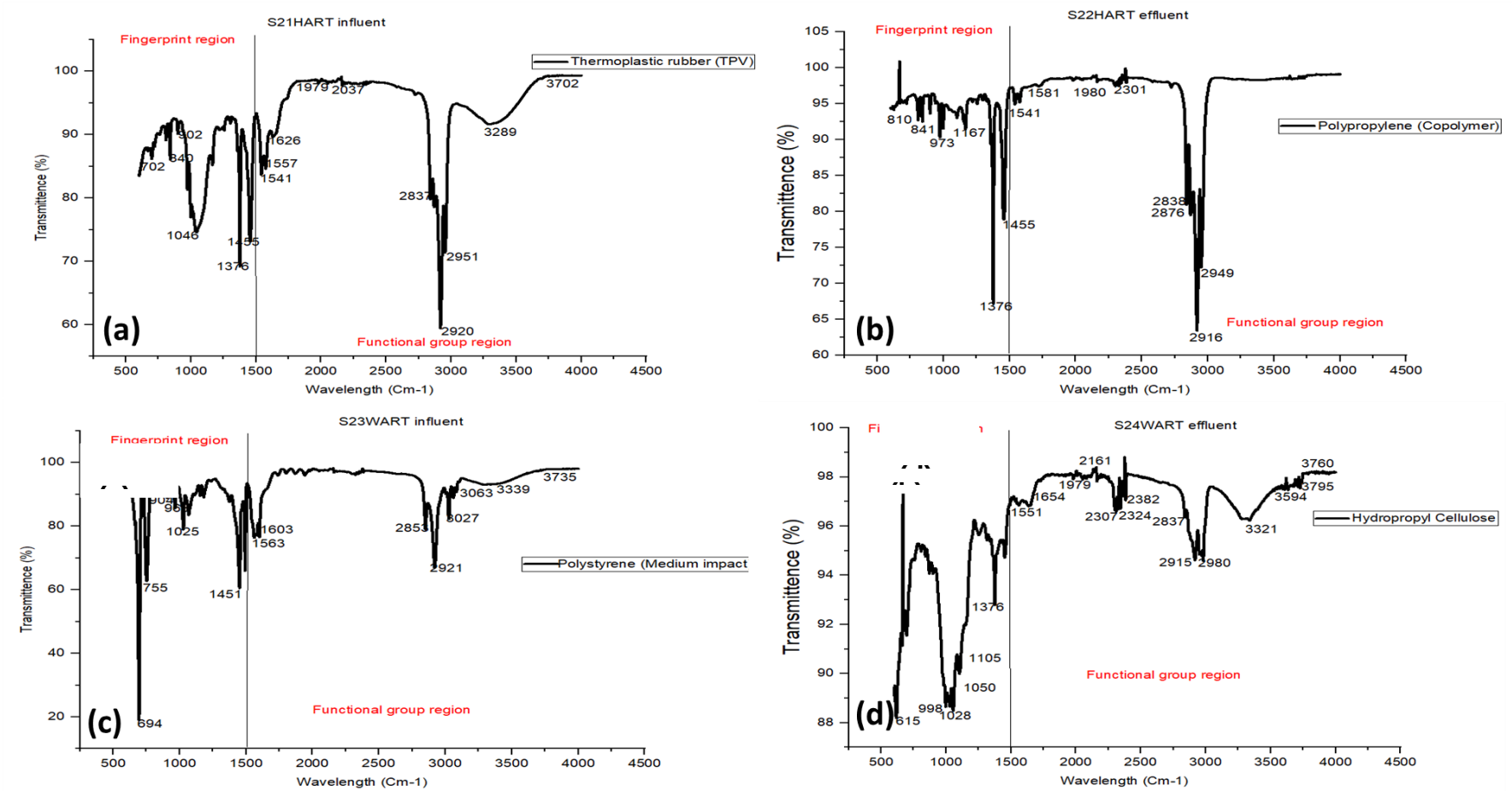

Figure 4-15: FTIR analysis, (a) S21HART-A influent, (b) S22HART-A effluent (c) S23WART-B influent, (d) S24WART-B effluent (winter)

During the spring season the influent at WWTW A was composed of PS (medium impact)-(PS-MI) and PET while WWTW B was composed of PAM and carboxyl modified. The influent FTIR spectra and functional groups are presented below in Figure 4-16 (a) and (c) and Figure 4-17 (a) and (c). The Effluent at WWTW A was composed of polycarbonate and PP (glass filled). The WWTW B effluent in spring was composed of cellulose and PS, which are completely different polymers from these identified in the influent. Figure 4-16 (b) and (d) and Figure 4-17 (d) shows the FTIR transmittance spectra of the polymers identified in the effluent and functional groups for both WWTW. The most common functional groups identified were similar to those identified in summer, autumn and spring with most prevailing being methylene ($>CH_2$), methyl ($–CH_3$), Methyne (>CH–) carbonyl compound, olefinic (alkene), alcohol and hydroxy compounds.

Across the four seasons it can be noted that winter was composed of different polymers as compared to summer, autumn and spring. This could be attributed to change in consumer behaviour due to seasonal change, which is likely to introduce different types of microplastics, for example consumers use more fiber clothes in winter as opposed to summer (Fältström *et al,.* 2021). The FTIR functional groups which are linked to polymers seems to be the same across the seasons. The functional group region was used to identify the compounds ranging from 1500-4000 cm^{-1}.

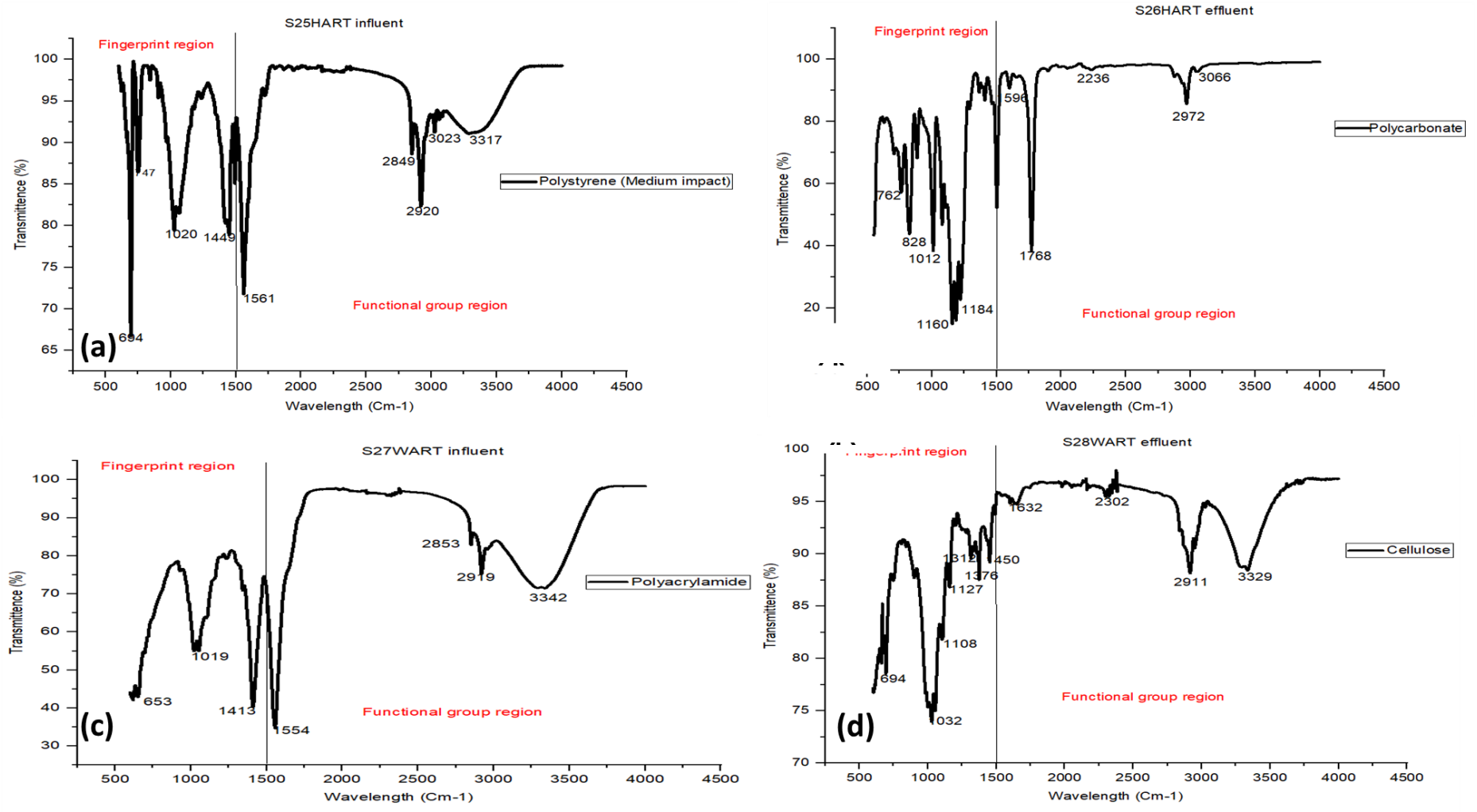

Figure 4-16: FTIR analysis, (a) S25HART-A influent, (b) S26HART-A effluent (c) S27WART-B influent, (d) S28WART-B effluent (spring).

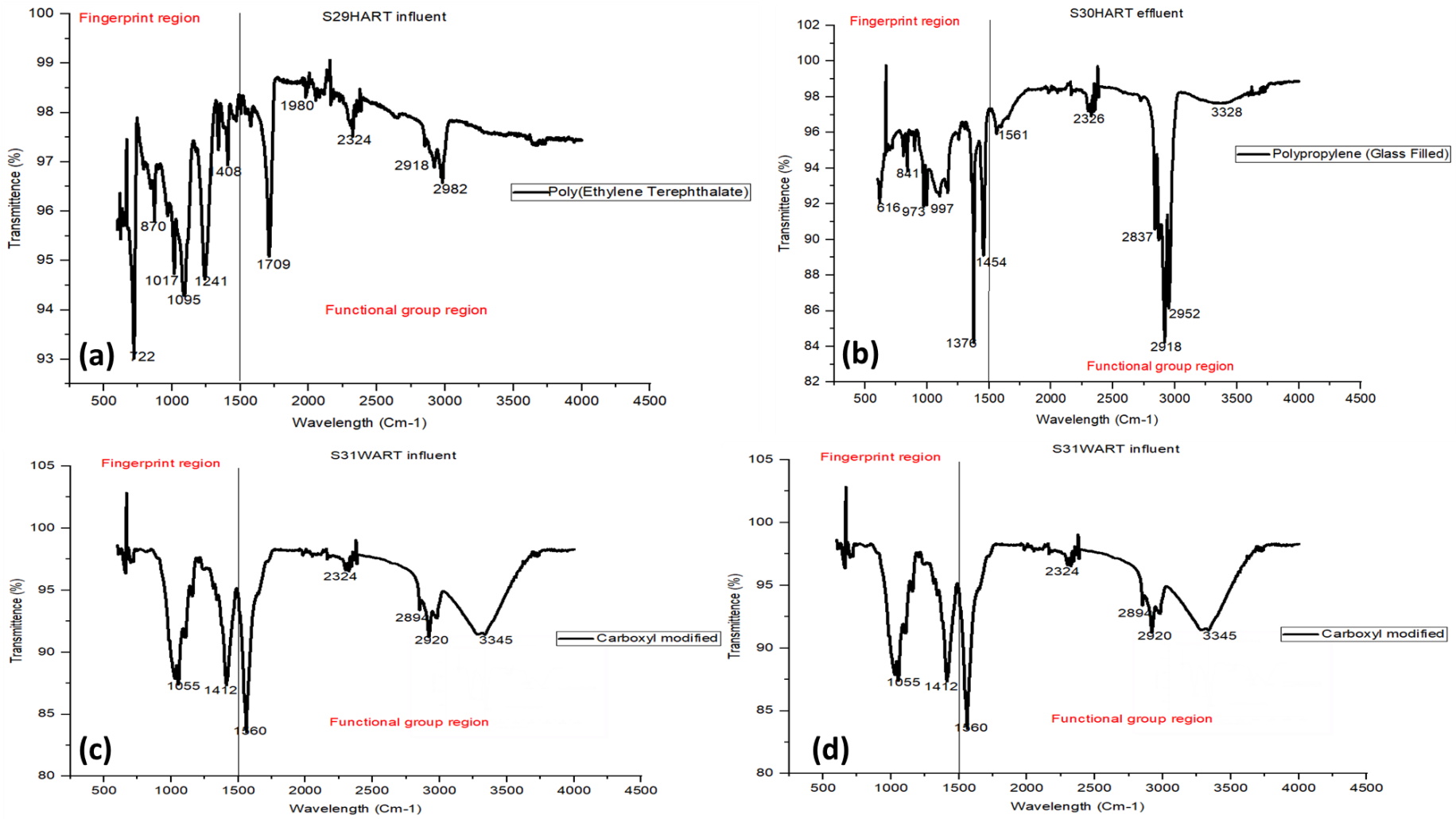

Figure 4-17: FTIR analysis, (a) S29HART-A influent, (b) S30HART-A effluent (c) S31WART-B influent, (d) S32WART-B effluent (spring)..

In the current study, different polymers were identified from the FTIR library while the spectra were used to identify the functional group region. According to Bayu *et al.* (2019) there are two regions of FTIR spectra which is the finger print region (between 600-1500 cm^{-1}) and the functional group region. However, the functional group is comprised of single bond region (2500-4000 cm^{-1}), triple bonds (2000-2500 cm^{-1}) and double bond region (1500-2000 cm^{-1}) wavelength. The finger print region is complex and is comprised of large number of peaks, therefore it was not used as opposed to functional group region (Aurelio Ramírez-Hernández & Universidad, 2019). In this study the fictional group regions were between 1500-3400 cm^{-1} as in the results obtained by FTIR.

Williams *et al.* (2020) conducted a study on microplastics quantification comparing two WWTWs. The findings of the study revealed that the two studied WWTWs contained polymers such as PP, PE, PET and silicon in the influent and effluent over 10 months of monitoring. The current study showed a complex range of polymers identified in the influent and effluent of WWTW A and B. The influent and effluent of two WWTWs in the current study were comprised of polymers such as PE, ABS, polyacrylamide (PAM), polymethyl methacrylate (PPM), PS, cellulose, acrylic, polypropylene carbonate (PPC), thermoplastic vulcanizates (TPV), PECA and PMM. Contrary to Williams *et al.* (2020), the current study showed that the microplastics polymer types observed in the influent and effluent tend to vary seasonally. Studies such as Olesen *et al.* (2017); Stockin *et al.,* 2021 and Yuvedha *et al.,* 2019 showed similar finding to the current study.

The functional groups identified represented various compounds such as ethylene ($>CH_2$), methyl ($-CH_3$), methyne ($>CH-$) carbonyl compound, olefinic (alkene), alcohol and hydroxy compound identified between 1500-4000 cm^{-1} wavelength. The functional groups identified are regarded as monomers from which polymers are derived. The monomers such as methyl and methyne are regarded as hydrocarbons and may contribute to water quality changes in the receiving environment. According to Shekoohiyan & Akbarzadeh (2022) microplastics particles can be consumed by fauna and may end up in human bodies through the hierarchy of food web and this is likely to affect the health of human beings. The identified saturated aliphatic, alcohol and

hydroxyl compounds as well as alkene in the current study forms part of polymer synthetic and are regarded as microplastics additives substances.

Kang *et al.* (2018) observed that the occurrence of such polymer types as PS in wastewater may indicate domestic sewage as the main source since it is largely fibres that disintegrate during washing of laundries. Although the currents studies did not monitor the industrial streams and domestic streams separately at WWTW A, the combined wastewater from both domestic and industrial showed diverse types of polymers as opposed to WWTW B which is only receiving domestic effluent. Fältström *et al.* (2021) further indicates that polymers such as acrylic, PE and PP may originates from laundries in WWTW fed from domestic sources.

4.5. Analysis of microplastics particles using (SEM/EDX)

Elemental analysis of particles was done to determine the composition of MPs using SEM coupled with EDX (SEM/EDX). Figures 4-18 below present the SEM images and graphs for elementary analysis for influent and effluent samples collected at WWTW A and B. Table 4-6 summarises the results obtained for elements identified. The SEM/EDX was performed for samples which were composed of PE low density PAM previously identified using the FTIR library of polymer standards.

Figure 4-18 (a) shows the SEM images for influent at WWTW A with particle sizes at 0.005 mm (5 µm). The sample was composed of PE low density showing fibrous shaped MPs. According to Figure 4-19 (a) and Table 4-5 below, a total of 13 elements were identified in the influent sample for WWTW A. The elements dominating the samples were C and O at 73.46% and 15.90%. Other peaks noticeable such as Cr, Mg, Cl, Fe, Al, Si, P and S were between the KeV ranges 0-4.

Figure 4-18 (b) Shows the SEM image for effluent at WWTW A with particle sizes at 0.05 mm (50 µm). The shapes of particles are long translucent fibres and fragmented materials containing PAM polymer. The elemental analysis shows peaks of high intensity between 0-4 KeV which are indexed to the elements C, O, P, Na, Mg and Cl. The most intense peak occurs in the range 2.5-3.0 KeV peak and is due to Cl followed the peaks due to Na and P between 1-2 KeV. The O and C peaks were noticeable between 0-1 KeV. The elements dominating the effluent sample at WWTW A were C at 58%, O at 26.9%, Cl at 8.73% and Na at 8.66% (See Table 4-6). The influent and effluent at WWTW A are composed of elements that can be linked to presence of microplastics, with heavy metals prevailing in the analysed samples which contain PE and PAM polymers. The influent shows higher percentages of O and C elements as compared to effluent.

Figure 4-18 (C) shows the SEM images for influent at WWTW B with particle sizes averaging at 0.025 mm (25 µm). The MPs appear to be thick fibres of PE low density. Elementa composition shows that the influent at WWTW B is composed of C (52.26%), O (19.92%), Cl (8.73%) and Na (8.6%) (Table 4-7). The most intense peaks were identified in the range 0-4 KeV indicating the abundance of C, O, Na, Si, S, Cl and P in the microplastics. Other elements identified at lower peaks include Fe, Mg and Ca.

The presence of C, O and Cl provide evidence of polymer presence in the samples and associated metal.

The SEM image for effluent at WWTW B confirms the presence of fibrous particles, but the fibres appear to be bio fouled, this could be attributed to microplastics having passed through different treatment stages in the activated sludge processes such as anaerobic zone, anoxic zone and aeration. Similar to the influent, the polymer composition of the effluent sample was PE low density as shown by the SEM image (See Figure 4-18 (c)) for reference. Figure 4-19 (d) present the elemental peaks in the samples, with the highest peaks occurring between 0 and 4 KeV. The highest peaks recorded in order are Cl between 2.8-3 KeV, Na between 1-1.5 KeV, Si between 1-2 KeV and the O and C between 0-1 KeV. Other elemental peaks recorded include Mg, Al, P and S. According to Table 4-8, the effluent at WWTW B was predominantly composed of elements such as C (58.31%), O (22.01%) and Cl (6.38%). Other elements such as Mg, Al, Si, P, and S were prevalent in smaller percentages.

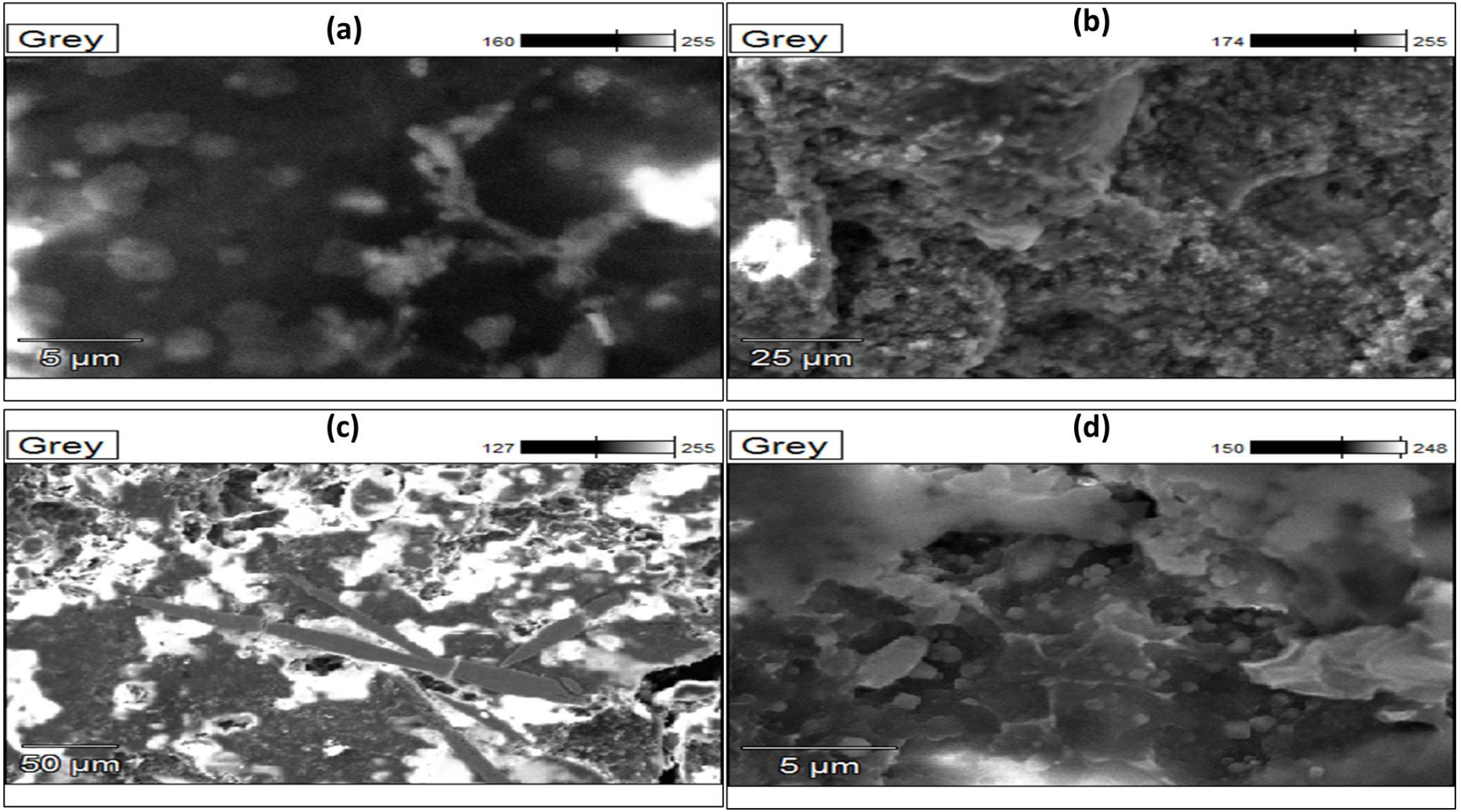

Figure 4-18 Shows SEM images for WWTW A & B, (a) HART-A Influent SEM image, (b) HART-A influent elementary analysis graph, (c) WART-B effluent SEM image, (d) WART-B effluent elementary analysis graph

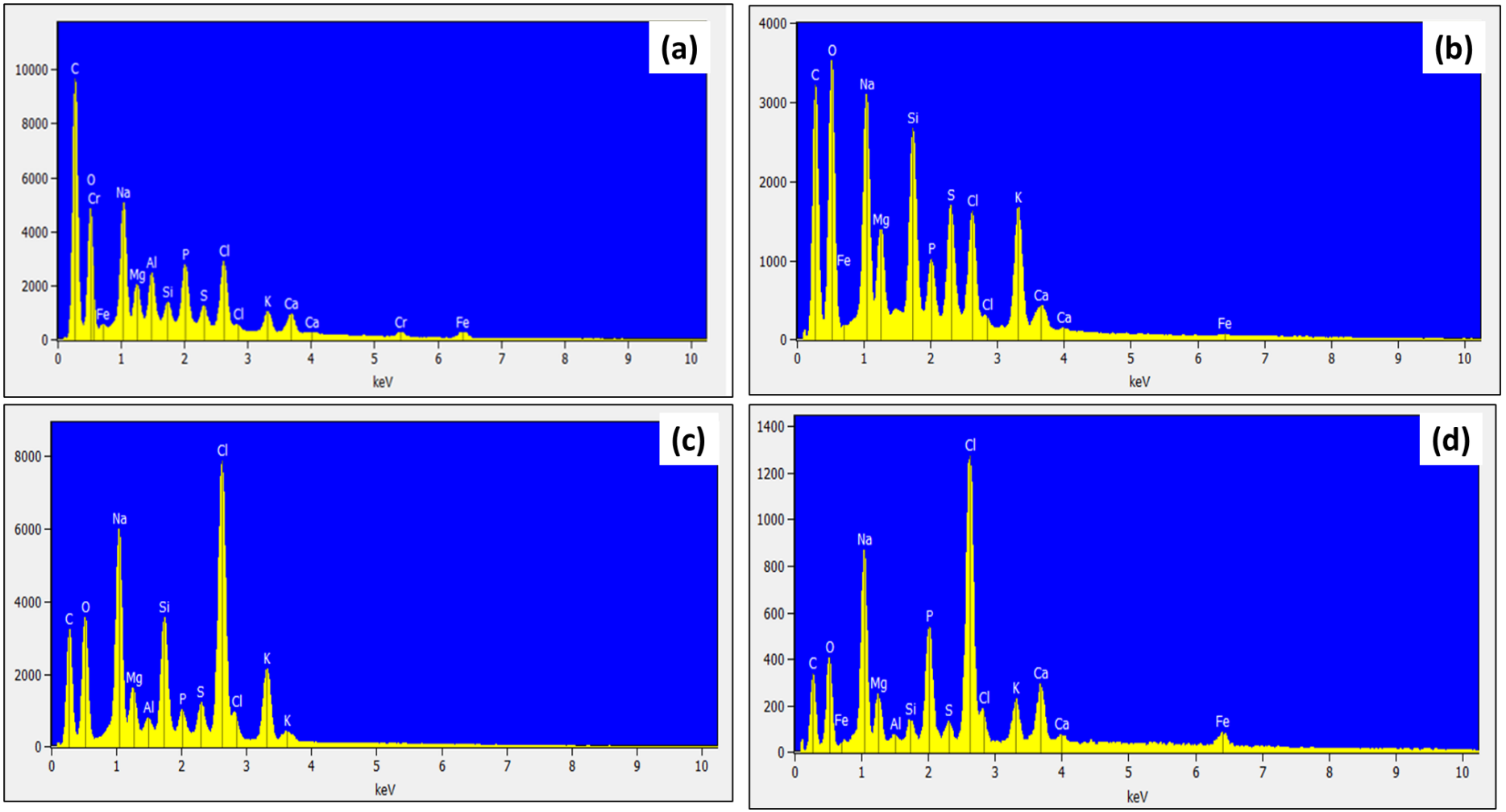

Figure 4-19: SEM/EDX analysis for influent and effluent at WWTW A & B, (a) HART-A Influent SEM image, (b) HART-A influent elementary analysis graph, (c) WART-B effluent SEM image, (d) WART-B effluent elementary analysis graph

Table 4-5: Details of microplastics particle composition from EDX analysis for Figure 4-19 (b) HART-A effluent. Details of microplastics particle composition from EDX analysis for figure 23 (a) HART-A influent.

Sampling point	Atoms	Weight % from EDX analysis	Error %	Reference spectrum
Raw Influent sample 1 HART	C	73.46	+- 0.35	Polyethylene low density (PE)
	O	15.90	+- 0.26	
	Cr	0.19	+- 0.01	
	Fe	0.41	+- 0.03	
	Mg	0.61	+- 0.03	
	Al	0.86	+- 0.03	
	Si	0.37	+- 0.03	
	P	1.23	+- 0.03	
	S	0.41	+- 0.01	
	Cl	1.47	+- 0.02	
	K	0.50	+- 0.02	
	Ca	0.55	+- 0.02	
	Na	4.02	+- 0.05	
Total		**100**		

Table 4-6: Details of microplastics particle composition from EDX analysis for Figure 4-19 (b) HART-A effluent.

Sampling point	Atoms	Weight % from EDX analysis	Error %	Reference spectrum
Final effluent sample 2 HART	C	58	0.42	Polyacrylamide (PAM)
	O	26.9	0.22	
	Fe	0.09	0.02	
	Mg	1.06	0.02	
	Si	2.26	0.03	
	P	0.77	0.01	
	S	1.66	0.03	
	Cl	8.73	0.03	
	K	4.41	0.03	
	Ca	0.40	0.02	
	Na	5.19	0.05	
Total		**100**		

Table 4-7: Details of microplastics particle composition from EDX analysis for Figure 4-19 (c) WART-B influent.

Sampling point	Atoms	Weight % from EDX analysis	Error %	Reference spectrum
Raw influent sample 3 WART	C	52.26	+- 1.34	PE low density
	O	19.92	+- 0.51	
	Fe	1.23	+- 0.09	
	Mg	1.21	+- 0.04	
	Al	0.18	+- 0.03	
	Si	0.52	+- 0.03	
	P	3.2	+- 0.08	
	S	0.49	+- 0.04	
	Cl	8.73	+- 0.12	
	K	1.39	+- 0.08	
	Ca	2.21	+- 0.10	
	Na	8.66	+- 0.05	
Total		**100**		

Table 4-8: Details of microplastics particle composition from EDX analysis for Figure 4-19 (d) WART-B effluent.

Sampling point	Atoms	Weight % from EDX analysis	Error %	Reference spectrum
Final effluent sample 4 WART	C	58.31	+- 0.43	PE low density
	O	22.01	+- 0.19	
	Mg	0.63	+- 0.03	
	Al	0.21	+- 0.01	
	Si	2.31	+- 0.02	
	P	0.48	+- 0.01	
	S	0.64	+- 0.01	
	Cl	6.38	+- 0.04	
	K	2.09	+- 0.02	
Total		**100**		

SEM imaging was used to generate high resolution micrographs which were used to differentiate the microplastics from organic materials. EDX was used to determine elements present in the MPs. Woo *et al., (2021*) further explained that elements such Al, Calcium, Mg, Na and Si are regarded as components of colorants, plasticizers and flame retardants in which microplastics are manufactured. In the current study, heavy metals related to microplastics additives were observed on polymers such as PAM and PE. The analysis of samples containing PE and PAM showed a high ratio of C and O which can be linked to fibres and fragments observed in the current study.

4.6. Microplastics removal efficiencies at WWTW A and B influent and final effluent.

The removal efficiencies of microplastics were obtained by getting the difference between influent and effluent concentrations. The removal efficiencies were calculated seasonal based on microplastics concentrations quantified. Figure 4-20 shows the seasonal variation in the removal efficiencies of microplastics by two WWTWs utilising activated sludge processes. During summer season, WWTW A MPs removal efficiencies ranged between 6-62% while WWTW B ranged between 22-73%. There were high removal efficiencies at WWTW B during summer season. During autumn season, WWTW MPs A removal efficiencies ranged between 28-41% while WWTW B ranged between 38-66%, once again WWTW B removal efficiencies for microplastics remained high. During winter season, removal efficiencies at WWTW A ranged between 6-46% while WWTW B was between 60-66%. During spring season, the removal efficiencies for both WWTWs dropped, with WWTW A ranging between 22-26% and WWTW B between 46-47%. The removal efficiencies across four seasons for WWTW A is highly variable while WWTW B shows similar trends. There is high removal rate of microplastics at WWTW B across four seasons as compared to WWTW B.

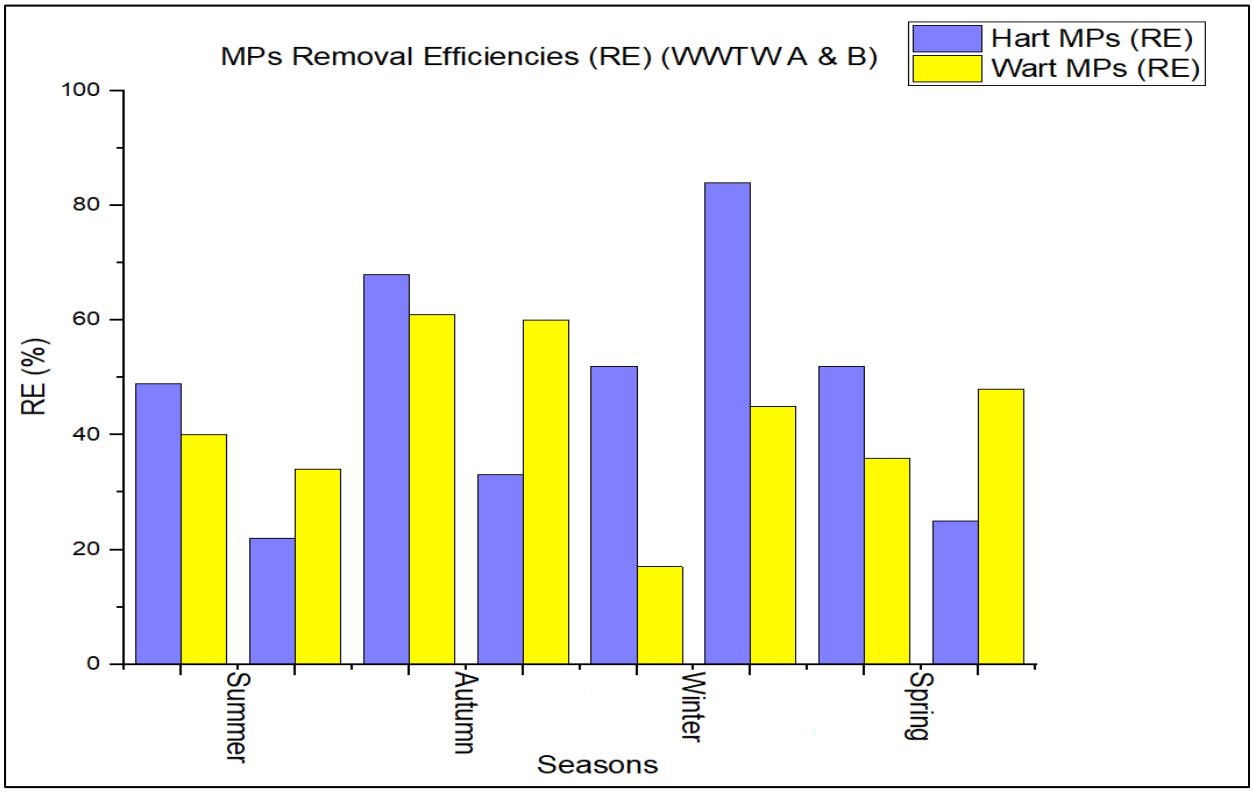

Figure 4-20: Seasonal microplastics removal efficiencies for WWTW A & B based on influent and effluent concentrations in MPs/L.

Microplastics removal in wastewater is key to limit the accumulation in the river and environment. The seasonal patterns in the microplastics removal efficiencies were determined at WWTW A and B. Figure 4-20 indicates that WWTW B has higher removal efficiencies than WWTW B across all the seasons attributed to hydraulic capacity and treatment configuration. A study done by Fernández *et al.* (2022) compared three different WWTWs, the WWTW 1 only do primary treatment, the water is then disposed to the sea while WWTW 2 and 3 has both primary and activated sludge treatment. The removal efficiencies for WWTW 1 were 58.2% while WWTW 2 and 3 was 90%, the finding suggest that different plant configurations have influence on the amount of microplastics removed. The WWTW A removed less MPs than WWTW B, the differences in the treatment technology are noted. The WWTW A is using Modified University of Cape Town (UCT) configurations while WWTW B uses 3 stage Pheredox.

The design of plant configurations with various treatment stages aid in removal of microplastics. The study done by Ho *et al.* (2021) identified that various treatment unit in the wastewater work plays an important role in the removal of MPs .

4.7. Physicochemical test and microplastics statistical analysis

The preliminary physicochemical test analysed were pH, temperature (T), COD and SS on the influent and effluent of WWTW. Microplastics concentrations and loadings were monitored and analysed across four seasons from 2021 December to 2022 September. The purpose of this section was to perform statistical analysis to determine statically correlations between physicochemical parameters and microplastics concentrations and loading rate in wastewater influent and effluent.

4.7.1. Statistical correlation between physicochemical test and microplastics concentrations

The strength of association between physicochemical parameters and MPs was determine through Pearson correlation at $p<0.05$ and the coefficient of variation (r) ranging between 0.1-0.3 (small correlation), 0.3-0.5 (Medium correlation) and 0.5-1 (large correlation) for all determined positive correlations. According to Figure 4-21 (a) there was a negative correlation between influent COD and influent MPs for WWTW A (r=0.04), however the effluent COD and effluent MPs showed a large correlation (r=0.6). There was also a medium correlation between influent COD and influent MPs for WWTW B (r=0.32). However, it can be noted that the effluent COD and MPs showed negative correlation (Figure 4-21 (b)). It can also be noted in Figure 4-22 (a) and (b) that for both WWTW A and B, there was a negative correlation in the influent and effluent SS and MPs concentrations. Figure 4-23 (a) and (b) also showed a negative correlation between MPs and pH in both WWTW A and B. The temperature and MPs correlated minimal with MPs (r=0.3) for WWTW A influent concentrations, however the effluent correlation was negative (see Figure 4-24 (a) and (b).

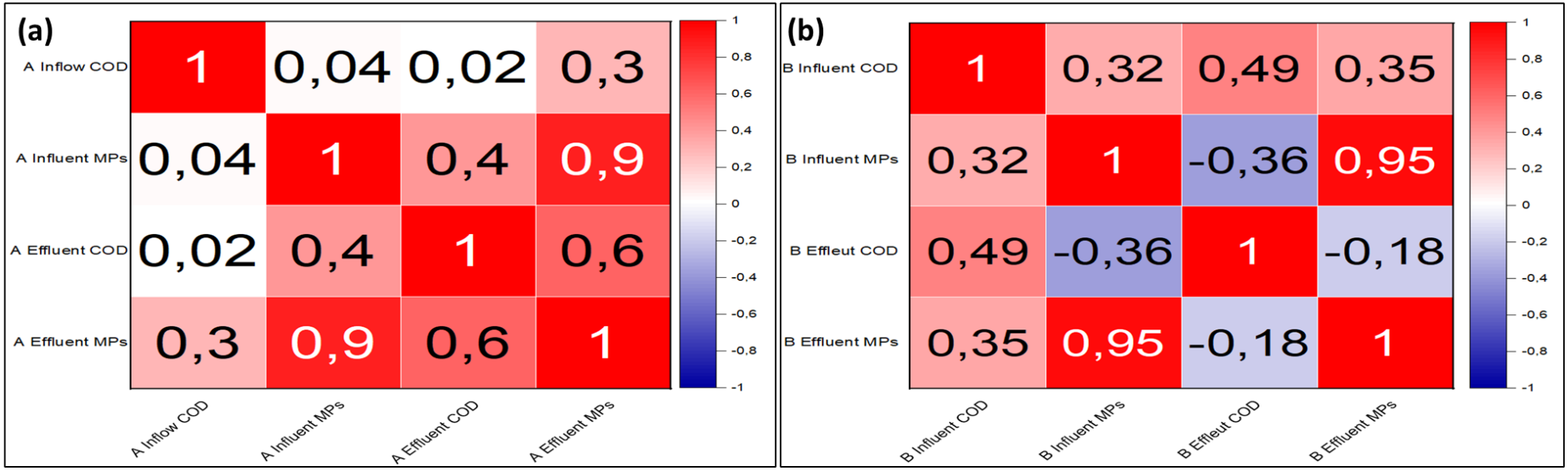

Figure 4-21: Correlation analysis between COD & MPs WWTW A & B, (a) WWTW A correlation, (b) WWTW B correlation

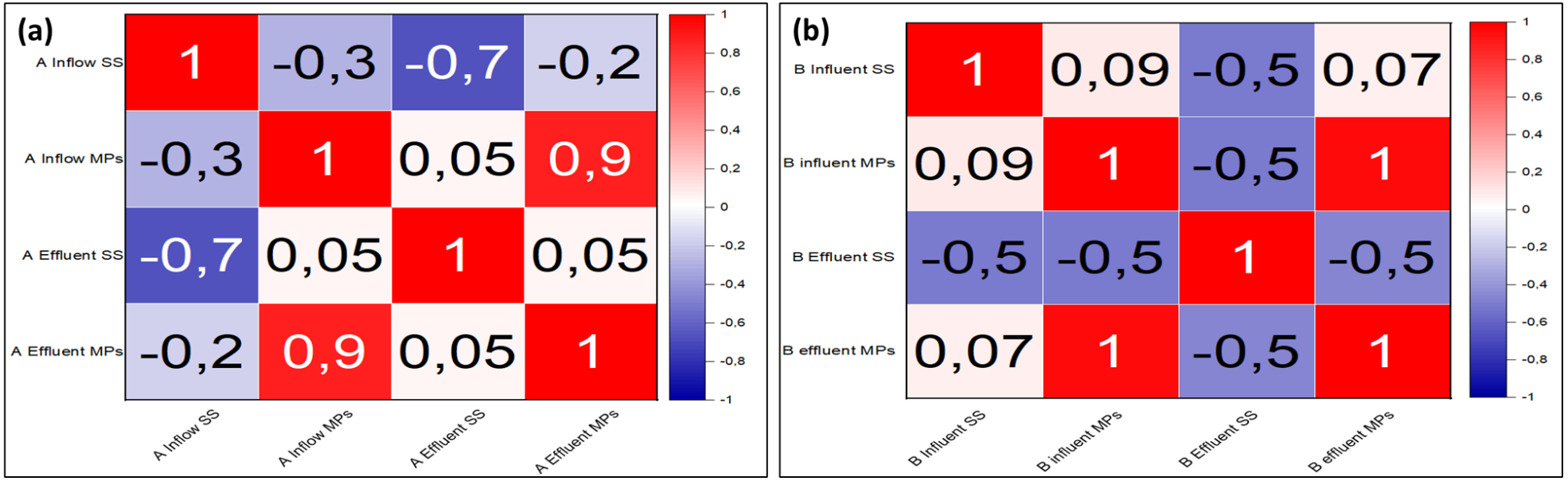

Figure 4-22: Correlation analysis between SS & MPs WWTW A & B, (a) WWTW A correlation, (b) WWTW B correlation

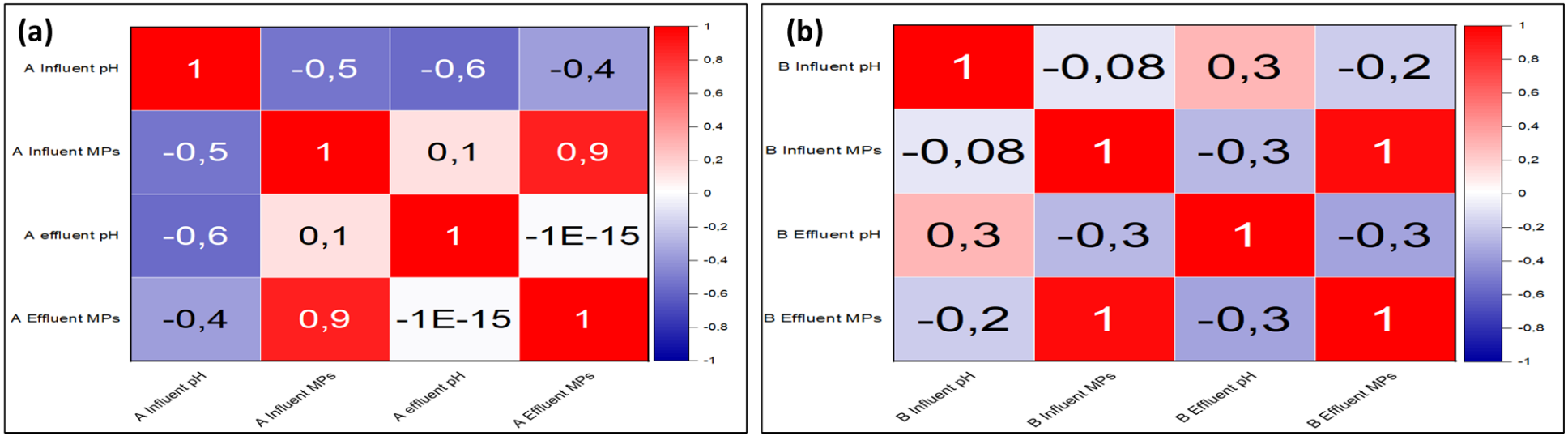

Figure 4-23: Correlation analysis between pH & MPs WWTW A & B, (a) WWTW A correlation, (b) WWTW B correlation

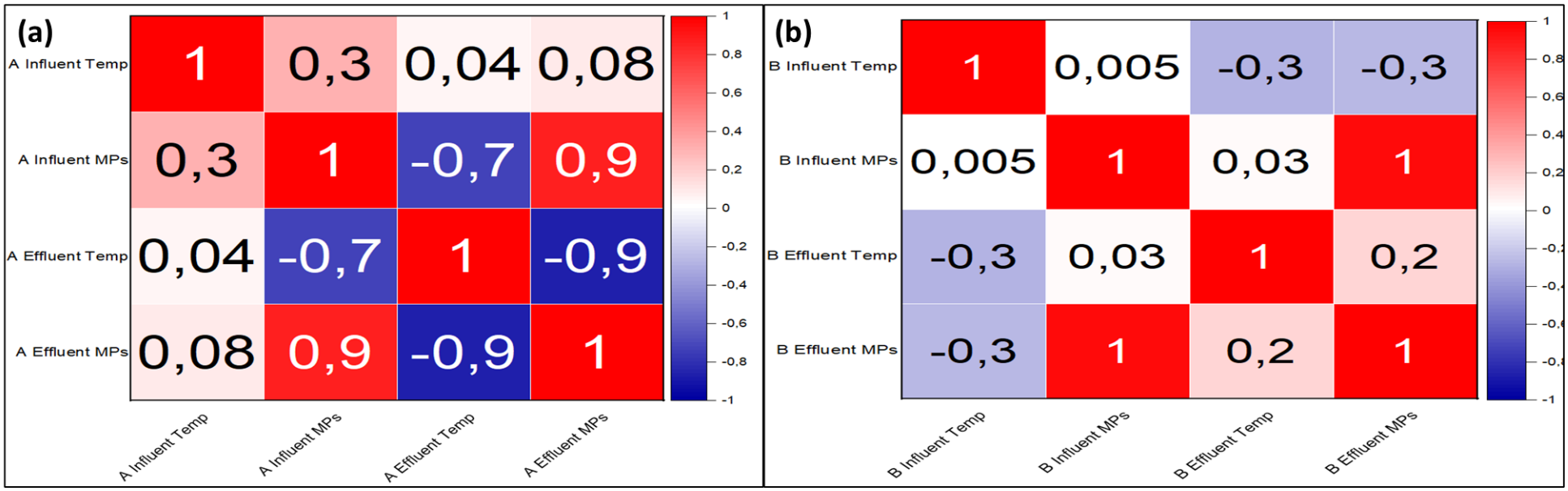

Figure 4-24: Correlation analysis between Temperature & MPs WWTW A & B, (a) WWTW A correlation, (b) WWTW B correlation

Microplastics in various studies have been observed to have positive correlations with physical and chemical parameters such as SS, COD and pH (Dalu *et al.,* 2021; Kwon *et al.,* 2022; Shekoohiyan & Akbarzadeh, 2022). The results of the study conducted by Kwon *et al.* (2022) using the IBM Statistical Package for Social Sciences (SPSS) shows that at 0.05 significance level, there is a positive correlation between COD, SS and MPs influent and effluent of WWTWs. In the current study COD had a positive correlation with MPs, but contrary to the study done by Kwon *et al*., (2022) there was a negative correlation between SS and MPs. Studies such as Dalu *et al.* (2021) and Seidensticker *et al* (2018) also confirmed that temperature and pH correlate with MPs concentrations. Contrary to the current study, temperature and pH in the influent and effluent did not correlate significantly. The poor correlation between pH and microplastics is likely to indicate a poor adsorption of polar and non-polar compounds in wastewater (Menéndez-Pedriza and Jaumot 2020).

The positive correlation between microplastics and organic parameters such as COD and SS suggest that higher microplastics are likely to be discharged with effluent containing high organic concentrations, this is likely to increase turbidity and toxicity in the receiving waters (Kim *et al* 2023; Kwon *et al* 2022).

4.7.2. Correlation between organic loading (COD & SS) and microplastics loading.

The COD, SS and MPs organic loading were obtained by multiplying the concentrations with the average flows received and discharged by WWTW A and B. From Figure 4-25 (a) and (b) it can be noted that there is a negative correlation between influent COD and microplastics loading (r=-0.2 and 0.003) for WWTW A and B. There was a smaller correlation between effluent COD loading and MPs loading (r=0.3 and 0.4) for WWTW A and B respectively. According to Figure 4-26 (a) there was a negative correlation between influent SS and MPs loading for WWTW A (r=-0.3). The effluent SS and MPs loading showed a smaller correlation (r=0.2) for WWTW A. The correlation for WWTW B is different from plant A, where the influent SS and MPs loading showed a smaller correlation and the effluent resulted in a negative correlation (See Figure 4-26 (b).

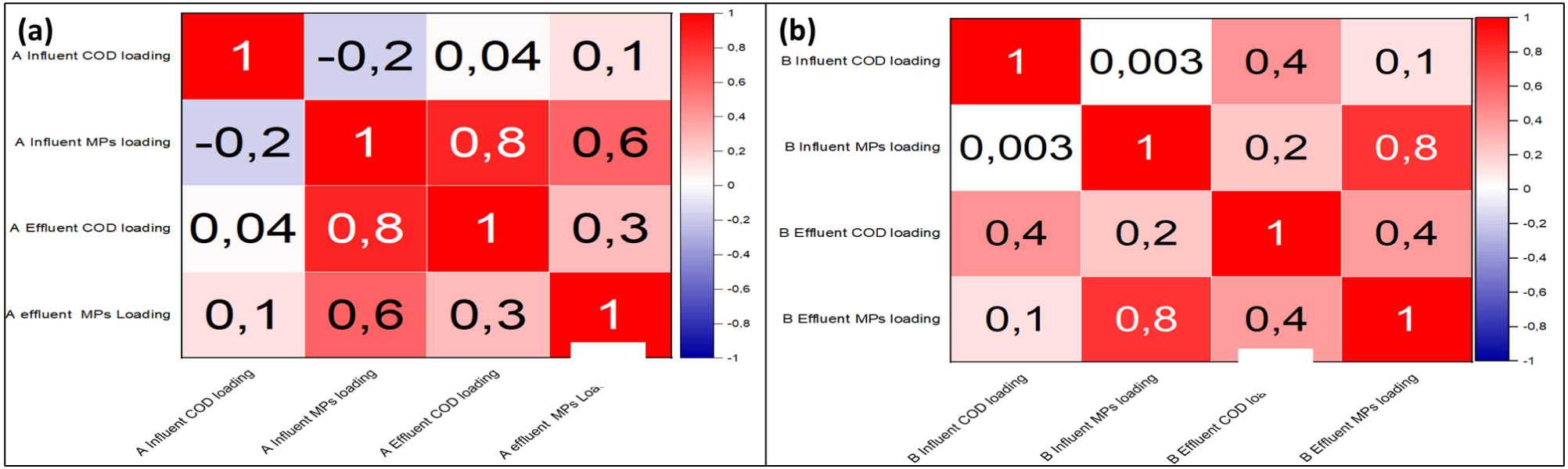

Figure 4-25: Correlation analysis between COD & MPs loading WWTW A & B, (a) WWTW A correlation, (b) WWTW B correlation

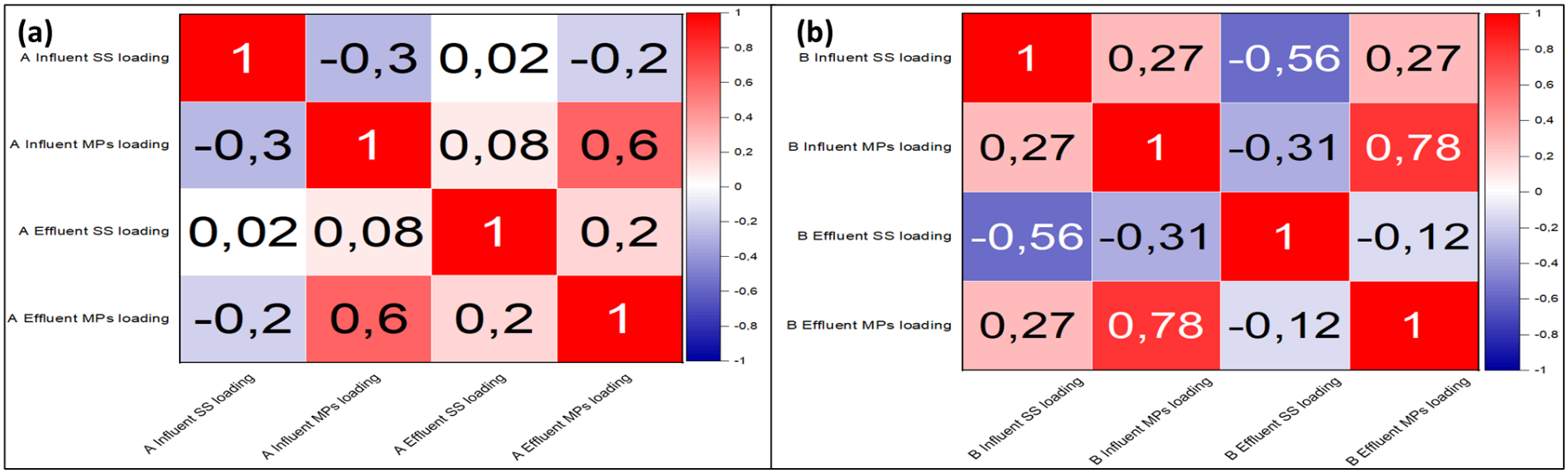

Figure 4-26: Correlation analysis between SS & MPs loading WWTW A & B, (a) WWTW A correlation, (b) WWTW B correlation

The COD and SS loading rate were obtained through recorded inflow rates which were also used to estimate MPs loading rate from the observed mass. The overall correlation analysis showed that there is no correlation between influent COD and MPs loading for most of the times, the effluent COD and MPs shows a smaller positive correlation while the influent and effluent SS loading showed a smaller correlation.

Although the COD and SS loading rate were correlated to MPs loading in the current study, there was limited literature correlating COD and SS loading in kg/d and MPs in kg/d. Most studies reported a correlation of microplastics in counts/d or items/d comparing it to COD and SS in mg/L, therefore the studies compared MPs loading and organic concentrations which is different to the findings of the current study. The examples of studies such as Kwon *et al.* (2022) and Long *et al.* (2019) did a comparison of COD and SS concentrations to microplastics load, however, did not consider the mass of MPs. The overall results are evidence that a holistic approach to microplastics quantification is required to get comparable results.

Although there is limited literature on SS and COD loading correlations, it is known that high COD and SS loading is associated with presence of microplastics in the surface water or receiving water bodies of wastewater effluent.

4.7.3. Statistical correlation between the concentration of microplastics, toxic metals, non-metallic parameters and their potential health effects.

The total of seven heavy metals and four non-metallic parameters were monitored and analysed at WWTW A and B and microplastics concentrations were determined at the influent and effluent each WWTWs. Microplastics are known to adsorb organic materials on their surfaces. The WWTWs has various sources of influent which include industries, storm water drainage, health facilities, pharmaceutical companies and domestic wastewater. The diverse source of influent makes the wastewater complex and contain different contaminants like heavy metals, microplastics and their additives. Therefore, it was essential to establish the statistical correlation between presence of microplastics and heavy metals.

Figure 4-27 (a) and (b) indicate that only chloride has a small positive correlation with influent and effluent microplastics concentrations (r=0,29 and 0.15) respectively. There was also a smaller correlation between Zn and Fe and MPs in the effluent for WWTW A (r=0.51 and 0.16). Other trace metals such as Al, Mn, Cd, Fe, Pb and non-metallic parameter SO_4^{2-} did not correlate with MPs concentrations, this is likely due to the less concentrations detected in the influent and final effluent of WWTW A. The correlation coefficient values are presented in Figure 4-28 (a) and (b). Overall, there is no positive correlation between trace metals and MPs at WWTW A. However, the metals are detected in lower concentrations and may accumulate in the surface of microplastics and accumulate in the rivers.

Figure 4-28 below present the correlation coefficient for non-metallic parameters such as Cl^-, S, SO_4^{2-} and F^- detected at WWTW B. Only Cl^- showed a small positive correlation (r=0.48 and 0.6) with influent and effluent MPs concentrations for WWTW B, non-metallic species such as Cl^-, S and SO_4^{2-} showed a negative correlation for WWTW B more specifically at the influent. Although S and SO_4^{2-} correlated negatively with influent MPs concentrations, the non-metallic species showed a small positive correlation on the final effluent. The correlation of non-metals on the final effluent is likely due to increased concentrations attributed to the breaking down of organic and inorganic materials that maybe composed of different chemical composition detected in wastewater as well as microplastics samples.

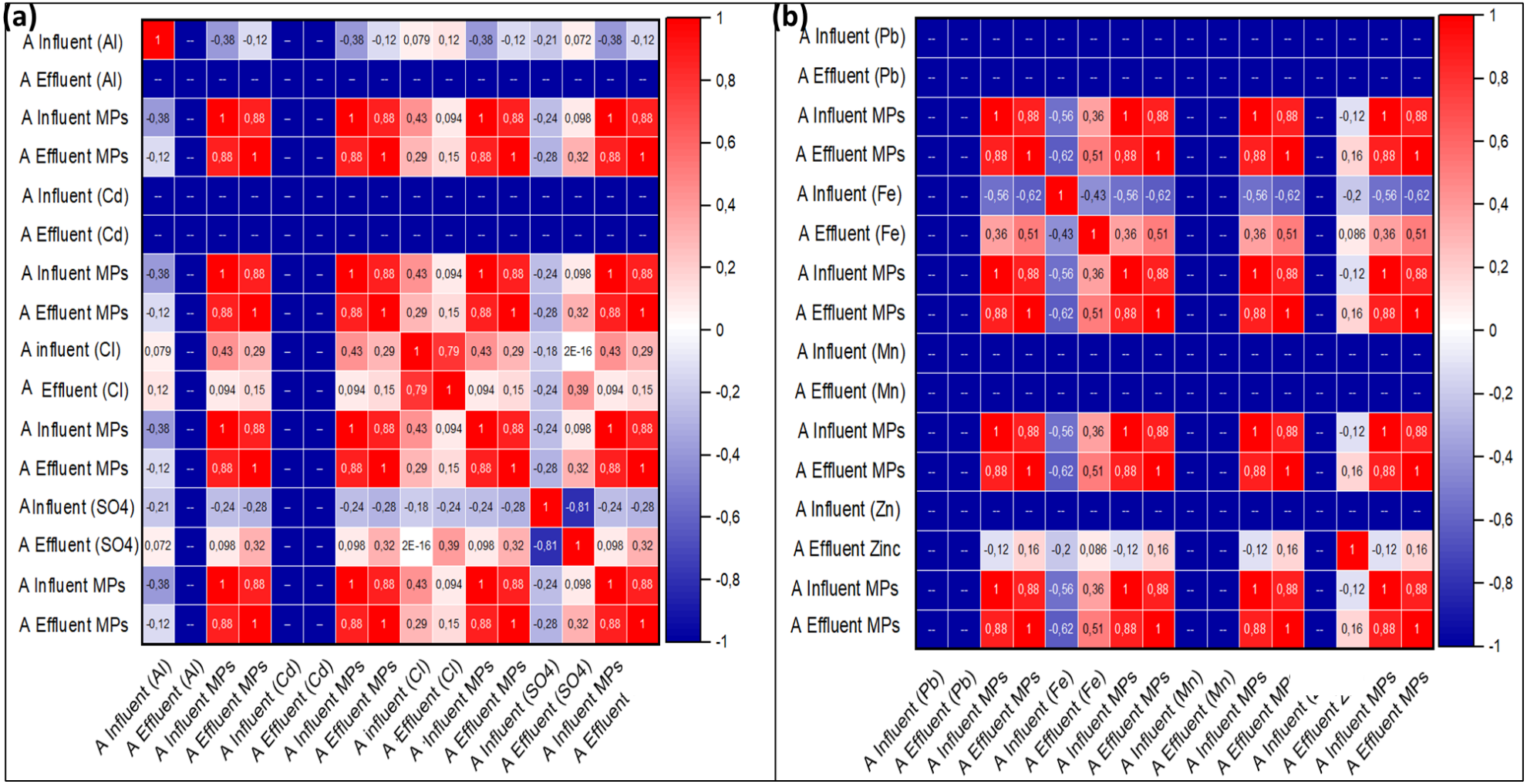

Figure 4-27: Correlation analysis between non-metallic parameter, metals & MPs concentrations WWTW A.

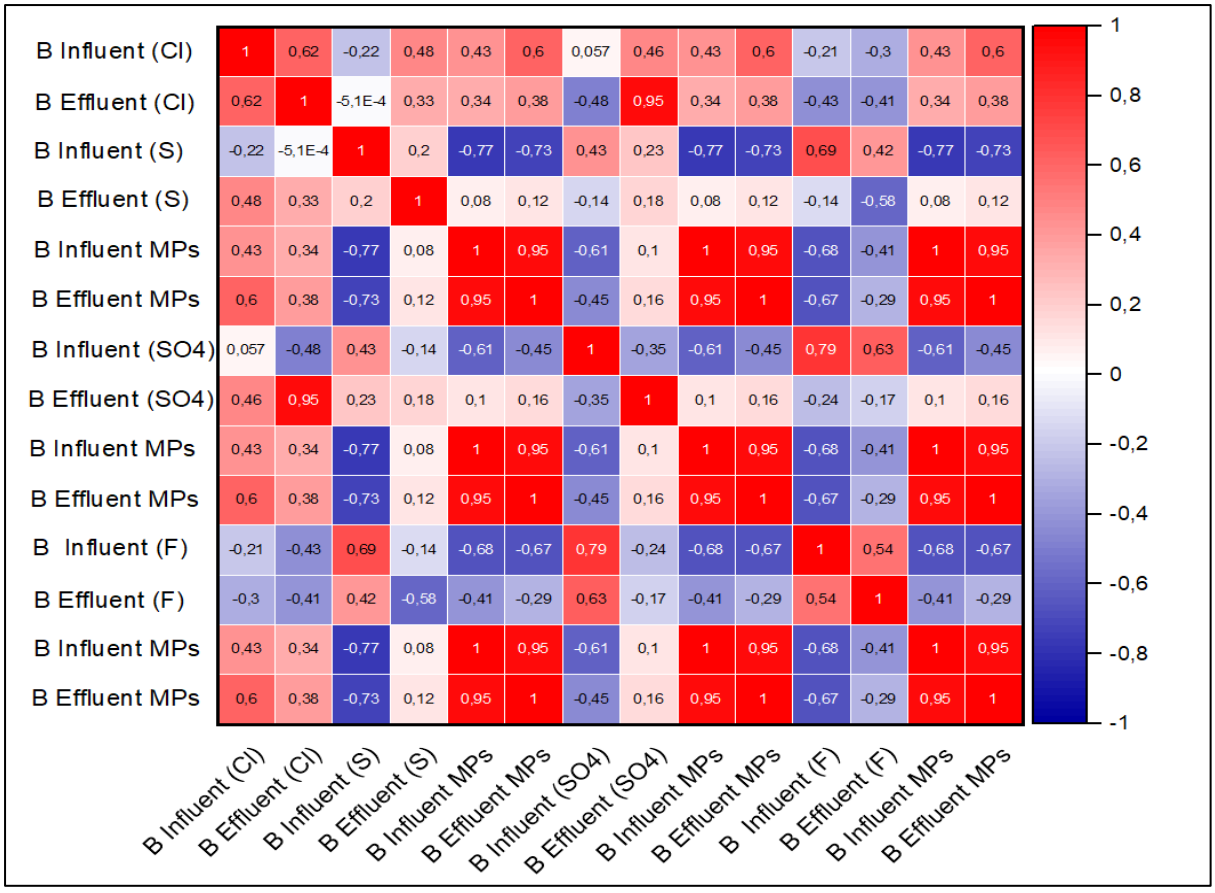

Figure 4-28: Correlation analysis between non-metallic parameter, metals and MPs concentrations for WWTW B

The heavy metals and non-metallic parameters were monitored in the influent and effluent for both WWTW A and B. According to the results obtained, it was evidence that only Cl^- correlated positively with both influent and effluent MPs concentrations, while S, SO_4^{2-} and Fe only showed positive correlation with effluent MPs concentrations. Although other parameters did not correlate statistically, their availability in water even in small concentrations can lead to accumulation and changes toxicity of the rivers (Ziajahromi, 2018).

The study done by Nkosi *et al.* (2022) confirmed the presence of heavy metals such as As, Cu and Zn on the MPs. The presence of heavy metals on the final effluent containing microplastics is an indication of contaminants distribution into the rivers and other receiving environment. The presence of microplastics and heavy metals can cause adverse effects on the ecological status of the river by changing the water quality and ingestion by aquatic species is more detrimental to their health (Kwon *et*

al., 2022). The accumulation of heavy metals in aquatic species and surface water present danger in the food chain (Liu *et al.* 2021). It is likely that human beings may consume aquatic food and drinking water from source heavily polluted by microplastics which poses health risk.

A number of studies have reported the presence of microplastics that have accumulated in fish species and other sea food (Beer *et al.,* 2018; J. Zhao *et al.,* 2018). There are various routes in which microplastics may find their way into the human body, the most common exposure routes is through eating food and inhaling air contaminated with microplastics (Yuan *et al.,* 2022). Accumulated microplastics in the human body may results in the deformation of cells, raising up the inflammatory levels in the body, this can lead to ultimate death to human beings (Bhuyan, 2022). The study done by Wu *et al.* (2019) showed that microplastics in the human body reduces the growth Caco-2 cells and affect cell membranes such as mitochondria, the reported polymer for such adverse effects was PS. The exposure of people with compromised immune systems to microplastics, may further affect their wellbeing. Many studies have reported the adverse effects of microplastics such as stomach disorders, bowel disorder, brain damages, metabolic disorders and cancer (Bhuyan, 2022; Wu *et al.,* 2019; Xu *et al.,* 2022; Yuan *et al*., 2022, 2022).

Bhuyan (2022) further cited that the combined effects of microplastics with contaminants such as heavy metals (Pb, Cd and Zn) also contribute to hormonal disorders. Heavy metals are usually adsorbed to the surfaces of microplastics leading to the distribution of the contaminants in the human body through the consumption of food and liquids containing microplastics. Leslie *et al.* (2022) confirmed the presence of PS, PE and PET in human blood, continuous research on microplastics and human health implications is necessary to prevent health risk to human beings.

CHAPTER 5:

CONCLUSION AND RECOMMENDATIONS

This chapter conclude the findings obtained from the investigation of the occurrence and removal options of microplastics in wastewater treatment processes. The overall objectives achieved, and results are summarised in this chapter and the detailed description of answered research questions is presented. Furthermore, based on the findings and discussions, recommendations are provided for future research and improvements in the monitoring and management of microplastics in WWTWs.

5.1. Summary of results

The physicochemical water quality test done such as pH, temperature, SS and COD showed a higher concentration in the influent and effluent, except for temperature which did not change across all seasons. Heavy metals were also monitored in the two WWTWs. However, the concentrations of metals detected did not show much difference in the influent and effluent. Statistically, the overall correlation between the physicochemical parameters and trace metals such as Al, Cd, Chloride, Pb, Fe, Mn, Na, and Zn showed a negative or smaller correlation for both WWTW A and B. Only COD and Cl concentrations showed high correlation with MPs concentrations. There was a small correlation between COD and SS loading and microplastics in kg/d.

Microplastics quantifications were done by determining the concentrations and loading in the influent and effluent of WWTWs. The influent concentrations and loadings of MPs were observed to be higher than the effluent MPs across summer, autumn, winter and spring. Lower concentrations were observed in the summer and autumn, while higher concentrations were observed in winter and spring. The removal efficiencies ranged between 6-66% at WWTW A and 46-66% at WWTW B. Although the two WWTWs removes microplastics, an estimated 34% of MPs are discharged into rivers.

The characterization of microplastics was done in order to determine the sizes, shapes colours and chemical properties of microplastics. The microplastics sizes ranged between 0.01-0.02 mm in the influent and 0.01-0.05 in the final effluent with majority of fibres, fragments and angular shaped particles observed with colours ranging from

white, blue, red and black. The chemical characterisation entailed the identification of polymers and elementary analysis. The different polymers identified in the influent and effluent of WWTWs include PE, PET, PAM, PPM, ABS, PS, PPC and TPV. The elementary analysis of microplastics confirmed the presence of additives such as Al, Mg, Na, Zn, S, Si and abundance of O and C atoms which can be linked to presence of polymers such as PE and PAM. The functional group indicated by the FITR spectra confirmed the –OH polymeric bonds and the presence of methylene, ethylene and esters which are linked to microplastics additives.

5.2. Conclusions

The occurrence and removal options of microplastics in the activated sludge processes at WWTW A and B was investigated. The physicochemical parameters such as pH, temperature, SS and COD are important in the determination of microplastics concentrations. Although temperature and pH did not show a significant correlation with MPs concentrations, it is important to continuously monitor them in wastewater as it is documented that they have influence of microplastics and transport of contaminants. The presence of SS and COD has influence on the microplastics loading rate and organic loading, the positive Pearson correlations suggest that the two parameters can be used as indicators of MPs pollution in WWTWs.

The analysis of heavy metals in the wastewater samples indicated the presence of trace metals such as Al, Cd, Cl^-, Pb, Fe, Mn, F^-, Sodium, and Zn. Although the overall correlation of metals and MPs concentrations is weak, their presence in the influent and effluent of WWTW A and B presence risk to the environment, as microplastics act as their vector to the environment.

The microplastics identified in WWTWs vary in terms of concentrations and loading rate across different locations, based on the current study WWTW A had smaller capacity than WWTW A. WWTW B showed high concentrations and loading of microplastics as compared to WWTW A. This conclude that WWTWs with high operational hydraulic and organic loading receive more microplastics. The removal efficiency of microplastics is higher in WWTWs with sufficient hydraulic design capacity, WWTW A had smaller design hydraulic capacity as compared to WWTW B which remove more microplastics. Although the design of WWTWs plays an important

role in MPs concentrations and loading, the seasonal changes showed that from summer to autumn concentrations are slightly low as compared to winter and spring, this is attribute to high organic concentrations during dry seasons and high dilutions factors of wastewater in the wet seasons.

The shapes, colours, and sizes of microplastics are not different across WWTWs, the physical properties of microplastics in WWTW A and B were similar across four seasons. However, the occurrence of diverse types of polymers at WWTW A indicates that wastewater connected to large industrial grids are prone to microplastics pollution as compared to WWTW B which only receives domestic sewage. The chemical composition of polymers were dominated by trace metals and organic compounds such as hydroxyl compounds, Alcohol, saturated aliphatic, polymeric –OH stretches which confirms the presence of plastics particles in the samples.

5.3. Recommendations

In line with the findings of the current study and empirical evidence gathered from literature, the following recommendations are suggested:

- The water quality monitoring programmes in the Water Use Licences (WUL) should make provisions for inclusion of microplastics to track their distribution into the environment.
- The current study was focused on the occurrence of microplastics in the influent and effluent of WWTWs, larger fractions of microplastics end up in the sludge process, it is therefore recommended that a broader study can be done focused on the assessments of microplastics in the sludge, soil, groundwater, influent, effluent, upstream and downstream in order to trace microplastics over the entire treatment process chain.
- The eco-toxicological impacts of microplastics discharged with WWWTs final effluent need further investigations to ascertain researchers on the toxicity associated with the particles.
- The municipal bylaws do not include the restrictions of disposal of plastics particles into the WWTWs-therefore municipalities should consider the

amendments of bylaws to reduce plastics loading which impact on the treatment processes.

- The designs for WWTWs should make a provision for microplastics removal to limit the high loadings and accumulations into the receiving environment.

APPENDIX A. *APPENDIX A: SEM/EDX IMAGES*

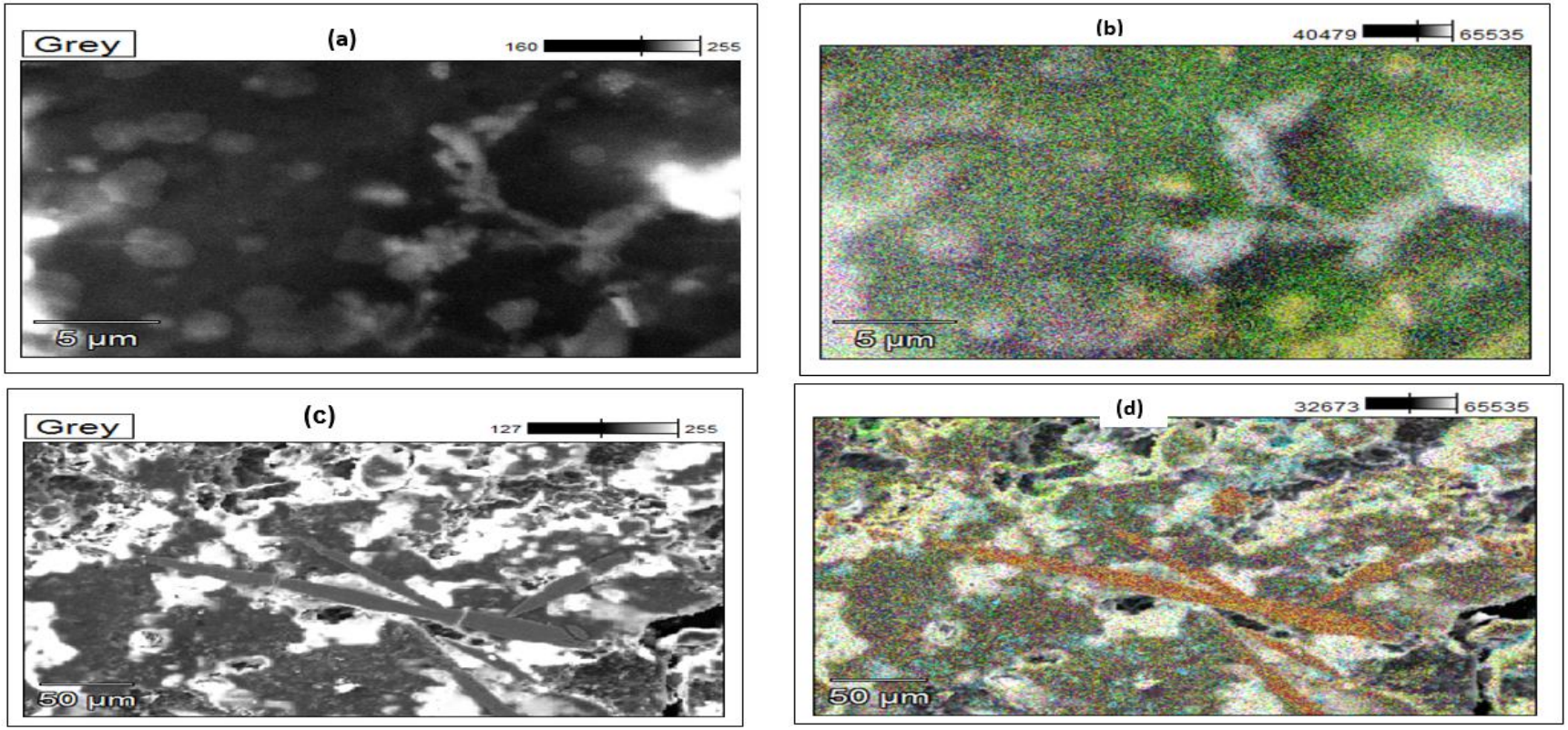

Figure A 1: ***SEM analysis for influent and effluent WWTW A:*** *(a) S1HART-A influent, (b) S1HART-A influent, (c) S2HART-A effluent and (d) S2HART-A effluent (summer).*

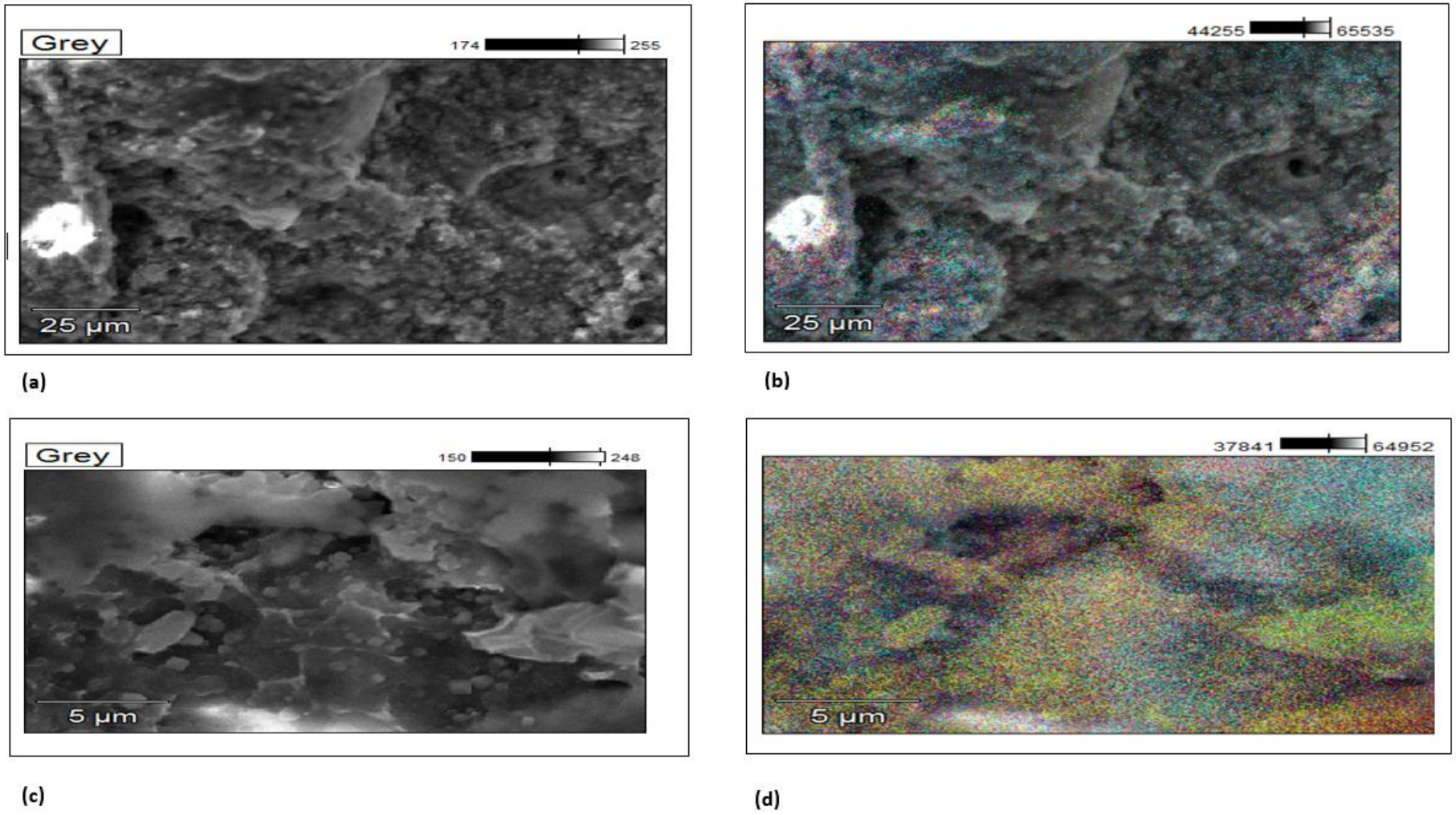

*Figure A 2:**SEM analysis for influent and effluent WWTW B:** (a) S3WART-B influent, (b) S3WART-B influent, (c) S4WART-B effluent and (d) S4WART-B effluent (summer).*

APPENDIX B. *APPENDIX B: POM IMAGES (SUMMER)*

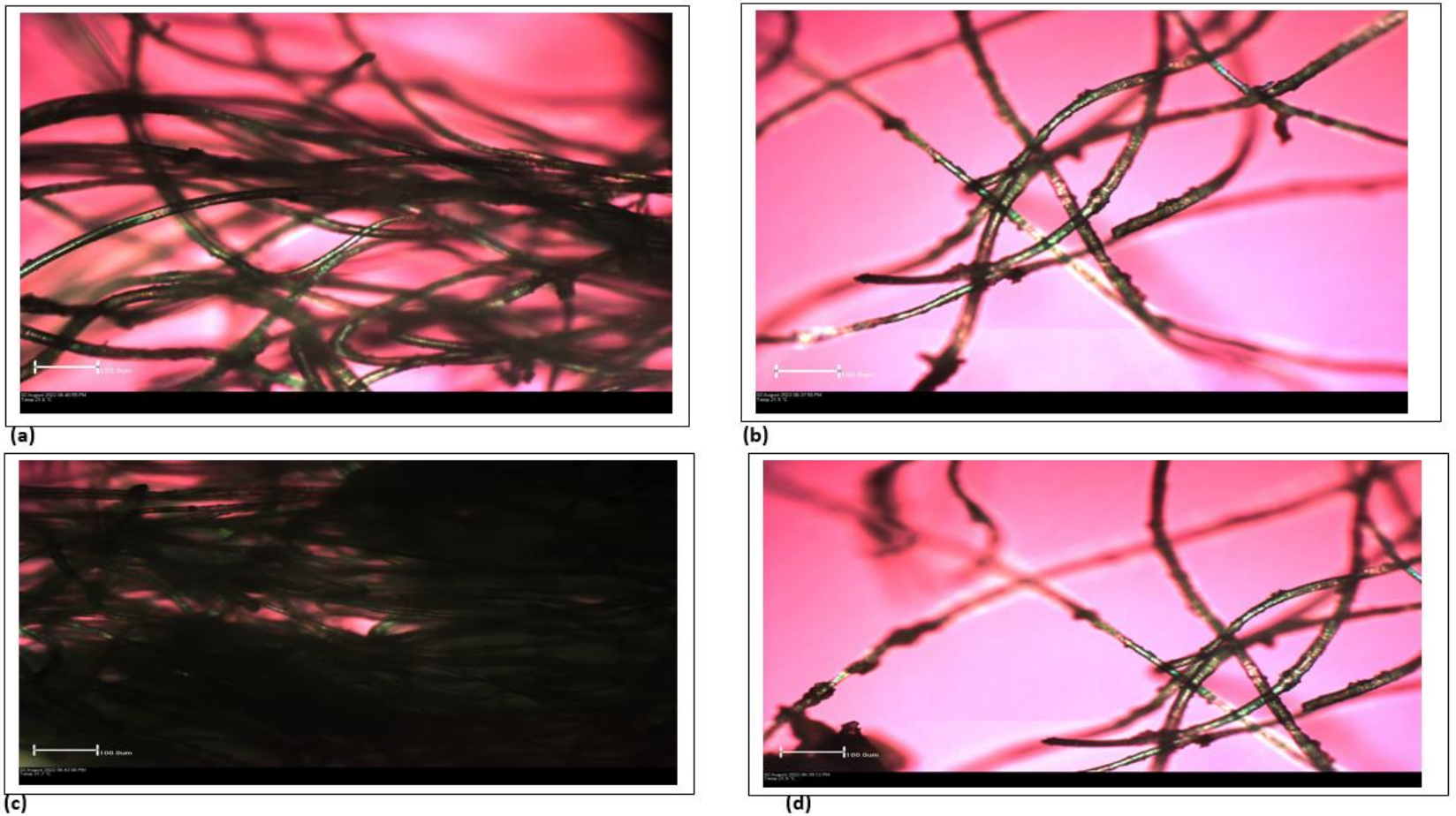

Figure A 3: ***POM analysis:*** *(a) S1HART-A influent, (b) S2HART-A effluent, (c) S3WART-A influent and (d) S4WART-A effluent (summer).*

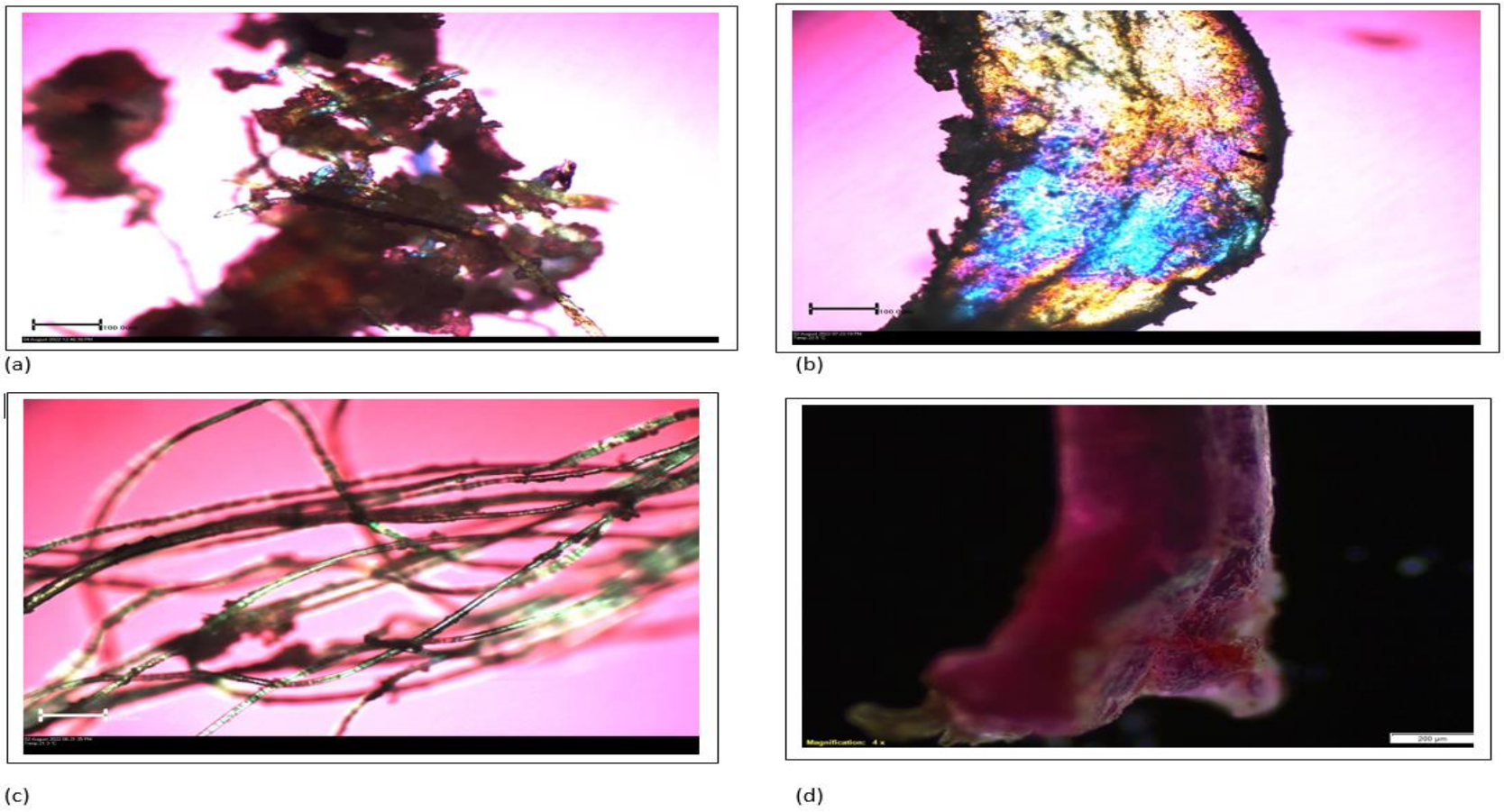

Figure A 4: **POM analysis:** (a) S5HART-A influent, (b) S6HART-A effluent, (c) S7WART-A influent and (d) S8WART-A effluent summer.

APPENDIX C. *APPENDIX C: POM IMAGES (AUTUMN)*

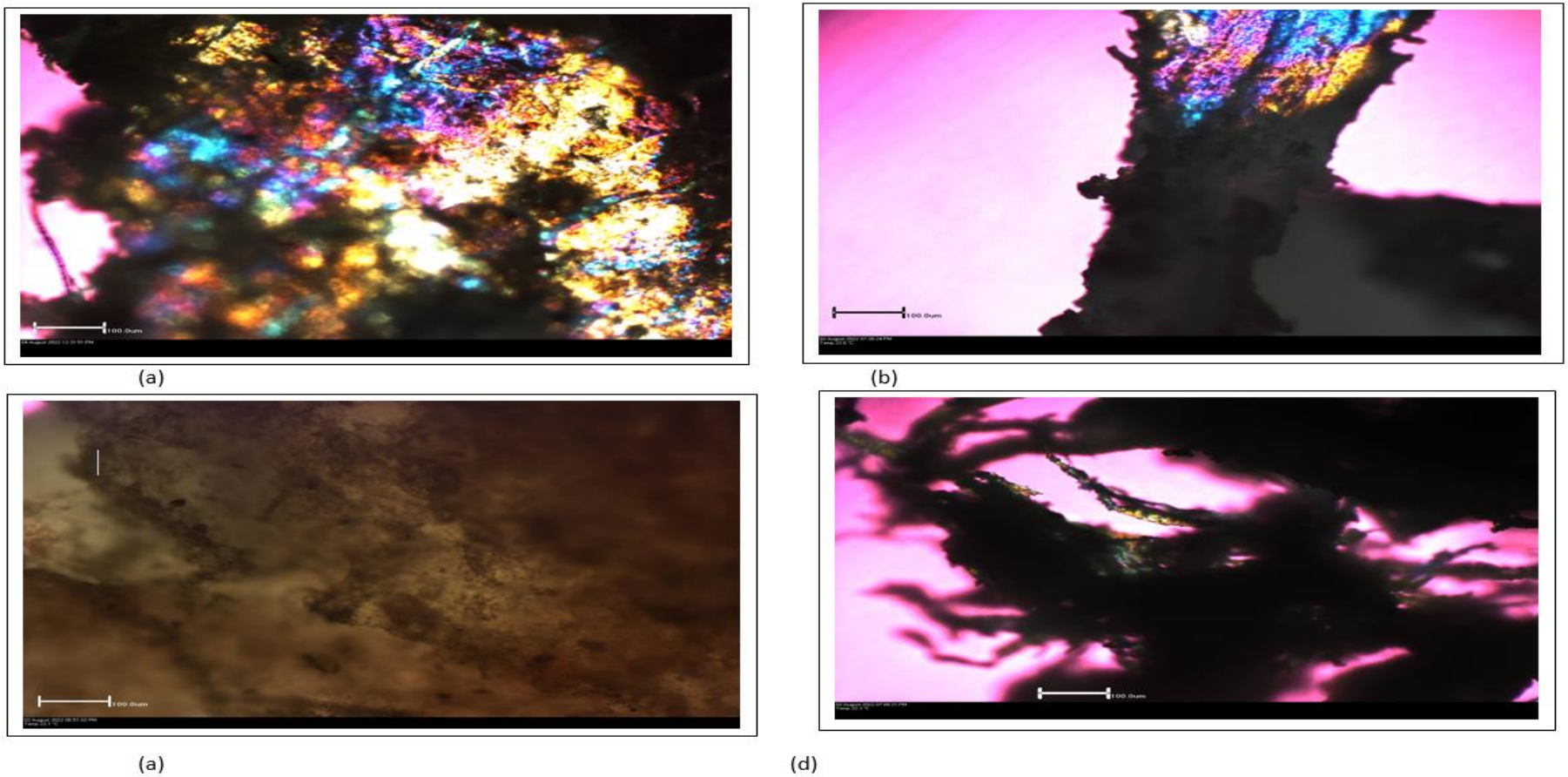

Figure A 5: ***POM analysis:*** *(a) S9HART-A influent, (b) S10HART-A effluent, (c) S11WART-A influent and S12WART-A effluent autumn.*

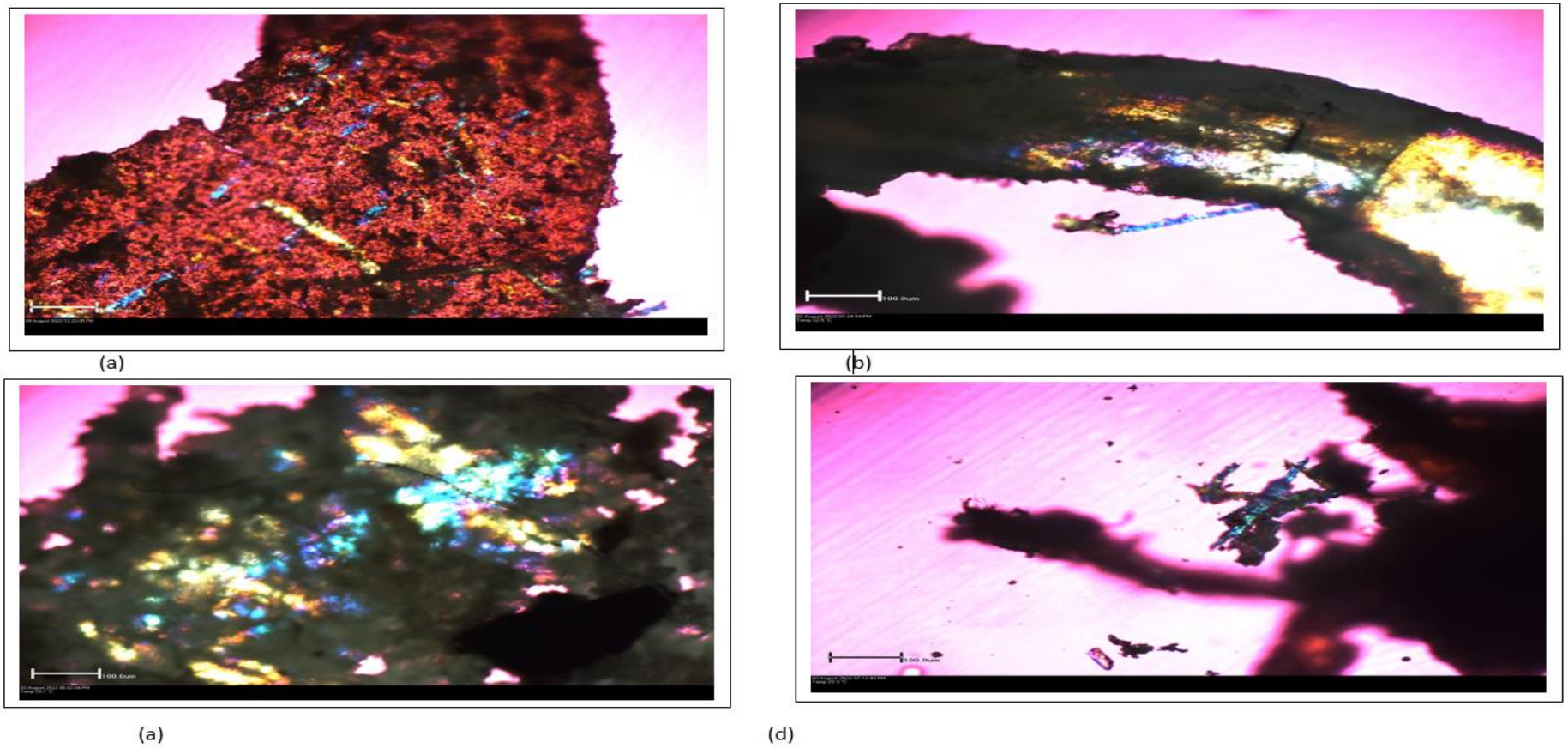

Figure A 6: ***POM analysis:*** *(a) S13HART-A influent, (b) S14HART-A effluent, (c) S15WART-A influent and S16WART-A effluent (autumn).*

APPENDIX D. *APPENDIX D: POM IMAGES (WINTER)*

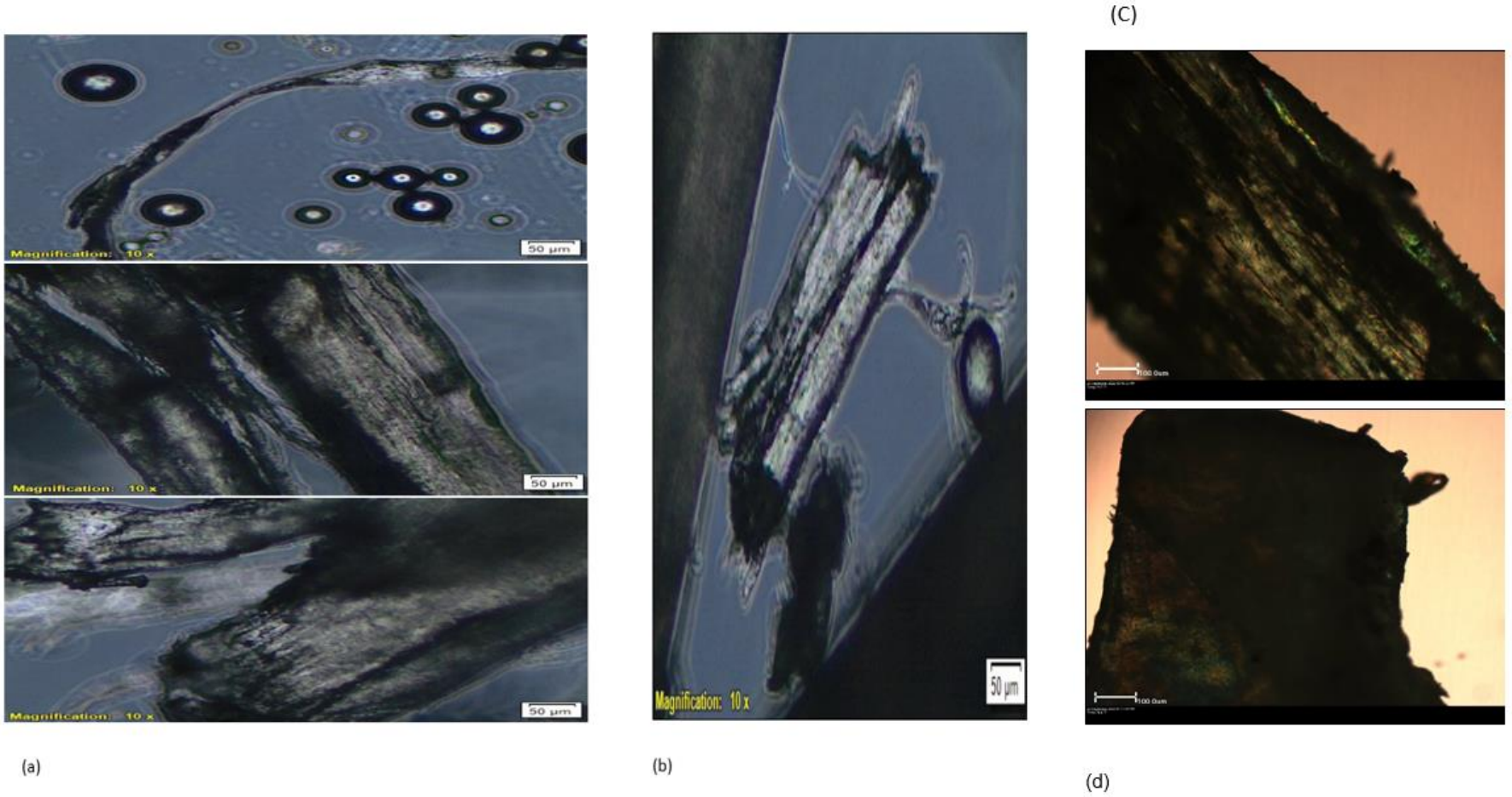

Figure A 7: ***POM analysis:*** *(a) S17HART-A influent, (b) S18HART-A effluent, (c) S19WART-B influent & (d) S20WART-B effluent (winter).*

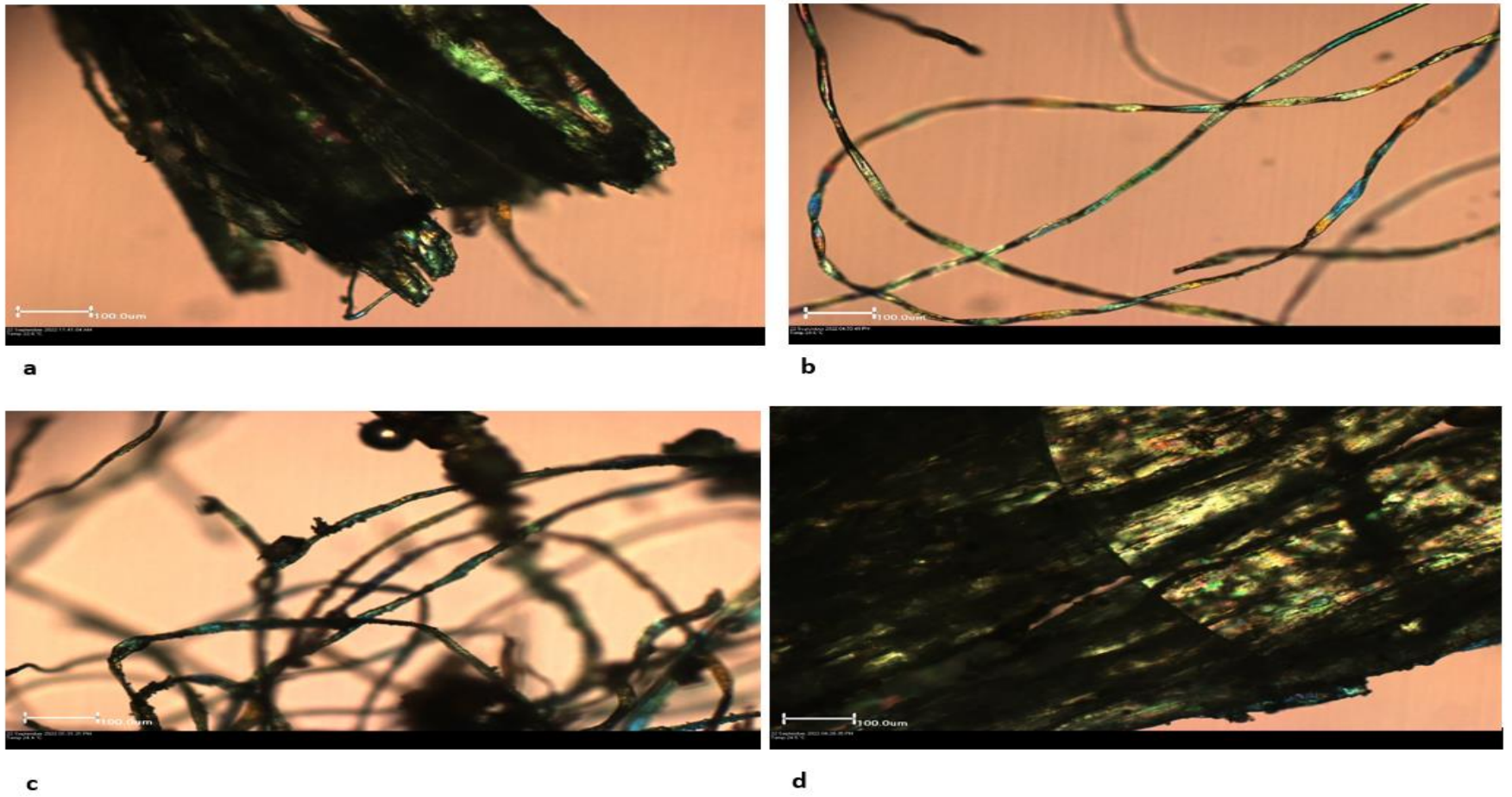

Figure A 8: ***POM analysis:*** *(a) S21HART-A influent, (b) S22HART-A effluent (c) S23WART-B influent, (d) S24WART-B effluent (winter).*

APPENDIX E. ***APPENDIX E: POM IMAGES (SPRING)***

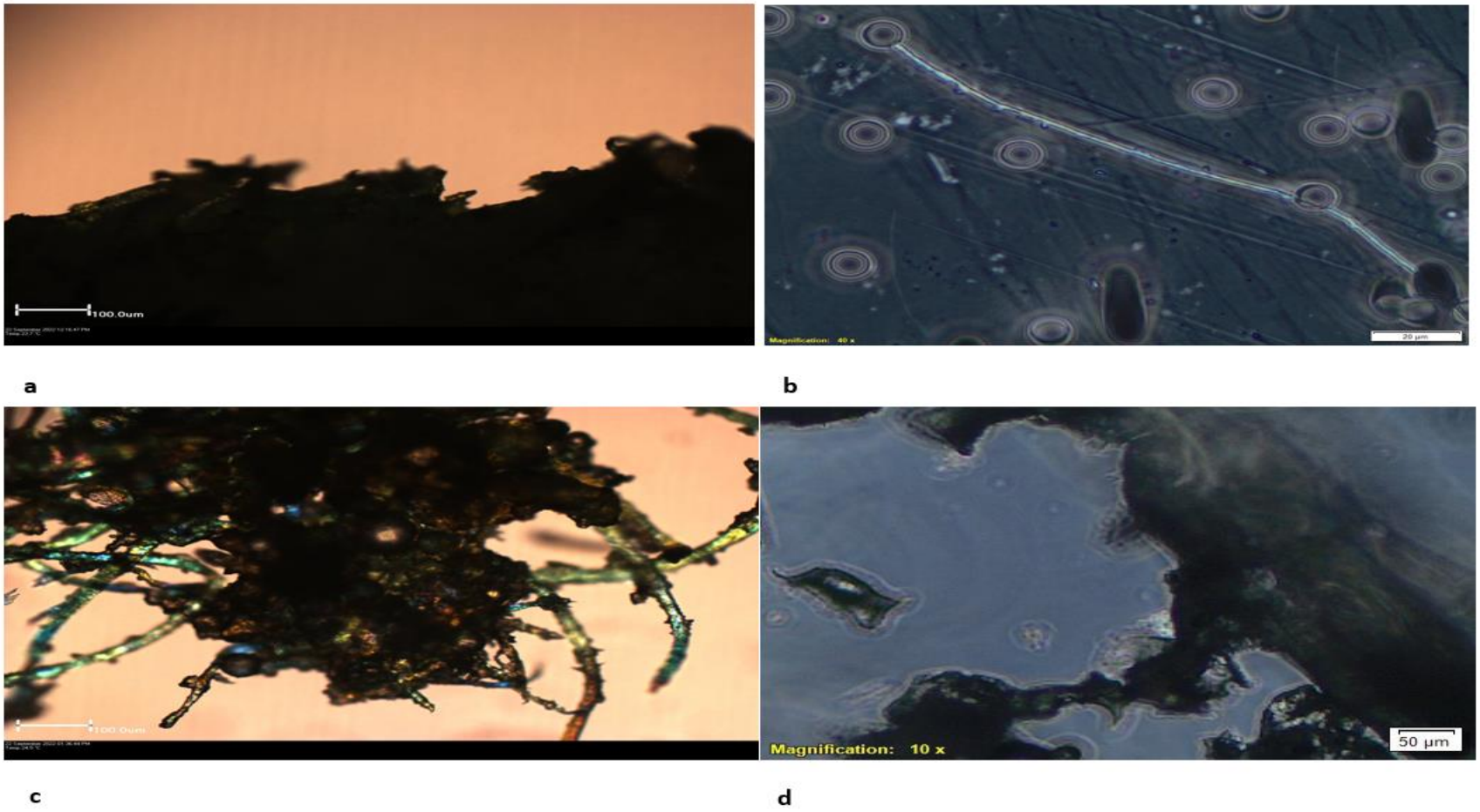

*Figure A 9:**POM analysis:** (a) S25HART-A influent, (b) S26HART-A effluent (c) S27WART-B influent, (d) S28WART-B effluent (spring).*

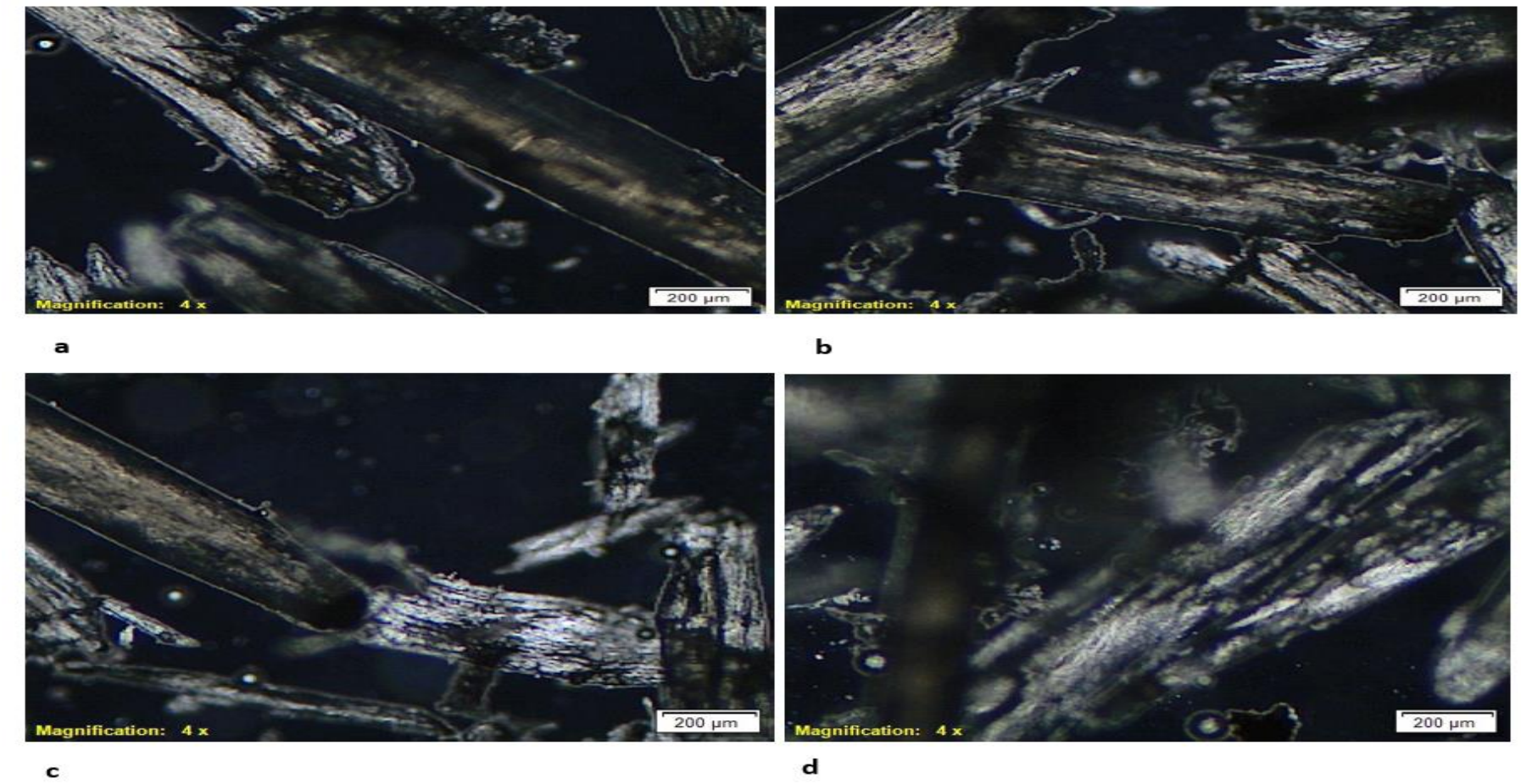

*Figure A 10:**POM analysis:** (a) S29HART-A influent, (b) S30HART-A effluent (c) S31WART-B influent, (d) S32WART-B effluent (spring).*

APPENDIX F. *APPENDIX F: CONSENT FORM*

UNISA university of south africa

CONSENT FORM

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Date 29 / 04 /2020.

TITLE OF RESEARCH PROJECT:

An Assessment Of The Occurrence and Removal Options Of Microplastics In Wastewater Treatment Processes At The City Ekurhuleni and Midvaal In South Africa

To: Ekurhuleni Water Care Company (ERWAT)

NATURE AND PURPOSE OF THE STUDY

I am Tendani Mphaga an MSc student at the University of South Africa (UNISA), College of Agriculture and Environmental Sciences, Department of Environmental Science. We wish to request your permission to undertake a Masters research project at designated plants at East Rand Water Care which involves site visit, provision of process infromation and sample collection. The student has been registered as from 28 April 2020 and would like to be granted approval for site visit to sampling sites for this study as from May 2020 for the period of the study. We will be sampling at allocated waste water treatment plants at the inlet works raw water and final effluent. Water samples will be collected once a month for a period of one (1) year. The physical parameters will be measured on site and 2L of water will be taken to UNISA for further analysis of Micro Plastics. We will appreciate if we can be allowed to conduct this study as micro plastics are one of emerging pollutants which has not been monitored and quantified in majority of waste water in South Africa yet literature has proven it has adverse effects on human health through various path which could be water and soil at most.

CONFIDENTIALITY

The identity of the property and personnel will be strictly confidential and only members of the research team will have access to the information. There will be no photographs taken during the course of sampling. Published work will not contain any information through which the participating supervisor/manager/respondent may be identified unless where written consent is signed.

Please note that you can withdraw your participation from this study when deemed necessary, without any penalties. If you have any questions concerning this research project, you can contact the supervisor Ms T.N Mhlongo, Department of Environmental Sciences.Science Campus, UNISA. Tel: 011 471 2655 or Email: eshongtn@unisa.ac.za

CONSENT

I, the undersigned ..... (Personnel Full name) have read the above information relating to the research and have also heard verbal version from the researcher, and therefore declare that I understand it. I hereby declare that I agree voluntary to participate to the research and indemnify the University and the researchers from UNISA against any liability that I may incur during the course of the project, unless resulting from negligence on the part of UNISA and the researchers.

Name of the researcher: Tendani Mphaga

Signature ..... Date 07 / 05 /2020

Supervisors Signature: ..... Date 07 / 05 /2020

Company ..... Designation of Personnel .....

Personnel Signature ..... Date 20 / 05 / 2020

APPENDIX G. *APPENDIX G: ETHICS CLEARANCE LETTER*

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 12/04/2021

NHREC Registration # : REC-170616-051
REC Reference # : 2021/CAES_HREC/066
Name : Mr T Mphaga
Student #: 69178240

Dear Mr Mphaga

Decision: Ethics Approval from 08/04/2021 to 31/03/2024

Researcher(s): Mr T Mphaga
69178240@mylife.unisa.ac.za

Supervisor (s): Ms TN Mhlongo
eshongtn@unisa.ac.za; 011-471-2655

Working title of research:

An assessment of the occurrence and removal options of microplastics in wastewater treatment processes at the City Ekurhuleni and Midvaal in South Africa

Qualification: MSc Environmental Science

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for three years, **subject to further clarification and submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for progress report: 31 March 2022

Please note the points below for further action:

1. Is there any risk to the researcher during the sample collection? The researcher should show an awareness of any possible risks to her own safety, and indicate what measures will be put in place to mitigate these.

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