OCCURRENCE, RISK ASSESSMENT AND ELIMINATION OF CHEMICALS OF EMERGING CONCERNS IN WASTEWATER TREATMENT PLANTS IN WESTERN, KENYA

BY

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree at any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

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DEDICATION

This thesis is dedicated to those who, in one way or another, have assisted in the preparation of this work.

To two supervisors, for your support, sharing of knowledge, and encouragement during the completion of this project. Your support for me has been very commendable, and your belief in my work has motivated me to continue my research.

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ABSTRACT

Chemicals of emerging concern (CECs) are a global threat due to their adverse effects on aquatic organisms and human health. Wastewater treatment plants (WWTPs) are a significant source of these compounds into the environment at varying concentrations ranging from ng/L to µg/L. However, data on the removal efficiencies of waste treatment technologies in Africa are limited. Therefore, this study aimed to assess the occurrence, removal efficiency, and potential toxic risk posed by CECs in wastewater treatment plants. The objectives were to: i) identify and quantify CECs from WWTPs, ii) evaluate the removal efficiencies of CECs by the selected WWTPs, and iii) perform ecotoxicological risk assessment of the detected CECs in the effluent of the four selected WWTPs in western Kenya. Solid-phase extraction and high-performance liquid chromatography coupled with high-resolution mass spectrometry were used for sample preparation and analysis, respectively. A target list of 795 compounds, including pesticides and biocides, pharmaceuticals, and industrial compounds, among others, was used. The toxic unit (TU) approach was applied to evaluate the risk posed by the contaminants present in the effluent of the WWPs. A total of 353 compounds were detected across influents and effluents of the sampled WWPs, with the most frequently detected compound classes being pharmaceuticals (102), pesticides (70), and industrial chemicals (72). The highest influent concentrations were detected for caffeine (830 µg/L), deoxycholic acid (719 µg/L), 2-oxindole (43 µg/L), ibuprofen (24 µg/L), and dichlorvos (14 µg/L). Notably, previously undocumented antiviral drugs such as emtricitabine and amantadine were reported for the first time at concentrations ranging from 4 ng/L to 536 ng/L. In the effluent, high concentrations were detected for caffeine (17 µg/L), TMDD (1.3 µg/L), cetirizine (2.3 µg/L), dichlorvos (1.3 µg/L), and sucralose (3.1 µg/L). Removal efficiencies of the compounds varied greatly, with 286 compounds having positive removals and 67 compounds with negative removal efficiencies. Compounds with > 80% removal efficiency included ibuprofen, trimethoprim, TMDD, diclofenac, and diazinon. WWTPs employing a combination of primary and secondary stabilization ponds, activated sludge, and trickling filters performed better in removing CECs. Risk assessment revealed crustaceans had the highest potential risk, with toxic units (TUs) up to 5.5, driven primarily by dichlorvos and diazinon. Algae and fish predominantly experienced chronic toxicity, with dichlorvos being the primary driver of toxicity for algae and didecyldimethylammonium for fish. This study underscores the substantial contribution of WWTPs to contamination of aquatic environments, with pesticides, pharmaceuticals, and industrial chemicals being the most persistent. It provides evidence-based data on the need for technological advancements in CEC removal, chemical use, and disposal, as well as robust monitoring and regulatory measures.

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LIST OF ABBREVIATIONS AND ACRONYMS

AES: Alkyl ethoxy sulfates

ARBs: Antibiotic-Resistant Bacteria

ARGs: Antibiotic Resistance Genes

BOD: Biochemical Oxygen Demand

CA: Concentration Addition

CECs: Chemicals of emerging concern

CLC: Capillary Chromatography

DEET: N, N-diethyl-meta-toluamide

DGT: Diffusive Gradients in Thin-Films

DO: Dissolved Oxygen

ECs: Emerging Contaminants

EDCs: Endocrine Disrupting Chemicals

PrEP: Pre-exposure Prophylaxis

ELDOWAS: Eldoret Water and Sanitation Company

EPA: Environmental Protection Agency

ERA: Ecological Risk Assessment

ESI: Electrospray Ionization

EU: European Union

FPs: Facultative Ponds

GC- Gas Chromatography

GC-MS: Gas Chromatography Mass Spectrometry

HLB Cartridges: Hydrophilic-Lipophilic Balance Cartridges

HPLC: High Performance Liquid Chromatography

HRT: Hydraulic Retention Time

IA:	Independent Action
LC-HRMS:	Liquid chromatography-high resolution mass spectrometry
LC-MS:	Liquid chromatography - mass spectrometry
LOEC:	Lowest Observed Effect Concentration
MAE:	Microwave Assisted Extraction
MEC:	Measured Environmental Concentration
MLSS:	Mixed Liquor Suspended Solids
NOEC:	No Effect Concentration
NSAIDs:	Non-Steroidal Anti-Inflammatory Drugs
NZOWASCO:	Nzoia Water Service Company
PAHs:	Polycyclic Aromatic Hydrocarbons
PCBs:	Polychlorinated biphenyls
PNEC:	Predicted No Effect Concentration
POCIS:	Polar Organic Chemical Integrative Samplers
PPCPs:	Pharmaceuticals and personal care products
PVCs:	Polyvinyl Chlorides
RQ:	Risk Quotient
SBR:	Sequencing Batch Reactors
SMX:	Sulfamethoxazole
SOCs:	Small Organic Compounds
SPE:	Solid-Phase Extraction
SPMDs:	Semi-Permeable Membrane Devices
SRT:	Solid Retention Time
SS:	Suspended Solids
TFs:	Transformation Products

TF-SCP:	Trickling Filters Solid Contact Process
TMDD:	2,4,7,9-Tetramethyl-5-decindol
TSS:	Total Suspended Solids
TU:	Toxic Unit
UFZ:	Helmholtz center for environmental research
UHPLC:	Ultra-High-Performance Liquid Chromatography
USGS:	United States Geological Survey
UV Filters:	Ultra-Violet Filters
WASREB:	Water Service Regulatory Board
WCX:	Weak Cation Exchange
WHO:	World Health Organization
WSPs:	Wastewater Stabilization Ponds
WWTPs:	Wastewater treatment plants

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

The global demand for chemicals has increased significantly to meet both industrial and agricultural needs (Shefali et al., 2021). Kenya's economy relies mainly on agriculture, which makes the use of synthetic and organic chemicals necessary to protect plants and animals. According to Islam et al. (2022), nearly a third of the world's agricultural products are sprayed with pesticides before consumption. Furthermore, the rise in disease outbreaks and pandemics has increased the production of pharmaceuticals, leading to greater consumption and disposal of these chemicals, which results in environmental exposure at various concentrations (Jemba, 2018). Unfortunately, in many developing nations, the emphasis on discovering and producing these chemicals has overshadowed their monitoring in the environment.

Many approved chemicals with significant effects on human health and plant growth are used today. However, due to inadequate monitoring and improper disposal practices, they are released uncontrollably into the environment (Hilawie Belay, 2022). This unregulated release often results in their presence in concentrations that exceed recommended guidelines, causing adverse environmental effects. These chemicals frequently enter rivers because of poor monitoring, where they accumulate and threaten aquatic ecosystems or bioaccumulate in the food chain (Hilawie Belay, 2022). Any artificial or naturally occurring chemical not regularly monitored in the environment can infiltrate and harm ecosystems and public health, according to the United States Geological Survey (USGS) (Klaper & Welch, 2011). As a result, these chemicals have become an emerging concern worldwide.

Chemicals recently identified in various environmental samples are called chemicals of emerging concern (CECs). However, limited knowledge exists about their potential risks to human health and aquatic ecosystems (Yadav et al., 2021a). These substances include pesticides, pharmaceuticals, personal care products (PPCPs), industrial chemicals, natural compounds, and transformation products (Parida et al., 2021a).

Water pollution from CECs results from human activities such as domestic waste, industrial waste, and agricultural practices, which release chemicals into aquatic environments and degrade water quality (García-Fernández et al., 2021). The expansion of water networks without upgrading sewerage systems worsens pollution and public health risks (Montgomery & Elimelech, 2007). There are various pathways for emerging concern chemicals (CECs) to enter the environment.

These include agricultural runoff, landfills, urban runoff, and wastewater treatment plants (WWTPs). WWTPs receive wastewater from hospitals, industries, slaughterhouses, and households where CECs are used extensively. Consequently, WWTPs become the primary sink and source of CECs to different environmental compartments.

Most wastewater treatment plants (WWTPs) in developing countries are designed to remove small organic compounds (SOCs) from wastewater (Rout et al., 2021a). To eliminate contaminants, these WWTPs combine physical, chemical, and biological processes (Y. Li et al., 2019b ; Rout et al., 2021b). However, due to the physicochemical properties and molecular structures of these chemicals, including solubility, water-octanol partition coefficient, and chemical and biological stability, many methods used in WWTPs are ineffective in entirely removing them. Most compounds are only partially removed or transformed into more toxic products (Di Marcantonio et al., 2023). This incomplete removal and transformation lead to the presence of CECs in the effluent, which are discharged into receiving water systems at various concentrations, ranging from ng/L to µg/L (Orata, 2020).

The release of these chemicals and their transformation products (TFs) into the environment could have different adverse effects on aquatic life and human health, depending on exposure levels (Parida et al., 2021a). Antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), acute toxic risks, and endocrine disruption have all been linked to the presence of CECs in aquatic environments (Christou et al., 2017; Krzeminski et al., 2019a; Luo et al., 2014). The World Health Organization (WHO) states that antibiotic resistance is a serious global health issue that makes treating infections and diseases more difficult (Vasilachi et al., 2021). Exposure to steroids in aquatic environments has been associated with cancer development, disruption of healthy reproductive systems, and the dysregulation of apoptotic mechanisms in humans (Albano et al., 2021). Pesticides have been shown to bioaccumulate in the food chain and cause both acute and chronic toxicity due to their high lipid solubility and low polarity (Islam et al., 2022). Industrial chemicals such as bisphenol A have been reported to act as endocrine disruptors, affecting the reproduction of freshwater gastropods (Huang et al., 2020a).

Nevertheless, the majority of these compounds' ecotoxicological effects and their harmful impacts on human health remain unknown, despite their presence and the potential risks they pose to the environment. Most risk assessments have been conducted in high-income countries, with few studies in developing nations. In Kenya, most research has focused on the occurrence of CECs in surface water and ecotoxicological risk evaluations. As a result, there is a significant knowledge gap regarding the hazards associated with WWTP discharges into receiving water systems. Additionally, most studies in Kenya have examined only a limited number of CEC classes (K'oreje et al., 2018). Currently, only five African countries, South Africa, Kenya, Ivory Coast, Tunisia, and Zimbabwe, have gathered data on the presence of most CECs in wastewater, mainly focusing on PPCPs (Necibi et al., 2021). This study, therefore, aims to address this knowledge gap by generating essential data for the regulation and protection of environmental health.

1.2 Problem statement

Pesticides, pharmaceuticals, personal care products (PPCPs), and industrial chemicals are major classes of emerging concern chemicals that enter the environment through various pathways. The primary route is through treated and untreated wastewater that contains excreted CECs and their metabolites (Astuti et al., 2023). Agriculture is the leading economic activity in Western Kenya. Besides agriculture, processing and manufacturing industries such as textiles, food processing plants, and hospitals also operate there (Were, 2016). These sectors use a range of chemicals, including pesticides, fertilizers, plasticizers, stimulants, and plastic additives. The consumption of pesticide-treated crops, the use of prescribed and over-the-counter medications, and the use of personal care products like soaps, hair dyes, nail polish, fragrances, emulsifiers, UV absorbers, acrylates, preservatives, and antioxidants lead to their release into wastewater systems as parent compounds or metabolites (Srinivasulu et al., 2022). Wastewater from industries, hospitals, households, and surface runoff from towns and agricultural fields enters wastewater treatment plants at different concentrations (Golovko et al., 2021a).

WWTPs employ treatment processes involving a combination of physical, biological, and chemical processes to eliminate solids, organic matter, and nutrients from the wastewater. The main biological processes employed are aerobic and anaerobic digestion(Anekwe et al., 2022). Additionally, chemicals can also be removed through sorption and deposition to the final sludge and volatilization. However, these methods prove ineffective for many CECs, with a considerable proportion remaining in the final treated effluent, resulting in their discharge onto surface water bodies. The primary rationale behind the partial elimination of CECs from wastewater by WWTPs is their physical-chemical attributes, which include a hydrophilic nature, low Dow (>1.0), and structural complexity(Ruan et al., 2023). Additionally, the chemical and biological stability of CECs makes them resistant to most conventional wastewater treatment processes, resulting in their incomplete degradation or transformation to more toxic chemicals and their discharge into aquatic environments at concentrations exceeding the WASREB and WHO effluent discharge guidelines(Zachariah et al., 2024). Even though these substances may not be prevalent in the environment, they may nevertheless have harmful impacts on human and environmental health, including increased risk of cancer, imbalances in hormones and metabolism, and difficulties with reproduction and development. However, there is little information on the prevalence of CECs and the degree to which the current conventional WWTPs have eliminated them since most developing nations, particularly those in Africa, lack sophisticated analytical techniques and technology to assess CECs in the various environmental matrices. As a result, few studies have been conducted in Kenya focusing on the potential toxicity risks that these chemicals bring to aquatic life and human health. This has led to inadequate monitoring and prioritizing of these chemicals in terms of their toxicity index.

1.3 Justification

Kenya's rapid population growth has led to increased consumption of contaminants of emerging concern (CECs) from domestic, agricultural, veterinary, and industrial sources. Following use, significant quantities of these bioactive and persistent compounds enter the environment, with wastewater treatment plants (WWTPs) acting as a significant pathway through effluent discharge. This situation poses considerable risks to aquatic and terrestrial ecosystems, as well as to human health.

Despite their documented potential to cause ecological and physiological harm, CECs remain unlisted in Kenya's environmental quality guidelines and are not routinely monitored. Limited research has examined the occurrence, fate, and removal efficiency of CECs in WWTPs, and such studies are particularly scarce in western Kenya. The region was selected for this study because it hosts several major WWTPs serving rapidly growing urban centers, agricultural zones, and industrial areas. However, many of these facilities operate with outdated infrastructure and discharge into rivers of high ecological and socio-economic importance.

This study seeks to address these gaps by determining the occurrence of CECs in WWTP effluents in western Kenya and evaluating the efficiency of WWTPs in their removal. In addition, the study will conduct an environmental risk assessment of the effluents to quantify the potential toxicity risks they pose to aquatic and terrestrial ecosystems. The findings will contribute to evidence-based policy development, inform regulatory frameworks, and guide future environmental monitoring strategies.

1.4 Objectives

1.4.1 Main objective

To investigate the occurrence, persistence, and environmental risks of contaminants of emerging concern (CECs) in wastewater treatment systems and their impact on receiving aquatic ecosystems in western Kenya.

1.4.2 Specific objectives

1. To identify and quantify CECs and their transformation products in WWTPs' influent and effluent.
2. To evaluate the removal efficiency of CECs in four WWTPs within the western Kenya region.
3. To perform ecological risk assessment on three standard test organisms (algae, crustaceans, and fish).

1.5 Research questions

i. What types of contaminants of emerging concern (CECs) and their transformation products are present in the influent and effluent of wastewater treatment plants (WWTPs) in western Kenya?

ii. To what extent are CECs removed by the existing treatment processes in the selected WWTPs?

iii. How do removal efficiencies of CECs vary across different WWTPs within the western Kenya region?

iv. Do the treated effluents from WWTPs pose ecological risks to aquatic organisms such as algae, crustaceans, and fish?

1.6 Hypotheses of the study.

i. CECs and their transformation products are present in both influent and effluent of WWTPs in the western Kenya region.
ii. There is a significant difference in the removal efficiency of CECs among the four WWTPs in western Kenya.
iii. Effluents containing residual CECs pose a significant toxicological risk to standard aquatic test organisms (algae, crustaceans, and fish).

1.7 Significance of the study

Pollution from contaminants of emerging concern (CECs), such as pesticides, pharmaceuticals, personal care products (PPCPs), and industrial chemicals, has become an urgent global environmental issue. These substances, often resistant to conventional treatment methods, are increasingly found in wastewater effluents and receiving water bodies worldwide. Their durability in the environment poses threats to aquatic ecosystems, disrupts hormonal systems in wildlife and humans, and endangers long-term water quality and public health.

Despite the global focus on this issue, developing countries like Kenya face significant data gaps regarding the occurrence, removal efficiency, and ecological risks of CECs. Most wastewater treatment plants (WWTPs) in Kenya, especially in western regions, rely on outdated technologies that are not designed to remove these complex pollutants. However, these WWTPs discharge into rivers that communities depend on for domestic use, agriculture, and fishing, increasing the risks to local populations.

This study adds to the global knowledge by providing region-specific data on the prevalence of CECs in four Kenyan WWTPs, evaluating their removal efficiency, and assessing the potential ecological risks of treated effluent. By producing quantitative evidence, the research supports international efforts to monitor and control environmental pollutants and offers valuable insights for enhancing wastewater treatment systems in low- and middle-income countries**.**

Locally, the findings will guide policymakers, environmental agencies, and water service providers on the urgent need to upgrade treatment technologies and implement preventative strategies. Ultimately, this research will benefit residents by promoting cleaner water systems, protecting aquatic biodiversity, and lowering exposure to potentially harmful pollutants, supporting Sustainable Development Goal 6: Clean Water and Sanitation and broader public health outcomes.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

Globally, the contamination of surface water systems by chemicals of emerging concern (CECs) has become a significant challenge (Yadav et al., 2021a). Chemicals that have recently been discovered in the environment at low quantities are referred to as chemicals of emerging concern (Pourchet et al., 2020). These compounds include: insecticides, pharmaceuticals, personal care products (PPCPs), and industrial chemicals. Prescription and over-the-counter medications, dietary supplements, disinfectants, UV filters, steroids, cosmetics, and other frequently used home items are all included in the category of pharmaceutical and personal care products (PPCPs) (Al-Baldawi et al., 2021). Their usage in various sectors of the economy, including agriculture, hospitals, animal husbandry, households, and industries, leads to their disposal in landfills, pit latrines, and sewage systems (Gwenzi & Chaukura, 2018). These chemicals have a complex, high molecular structure, a high water-octanol partition coefficient, and are resistant to biodegradation (X. Li, 2016). Their physicochemical properties make them persistent in sewerage systems and their release to conventional wastewater treatment plants (WWTPs)(N. H. Tran et al., 2018).

It has been observed that most African conventional WWTPs, which utilize wastewater stabilization ponds and physical and biological processes in treatment, are inefficient in eliminating CECs from the wastewater before their discharge into the environment (Necibi et al., 2021). The concerning levels of these chemicals found in most European nations have sparked concerns about the detrimental impact they may have on aquatic life as well as the increased risk of health problems for humans who may come into contact with polluted water (Morin-Crini et al., 2022). However, these issues are often overlooked in Africa due to socio-economic problems, resulting in poor sanitation facilities and a lack of well-designed WWTPs in many regions (Amaefule et al., 2023). This has resulted in the release and accumulation of CECs in different environmental compartments and at varying concentrations, posing both chronic and acute toxicity to aquatic organisms and human health (Espíndola et al., 2024).

Previous studies have shown that treated effluent released from WWTPs is the major contributor to the highest concentrations of CECs (Sengupta et al., 2014). Pesticides and personal care products (PPCPs) have been found in aquatic environments at rising concentrations, potentially posing a threat to aquatic life (Narayanan et al., 2022). Despite the mechanical and biological techniques employed in conventional WWTPs to remove waste and solid fractions, these treatment methods are ineffective in removing pesticides and PPCPs, allowing them to find their way into the environment through treated effluents (Kumar et al., 2022).

The removal of suspended particles and the biochemical oxygen requirement are the main engineering goals of WWTP design. Pesticides, as well as industrial chemicals, pharmaceuticals, and personal care products, are frequently partially treated, completely untreated, or adsorbed on biosolids, with some of them becoming metabolites (Chacón et al., 2022). While certain biodegradable chemicals can be effectively eliminated through biological treatment, others, like Triclosan, may get adsorbed onto particles. The release of treated wastewater back into the ecosystem through irrigation is another way that PPCPs are introduced. Nutrient-rich treated wastewater is frequently utilized as irrigation water in agriculture (Ofori et al., 2021). Furthermore, it has been documented that the exposure of pharmaceutical residues to bacteria and fungi can cause bacterial mutation, leading to the emergence of antibiotic-resistant bacteria and genes (Mutuku et al., 2022).

The consequences of CECs and their transformation products on aquatic life and human health should not be understated, even if they may be released to receiving water systems at low quantities. Most of these chemicals are biologically active, and their exposure to aquatic organisms might interact with non-target organs. Therefore, they potentially cause detrimental effects such as endocrine disruption and changes in reproductive systems (V. Kumar et al., 2023). Substances that alter sexual function and the hormonal system, including steroid hormones, natural and synthetic, food additives, and industrial chemical bisphenol A, can have an impact on the reproductive system (Amir et al., 2021). Furthermore, the majority of these substances have lipophilic properties, which means they may bioaccumulate in organisms' fat sites and biomagnify at higher trophic levels of the food chain. These chemicals' interactions with wastewater during treatment operations may result in the production of a more hazardous complex or environmental mixture that may have more harmful effects than the individual compounds since they have distinct functional groups and reactive sites(Kelly et al., 2004).

2.1 Chemicals of Emerging Concern

Natural or artificial substances detected in the environment that have detrimental effects on human and ecological health are known as chemicals of growing concern (CECs) (Yadav et al., 2021b). These compounds consist of chemicals that have recently been identified in several environmental compartments, such as pharmaceuticals, personal care products, pesticides, industrial chemicals, surfactants, heavy metals, and perfluorinated compounds(Boxall et al., 2012).

A wide range of chemicals is classified as pharmaceuticals and pesticides, including vitamins, nutritional supplements, prescription and over-the-counter medicines for treating humans and animals, biopharmaceuticals, growth-promoting chemicals, and vitamins (Vlachogianni & Valavanidis, 2013). All these chemicals are helpful in various human activities and, therefore, are obliged to be disposed of and released into the environment distinctively after consumption (Chakraborty et al., 2023)

Most of these substances have been categorized as endocrine disruptors based on their possible impacts on aquatic life and human health. Endocrine disruptors are a class of substances that have an impact on an organism's development, reproduction, metabolism, and hormonal balance. Endocrine-disrupting chemicals comprise various classes of CECs, including pesticides, fungicides, pharmaceuticals, and industrials, with most being lipophilic and thus bioaccumulating in the fat tissues(Czarnywojtek et al., 2021). Bisphenol A, Nonylphenol, nonylphenol ethoxylates, Octylphenol, and 17α-ethinylestradiol, which are active compounds present in birth control pills, have been reported to cause endocrine disruption. (Pironti et al., 2021).

The emergence of pandemics, the increased need for agricultural and industrial output, and the increased use of pharmaceuticals have all led to a rise in the use of these chemicals in the environment, resulting in increased consumption and discharge at varying concentrations (Kandie et al., 2020). Some of these substances, including hormones and steroids, can be hazardous to aquatic life at trace amounts and can also bioaccumulate and biomagnify in the food chain. Although some of these chemicals may not have an immediate effect at low concentrations, they have deleterious effects when they magnify in higher trophic levels (Zenker et al., 2014).

2.1.1 Pharmaceuticals

Pharmaceuticals are the largest and most diverse group of chemicals, which are widely used in treating medical conditions in both humans and animals(Aus der Beek et al., 2016). Steroids, antiinflammatories, beta-blockers, antibiotics, and antiepileptics are the commonly detected classes of pharmaceuticals(Yuan et al., 2014). The Kenyan pharmaceutical consumption patterns lie primarily on the use of antibiotics, analgesics, anti-inflammatory drugs, antimalaria compounds, and anti(retro) viral drugs (Kandie et al., 2020). However, a large percentage of these compounds are not completely metabolized or eliminated from the organism, thereby being excreted into the environment in the form of parent compounds or as metabolites in urine or feces(Orhan, 2021). The continuous consumption of these compounds due to a surge increase in diseases and emerging of pandemics among the population and the existence of diseases such as HIV/AIDs, respiratory diseases, and malaria has increased their persistence in the environment, with many of the classes of pharmaceuticals been detected in receiving rivers, drinking water and groundwater (Sengupta et al., 2014). Pharmaceuticals are inefficiently eliminated by most of the existing conventional wastewater treatment plants (WWTPs), and they have consequently been detected in effluents and sewage sludge at ng/L concentrations (K'oreje et al., 2018). Despite being present in the environment at low concentrations reported by previous studies, which are below the toxicity threshold to induce any acute effects, their long-term impacts on their accumulation in the environment on human health and aquatic organisms are still unknown (Nilsen et al., 2019).

Antibiotics, anti-inflammatory compounds, and antiviral drugs are crucial pharmaceutical classes extensively used in human and veterinary medicine, contributing to the emergence of antibioticresistant bacteria and posing significant public health and ecological risks(Muteeb et al., 2023a). Antibiotics are frequently detected in wastewater treatment plants (WWTPs) globally, with concentrations ranging from nanograms to micrograms per liter. Common antibiotics such as tetracyclines, sulfonamides, macrolides, fluoroquinolones, and beta-lactams are detected using methods like liquid chromatography-tandem mass spectrometry (LC-MS/MS), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS)(PerisVicente et al., 2022). Advanced treatment processes such as activated carbon adsorption, ozonation, and membrane filtration achieve higher removal rates, though conventional treatments are less effective(Guillossou et al., 2019). Antibiotics' ecological risks include promoting antibioticresistant bacteria, toxicity to aquatic organisms, and disruption of microbial communities essential for wastewater treatment(W. Wang et al., 2023).

Anti-inflammatory compounds, including non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are commonly used to treat pain and inflammation. These compounds are extensively excreted and detected in WWTPs(Izadi et al., 2020). NSAIDs like ibuprofen, diclofenac, and naproxen, as well as corticosteroids such as prednisolone and dexamethasone, are identified using similar analytical methods. During wastewater treatment, these compounds undergo transformations resulting in metabolites that may be more persistent and toxic than the parent compounds(Patel et al., 2019). Removal efficiencies vary, with conventional activated sludge processes achieving moderate success and advanced processes like membrane bioreactors achieving higher efficiency. Anti-inflammatory compounds pose risks to aquatic organisms by bioaccumulating and affecting endocrine systems, behavior, and reproduction(Almeida et al., 2020).

Antiviral drugs, used to treat infections like HIV, hepatitis, and influenza, are also detected in WWTPs. Studies have identified antiviral compounds such as acyclovir, lamivudine, and zidovudine, highlighting their entry into the environment through wastewater discharge. These drugs undergo transformation processes in WWTPs, forming more persistent and toxic transformation products (TPs)(Nannou et al., 2020a). Conventional treatment processes are generally ineffective at removing antiviral drugs due to their resistance to biodegradation and adsorption. Ecological risks associated with antiviral drugs include toxicity to aquatic organisms, disruption of endocrine systems, and the development of antibiotic resistance(Loffler et al., 2023).

2.1.2 Personal Care Products

Personal care products (PCPs) encompass a wide range of substances used for personal hygiene and cosmetic purposes, such as soaps, shampoos, lotions, and perfumes. Each product includes a range of chemicals that can wind up in wastewater treatment plants (WWTPs) when wastewater from residential and commercial sources is discharged into the sewer system and later into the environment.

The existence of PCPs in WWTPs in various geographical areas have been the subject of several investigations. As an example, a European study looked at the presence of PCPs in influent and effluent samples from several WWTPs(Tran et al., 2018). The results revealed the widespread presence of PCPs, including fragrances, preservatives, UV filters, and surfactants (Mishra et al., 2023). Comparably, an American research that evaluated the presence of PCPs in WWTPs discovered a wide variety of substances, including triclosan and synthetic parabens (Khalid & Abdollahi, 2021).

The entry of PCPs into WWTPs are primarily through domestic sources, such as residential wastewater from households, which contains residues from personal care product use. Additionally, industrial effluents from facilities involved in the manufacturing of personal care products can contribute to the presence of PCPs in WWTPs.

2.1.3 Pesticides and biocides

Chemicals known as pesticides are used to suppress or eliminate rodents, weeds, and insects. They are extensively utilized in homes and farms to safeguard crops and manage pests (Poudel et al., 2020). Pesticides, however, can also infiltrate the ecosystem by air deposition, runoff, leaching, and deposition, and they can build up in the food chain. Because they are not eliminated during traditional treatment procedures and may have detrimental ecological effects, pesticides are regarded as emerging pollutants in WWTPs (Poudel et al., 2020).

Pesticides are detected in WWTPs worldwide, indicating their widespread use and environmental persistence. Pesticides can enter WWTPs through different pathways, including domestic and industrial wastewater, urban runoff, and agricultural drainage. Studies have shown that pesticides can be detected in influent, effluent, and biosolids of WWTPs (Rousis et al., 2021). The types of pesticides detected in WWTPs vary depending on the location, but commonly detected pesticides include herbicides, insecticides, and fungicides.

Pesticides can undergo various transformation processes during WWTP treatment, including biodegradation, hydrolysis, and photolysis. The transformation products of pesticides can have different properties and toxicity from the parent compounds(X. Wang et al., 2020). According to some research, pesticide transformation products might linger in the environment and pose ecological hazards(X. Wang et al., 2022). For instance, desethylatrazine, the transformation product of the herbicide atrazine, is more hazardous and persistent than its predecessor substance (Chang et al., 2022).

2.1.4 Industrial Chemicals

A vast variety of substances are produced or acquired for use in the manufacturing and retailing of goods, and they are referred to as industrial chemicals. They are used in many different industries and on a wide range of consumer goods. Industrial chemicals are essential to modern manufacturing and consumer goods because they offer benefits such as increased functionality, durability, and performance(Chandel et al., 2020). Nevertheless, the manufacture and utilization of these substances also present significant environmental and health obstacles. Several industrial chemicals can persist in the environment, accumulate in living creatures, and potentially lead to harmful health consequences. According to Morin-Crini et al. (2021), substances consist of flame retardants, surfactants, plasticizers, perfluorinated compounds, colors, and fragrances.

Surfactants are a heterogeneous collection of compounds that possess the ability to clean and/or dissolve substances. They are widely used in personal care products, household cleaning detergents, and other industries like the paper, textile, paint, and plastics industries (Jena et al., 2023). Surfactants are molecules that have both a hydrophilic (polar) head and a hydrophobic (nonpolar) hydrocarbon tail. This unique structure allows them to dissolve in both polar and nonpolar liquids.

They can be classed according to the ionic charge of the hydrophilic component of the molecule (nonionic, anionic, cationic, amphoteric) in the aqueous solution, with anionic and nonionic surfactants accounting for the most significant production quantities. Global surfactant output has been reported to reach 17.6 million tons in 2015. Among the numerous types of surfactants, Alkyl ethoxysulfates (AES, also known as alkyl ethoxylated sulfates, alcohol ethoxysulfates, or alcohol ethoxylated sulfates) are another major class of anionic surfactants(Sasi et al., 2021). They are commonly used in numerous consumer products, such as shampoos, hand dishwashing liquids, and laundry detergents, as well as in industrial cleaning operations, as industrial process aids in emulsion polymerization, and as additives in the plastics and paint manufacture. Raw wastewater discharge and discharge from WWTPs are two ways that surfactants might end up in the environment. Wastewater from homes, businesses, and municipalities is where surfactants are primarily found in the environment (Badmus et al., 2021). Various surfactants have been discovered in both influents and effluents of WWTPS at varying quantities. In a study conducted in Germany by Guedez & Püttmann. (2011a), both influents and effluents from WWTPs exhibited high concentrations of TMDD, ranging from 134 ng/L to 5846 ng/L. Flame retardants are widespread compounds that are utilized extensively for industrial and home uses. They are chemicals applied to manufactured materials such as plastics, fabrics, and surface coatings to block, suppress, or postpone production and prevent the spread of fire. There are three basic forms of organic flame retardants, which include bromine, chlorine, and phosphate flame retardants. Tris (2-chloroethyl) phosphate, tris(2-chloro-propyl) phosphate, and tris (1,3-dichloro-2-propyl) phosphate are typically employed as flame retardants in electronic or electric equipment, textile coating, and furniture(L. Zhang et al., 2021). The global usage of flame retardants was reported to be more than 2.25 million tons per year in 2017(Zheng et al., 2021; Zapata-Corella et al., 2023).

Plasticizers are low molecular-weight compounds added to polymer solutions to increase their plasticity and flexibility. They are a key component of many common products, including packaging bags, medical appliances, and building materials (Ma et al., 2020). Most of these plasticizers include phthalates, adipates, citrates, camphor, and acetates that boost the adaptability of plastics. Non-chlorinated organophosphate esters such as tri-n-butyl phosphate, triphenyl phosphate, and tritoyl phosphate are employed as plasticizers or additives in plastic products, hydraulic fluids, lubricants, and motor oils. Tris(-2-butoxyethyl) phosphate is widely applied to plastics, floor polish, and PVCs(Hu et al., 2021). Phthalates are a typical class of plasticizers that do not form a covalent bond with polymers. They are members of the phthalic anhydride group. For instance, phthalates can seep into the environment from items when they come into contact with liquids or heat (H. T. Tran et al., 2022). The majority of the aqueous and solid phase samples of full-scale WWTPs in China contained dimethyl phthalate, diethyl phthalate, di-n-butyl phthalate, and butyl benzyl phthalate. Bis(2-ethylhexyl) phthalate is the most common compound (Gao et al., 2014). Di(2-ethylhexyl) phthalate, due to extensive usage, has been identified in wastewater at a concentration range from 0.716-122µg/L (Takdastan et al., 2021; Zolfaghari et al., 2014). Industrial chemicals have an excellent potential for bioaccumulation and biomagnification, and they are also quite persistent. In humans and aquatic organisms, detrimental impacts have been noted, where they have been linked to immunological, neurological, endocrine, reproductive, developmental, and cancer risks, as well as hormone disruption and other adverse effects. Industrial chemicals in industrial and domestic wastewater can be released into the environment via the outflow of wastewater plants, particularly for those serving urban centers. WWTPs have been characterized as key sources of industrial chemicals to the aquatic environment, by their discharge of effluents into receiving water systems.

2.2 Chemicals of Emerging Contaminants in African WWTPs

African scholars have begun investigating this new environmental problem, focusing on its detrimental impacts on aquatic life, animal health, and human health, in response to the growing global awareness of CECs and their detection and ecotoxicological consequences in aquatic environments. Investigating the current state of affairs about the occurrence of CECs in wastewater, sludge, surface water, sediment, groundwater, and drinking water sources in various African nations is the first step in such a complicated endeavor(K'oreje et al., 2020). The lack of improved methodologies and tools for monitoring chemicals of emerging concern in Africa results in a significant information gap regarding the fate and occurrence of these chemicals, with most research focusing on surface water. Nevertheless, researchers have done numerous studies on new contaminants, despite the detection of most of them in minuscule concentrations. Their fate and occurrence were determined, and even risk evaluations were made to determine their toxicity levels on both aquatic organisms and human health.

The existence and fate of pesticides and PPCPs have been investigated in various studies in Africa, with findings mostly indicating their deposition in sediments and aquatic ecosystems. Pharmaceuticals have been the most commonly observed class of CECs in most African wastewaters. The most predominant groups of drugs include antibiotics, analgesics/antiinflammatory agents, beta-blockers, and antiretroviral agents. Beta-blockers, which are a type of therapeutic drug typically suggested for the treatment of cardiovascular problems and hypertension, have been detected in various investigations in Africa. In a study done in North West Province, South Africa, by Kanama et al.(2018), atenolol, a common chemical, was detected with a range of 1.08–8.34 µg/L. Triclosan and triclocarban, on the other hand, were detected with a mean concentration of 0.11 µg/L.

With the increasing incidence of infections in most African countries, there has been a massive increase in the consumption of antibiotics and analgesics. In a survey done in

West Africa, a range of PPCPs, including antibiotics and analgesics, were detected mainly, with a total concentration ranging from 8.5 to 121,310,00 ng/L in wastewater (Cangola et al., 2023). In addition to antibiotics, analgesics, and anti-inflammatory drugs have also been shown to have high maximum concentrations. Naproxen, ibuprofen, diclofenac, and ketoprofen are the most predominant NSAIDs detected in WWTP influents in Africa. In a study done by K'oreje et al.(2018) in four wastewater stabilization ponds in the Nzoia Basin, Kenya, a concentration of paracetamol and ibuprofen was recorded at up to 100 µg/L. Additionally, naproxen, ibuprofen, diclofenac, and ketoprofen have also been detected in WWTP influents, up to 20.4 µg/L of naproxen in South Africa, 8.02-43.22 µg/L in Tunisia, and 1.6 µg/L of diclofenac in Algeria. Bisphenol A is a frequently detected pollutant at high concentrations up to 210 µg/L, according to research done in Cape Town, South Africa, to assess the presence and quantify numerous CECs, including six perfluorochemicals, bisphenol, and acetaminophen in WWTPs. About the evaluation of acetaminophen, the findings indicated that the average concentration in the WWTP influents varied from 27.9 to 175 µg/L (Necibi et al., 2021).

In a study conducted in South Africa by Mhuka et al.(2020), which targeted 156 compounds, 120 compounds were detected, with ibuprofen, caffeine, and estradiol being the major contaminants in both influents and effluents. Acetaminophen, sulfamethoxazole, and propanol were detected in wastewater treatment plant effluents in a study done by Mayoudom et al.(2018). (at a wastewater treatment plant in Cameroon, which receives wastewater from a hospital. Perfluorinated substances possess amphiphilic properties, making them widely used in most industries, combined with their anti-wetting and surfactant properties. These properties enable their use in textiles, paper, household cleaning products, and pesticides (Zahid et al., 2019).

By focusing on wastewater discharge locations, Ngumba et al.(2016a) conducted a study to examine the prevalence of certain antibiotics and antiretroviral medications in the Nairobi River basin. Depending on the kind of settlement, different amounts of ciprofloxacin, trimethoprim, and nevirapine were found, although sulfamethoxazole was found at high concentrations.

2.3 Occurrence of metabolites and transformation products in Wastewater Treatment Plants.

The intermediate result of metabolic processes, known as metabolites, is facilitated by a variety of naturally occurring enzymes found in cells. The majority of metabolites are created by an organism's metabolic processes, which frequently involve the breakdown of other chemicals, medications, and personal hygiene items(Daughton, 2001). The body creates metabolites of medications to make them more polar and easier to excrete. Most medications transform the human body after entering the systemic circulation, with the liver serving as the primary site of metabolism. The metabolites produced when the medication is metabolized in the body are often more hydrophilic, which makes them easy to excrete in urine and end up in urban wastewater treatment facilities. It is essential to identify and comprehend the presence of metabolites in WWTPs.

Transformation products (TPs) and metabolites are a topic of concern due to their presence in wastewater treatment plant (WWTP) effluents and receiving waters. The biological treatment processes employed in WWTPs can lead to the transformation of PPCPs into various TPs, which may possess different properties and environmental impacts compared to their parent compounds (Luo et al., 2014).

In wastewater samples from Norway, metabolites of the anti-inflammatory medication ibuprofen that are hydroxylated and carboxylate were found. Three metabolites of the analgesic medication phenazone have been found in conventional wastewater treatment plants, according to a German study. Acetylsalicylic acid, another medication that breaks down quickly into salicylic acid, salicyluric acid, and glucuronide conjugates, is another medication that creates metabolites.

Studies have reported the transformation of ibuprofen into its primary TP, 2-hydroxyibuprofen, during WWTP treatment. Further oxidation of 2-hydroxy ibuprofen can result in the formation of additional TPs such as carboxyibuprofen and dihydroxyibuprofen. The transformation of diclofenac, another NSAID, can lead to the formation of TPs like 4'-hydroxydiclofenac and 5hydroxydiclofenac (Kasprzyk-Hordern et al., 2008).

Antibiotics are another class of PPCPs frequently detected in wastewater effluents, and their biological treatment in WWTPs can give rise to various TPs. Antibiotic degradation into metabolites, as well as the formation of new compounds through hydrolysis and oxidation, are common transformation pathways. For example, the transformation of sulfamethoxazole (SMX) during WWTP treatment can lead to the formation of TPs such as 3-amino-5-methylisoxazole and N4-acetylsulfamethoxazole (Boreen et al., 2003)

Personal care products (PCPs) are also commonly found in wastewater and can undergo transformation during WWTP treatment. Triclosan, an antimicrobial agent used in PCPs, can be transformed into TPs such as 2,4-dichlorophenol and 2,8-dichlorodibenzo-p-dioxin (Lindström, 2002).

For ibuprofen, TPs like 2-hydroxyibuprofen, carboxyibuprofen, and ibuprofen lactone have been identified, which can be formed through hydrolysis, oxidation, and other chemical reactions (Pérez & Barceló, 2007). Similarly, for naproxen, transformation products such as 6-O-desmethyl naproxen, 6-O-desmethyl-2-hydroxy naproxen, and 6-O-desmethyl-2,3-dihydroxy naproxen have been identified. These TPs can also be formed through hydrolysis, oxidation, and other chemical reactions (Sui et al., 2016). For carbamazepine, some of the identified TPs include 10,11-dihydro-10,11-trans-dihydroxy carbamazepine and carbamazepine-10,11-epoxide. These TPs can be formed through hydrolysis, oxidation, and other chemical reactions (Mohapatra et al., 2014).

2.4 Wastewater treatment plants (WWTPs)

Wastewater treatment plants (WWTPs) are facilities constructed to treat wastewater by eliminating contaminants from the wastewater to suitable levels before discharging the wastewater into receiving water systems. WWTPs receive millions of gallons of wastewater from households, industries, hospitals, urban surface run-offs, and stormwater. The wastewater received contains waste ranging from suspended solids, fecal matter, pharmaceutical residues, pesticides, personal care products, industrial chemicals, surfactants, organic matter, disinfectants, soaps, and detergents, at different concentrations(Mahapatra et al., 2022). These chemicals differ in their structures and physicochemical characteristics such as solubility, adsorption, and biodegradability, which determine their mode and rate of removal by the various treatment processes employed by wastewater treatment plants(Cirja et al., 2008).

There are three categories for the treatment procedures used in WWTPs: mechanical, aquatic, and terrestrial. To achieve treatment goals, mechanical systems combine chemical, biological, and physical processes. To treat wastewater, these systems use a sequence of tanks coupled with pumps, blowers, screens, grinders, and other mechanical parts in a regulated artificial environment (G.-H. Chen et al., 2020). The instruments manage wastewater as it moves through the system. Process modifications for activated sludge that serve as suspended-growth systems include sequencing batch reactors (SBR), oxidation ditches, and extended aeration systems (Ogunwumi et al., 2024). Conversely, the trickling filter solids contact process (TF-SCP) is the attached growth system. Artificial wetlands and facultative lagoons are the most prominent lagoon technologies used in aquatic treatment systems nowadays. In facultative lagoons, the lower layer of water, which contains sludge deposits, is anaerobic while the upper layer of water is aerobic. It is anaerobic toward the bottom of the intermediate layer and aerobic near the top, known as the facultative zone.

Aerated lagoons differ from facultative lagoons in that they are smaller and deeper (Awad et al., 2023). Aquatic vegetation is employed in terrestrial systems, including artificial wetlands, to clean wastewater.

These processes are further divided into physical, chemical, and biological treatment processes.

Physical processes involve the removal of suspended solids, oils, and grease from wastewater (Y. Li et al., 2019b; H. Zhang et al., 2023). These processes include screening, grit removal, and sedimentation. Physical processes do not remove dissolved contaminants, such as CECs. Microorganisms are used in biological processes to break down organic materials in wastewater (Ferreiro et al.2017.; Pabbati & Reddy, 2021). These processes include activated sludge, trickling filters, and oxidation ponds. Biological processes can remove some CECs, but their removal efficiency depends on the biodegradability of the compounds. Advanced treatment processes include membrane filtration, adsorption, and ozonation (Samal et al., 2022). These processes are used to remove trace contaminants, including PPCPs and pesticides, from effluent. Membrane filtration is effective for removing small molecules, such as hormones and antibiotics, while adsorption is effective for removing hydrophobic compounds, such as pesticides.

Despite advancements in treatment technologies aimed at increasing the removal efficiencies of CECs, Kenya employs three primary wastewater treatment techniques: wastewater stabilization ponds (WSPs, lagoons), trickling filters, and constructed wetlands (Kilingo et al., 2021; Rayori, 2023). However, the selection of a particular treatment technique mainly depends on the mass load, the cost of the treatment technique, and climatic conditions. Overall, WSPs are the most dominant technique in Kenya due to their low cost, ample land availability, and favorable climatic conditions, which include temperature, pH (>9.0), and high light intensity(Rayori, 2023).

2.5 Wastewater treatment plants: treatment stages and processes in wastewater treatment

2.5.1 Wastewater Stabilization Ponds (WSPs)

Wastewater stabilization ponds, or WSPs, are biological treatment systems that rely heavily on environmental factors, including temperature, wind speed, and light intensity, all of which are specific to a given area and highly variable (Mahapatra et al., 2022).WSPs offer several advantages, including simplicity of use, low energy requirements, minimal equipment maintenance, and enhanced sludge thickening. However, in terms of total suspended solids (TSS) and biochemical oxygen demand (BOD), the effluent quality from fixed film systems is comparatively lower than that from suspended growth (Waqas et al., 2020). The pond systems are designed to cultivate anaerobic and aerobic bacteria and green micro-algae to decompose water-borne organic waste effectively and efficiently. The stabilization process consists mainly of the interactions of bacteria and algae(Mahapatra et al., 2022). The components of sewage are broken down and oxidized by the bacteria. The algae provide the oxygen required to keep the bacteria alive during the treatment process by using carbon dioxide and other materials produced by the bacterial activity, as well as through photosynthesis (Saravanan et al., 2021). Large and heavy solids are removed from wastewater by first putting it through grit removal and preliminary treatment screening in the WSP setup. This is followed by the anaerobic pond's primary treatment, the facultative ponds' secondary treatment, the maturation ponds' tertiary treatment, and so on. Fermentation ponds are used to remove nutrients (nitrogen and phosphorus) and fecal viruses and bacteria, whereas anaerobic and facultative ponds are used to remove organic matter (BOD) (Mahapatra et al., 2022).

2.5.2 Anaerobic ponds

High organic load wastewater is fed into anaerobic ponds, which are built at a depth of roughly 2 to 5 meters. Dissolved oxygen (DO) and algae are absent from the ponds because their sole function is to remove BOD (Ghangrekar, 2022). In anaerobic ponds, BOD is typically removed by sedimentation of solids followed by digestion in the resultant sludge. According to Uddin & Wright (, Anaerobic digestion is a biological process that releases methane and ammonia when organic matter is broken down in the absence of oxygen. When the temperature rises above 15°C, the digestive process becomes vigorous and swift. Anaerobic bacteria often have a pH of less than 6.2 and a residence period of two to five days in ponds (OKALIWE, 2022).

2.5.3 Facultative ponds

Facultative ponds (FPs) feature a distinct stratification with an upper aerobic zone and a lower anaerobic zone, facilitating active purification in both regions. Designed for Biochemical Oxygen Demand (BOD) removal, facultative ponds are sized based on volumetric BOD loading (g BOD/$m^2 \cdot d$)(Ansa, 2013). These ponds can be categorized as either primary or secondary, depending on whether they treat raw or settled wastewater, respectively. When organic matter enters the pond, settleable and flocculated colloidal matter sinks to the bottom, forming a sludge layer where anaerobic decomposition occurs(Izdori, 2020). The remaining soluble or suspended organic matter remains in the water column, where it undergoes primarily aerobic or facultative decomposition, with occasional anaerobic activity.

Facultative ponds consist of three distinct zones:

i. A surface zone where aerobic bacteria and algae coexist symbiotically.
ii. An anaerobic bottom zone where anaerobic bacteria decompose accumulated solids.
iii. A zone in between that is partially anaerobic and partially aerobic, where facultative bacteria break down organic waste. The ponds get their name from this intermediary zone.

Facultative ponds typically follow anaerobic ponds in a Waste Stabilization Pond (WSP) system, and they usually have a depth of 1.5 to 2.0 meters(Izdori, 2020).

2.5.4 Maturation ponds

Maturation ponds are a standard tertiary treatment method used worldwide to enhance the effluent quality following secondary biological treatments, such as facultative ponds (Kaid et al., 2022). Mphuthi. (2021) state that the size and number of maturation ponds, which collect wastewater from facultative ponds, are dictated by the intended bacteriological quality of the final effluent. These ponds are usually only one to 1.5 meters deep, with one meter being the ideal depth; they are shallower than facultative ponds. Less than one meter of depth can stimulate mosquito breeding and the growth of rooted macrophytes (Shilton, 2006).

2.5.5 Trickling filters

These are aerobic biofilm reactors that are not submerged and are made of a highly permeable material tank that receives wastewater application via a distribution (Komolafe et al., 2021). Air naturally travels upwards or downwards, and the liquid trickles downhill, permitting bacterial development (biofilm) on the packing material's surface. To extract organic stuff from the wastewater, they use microorganisms affixed to a medium. As ambient air permeates wastewater, it provides the oxygen required for organic components to undergo biochemical oxidation and for ammonium to undergo enhanced nitrification (Kumwimba & Meng, 2019).

The organic material in the wastewater is adsorbed by a colony of microorganisms that attaches to the medium and forms a biofilm or slime layer that is between 0.1 and 0.2 mm thick. The colony includes facultative, aerobic, and anaerobic bacteria as well as fungi, algae, and protozoa (Saini et al., 2023). Microorganisms in the water gradually stick to the surface of the medium as the wastewater passes over it, creating a film. These organisms then oxidize the organic load into carbon dioxide and water, generating new biomass. Trickling filters require a continuous supply of power and wastewater, and can only be used after primary clarification, as high solids loading can cause clogging(Daigger & Boltz, 2011).

2.5.6 Constructed wetlands

These are treatment systems that utilize microbial assemblages linked to vegetation, soils, and other natural processes to improve water quality. According to Gorgoglione & Torretta. (2018) (these are often shallow basins that are planted with plants that can withstand saturated conditions and filled with filter material (substrate), typically sand or gravel. In artificial wetlands, vegetation is essential because it offers surfaces and a favorable habitat for microbial development and filtration. In general, wetlands remove pollutants through an intricate network of physical, chemical, and biological processes. According to Gorgoglione & Torretta (2018), wastewater is injected into the basin and either flows over the surface or through the substrate. The use of natural processes, ease of building, ease of operation and maintenance, cost-effectiveness, and process stability are some of the benefits of artificial wetlands.

Filtration and sedimentation in wetlands effectively remove suspended and settleable particles that are not eliminated during the first treatment. Particles are trapped in flow structures or remain stationary in micro-pockets (Thamke & Khan, 2021). Organic molecules that dissolve are eliminated by attached and suspended microbial growth. Both aerobically and anaerobically, in the presence or absence of dissolved oxygen, these compounds undergo biological annihilation (Boyd & Boyd, 2020). Through diffusion or oxygen leakage from the roots of vegetation, the oxygen needed for aerobic degradation is directly provided by the atmosphere.

Adsorption, complexation and precipitation, storage, plant uptake, and biotic assimilation are the processes by which phosphorus is removed from artificial wetlands (Malyan et al., 2021). Nitrogen is eliminated using matrix adsorption, volatilization, ammonification, nitrification, and plant uptake. Bacteria in aerobic zones nitrify ammonia to produce nitrate. In anoxic and anaerobic zones, denitrifying microorganisms transform nitrates into dinitrogen gas (Vymazal, 2007).

2.6 Mechanisms in the removal of CECs in wastewater treatment plants

WWTPs play a significant role in the removal of a variety of contaminants from wastewater, such as pathogens, dissolved organics, nutrients, and suspended and colloidal particles. (Chahal et al., 2016). Despite most conventional WWTPs not being designed to eliminate CECs from wastewater, the primary, secondary, and tertiary treatment processes, to some extent, result in the elimination of CECs at varying concentrations. Upon entry to the treatment plant, wastewater is first subjected to primary treatment processes, which are mainly dominated by the sorption of compounds into the primary sludge. This is followed by secondary treatment processes, which are comprised of sorption, biodegradation, photodegradation, and volatilization, with organic matter being obliterated (Gruchlik et al., 2018). CECs in this stage are mainly eliminated using biodegradation and sorption processes. In the tertiary treatment process, nutrients, suspended solids, and pathogens are removed, together with CECs, which are recalcitrant through ozonation-like conventional oxidation processes(Rout et al., 2021b). The removal effectiveness of CECs is expected to be between 20 and 50 percent in the first treatment step and 30 and 70 percent in the secondary treatment phase. The removal rate of CECs changes across the three stages of the treatment procedures (Rahman et al., 2018).

2.6.1 Sorption

The chemical composition of the sorbent solids, the prevailing conditions in the environment, and the structural properties of the emerging contaminants (ECs), such as their hydrophobicity and availability of functional groups for chemisorption, all have a significant impact on the adsorption of ECs onto the solids (i.e., the sorbent) present and generated during wastewater treatment (Mahmood et al., 2022). Antibiotic classes such as sulphonamides, aminoglycosides, and tylosin typically exhibit weak sorption to activated sludge, whereas quinolones and tetracyclines demonstrate high sorption affinities. Estrogens also exhibit high sorption affinities compared to many pharmaceuticals. However, their removal via sorption is often low because they typically undergo biodegradation in aerobic sludge(Mejias et al., 2021).

2.6.2 Biodegradation

The biodegradation treatment mechanism is mainly employed in the secondary treatment process step, which utilizes both anaerobic and aerobic microorganisms to break down parent compounds into their metabolites(Doukani et al., 2022). The process is highly dependent on the structural complexity and biodegradation characteristics of the compound. The functional groups and the parent chains of the compounds play an important role in their degradation by microorganisms. In contrast to molecules with long chains, branching structures, and saturated structures, those with short straight chains and unsaturated structures are more likely to biodegrade. This is mainly due to their structural complexity, which poses some strain and complications to the microorganisms in degrading them(Y. Liu et al., 2020).

In treatment plants, biodegradation is the primary strategy used to remove CECs, particularly PPCPs, endocrine disruptors, plasticizers, and surfactants. CEC biodegradation is mainly achieved through co-metabolism, in which the degrading microorganism utilizes one compound as the carbon source and another as energy. PPCP and pesticide removal effectiveness in WWTPs vary based on the compounds, WWTP design, and treatment procedures used (Mahmood et al., 2022; H. Zhang et al., 2023). Removal efficiencies for individual compounds can range from 0% to over 99%.

The presence of pesticides and PPCPs in WWTPs across the world has been the subject of several studies. Five pesticides and 27 PPCPs were discovered in wastewater samples taken from 50 WWTPs in an American investigation (N. Liu et al., 2020a). Similar findings were made by another Chinese research that examined wastewater and sludge samples taken from six WWTPs and found 28 PPCPs and 22 pesticides (Y. Zhang et al., 2021). Several classes of substances have been identified, such as hormones, insecticides, herbicides, hormone-like compounds, non-steroidal antiinflammatory drugs (NSAIDs), and antibiotics(Peysson & Vulliet, 2013).

Recent studies have detected a wide range of PPCPs and pesticides in WWTPs. For example, a study conducted in Spain detected 40 PPCPs and 20 pesticides in influent and effluent samples from a WWTP (Mejias et al., 2021). The detected compounds included antibiotics, NSAIDs, hormones, fragrances, insecticides, and herbicides. Another study conducted in India detected 29 PPCPs and six pesticides in effluent samples from a WWTP (SUMIDA et al., 2024). The detected compounds included antibiotics, hormones, and fragrances.

PPCPs and pesticides are released into the environment via WWTPs, which are significant sources. These substances can harm aquatic life, such as fish and invertebrates, and may also cause germs to become resistant to antibiotics when released into the environment. Furthermore, several pesticides and PPCPs can persist in the environment and build up in sediments and biota, which could be harmful to human health.

2.7 Occurrence of chemicals of emerging concerns in WWTPS

Numerous research works have documented the existence of CECs in various kinds of WWTPs. As an illustration, 47 CECs were discovered in the influent, effluent, and sludge of a WWTP in China by (Xie et al., 2023). The substances that were found most commonly were naproxen, ibuprofen, caffeine, and carbamazepine. (Yashas et al.,

2022) discovered 38 CECs in the wastewater of a WWTP in Switzerland. Three substances were found the most often: metformin, atenolol, and gabapentin.

One of the most frequently observed categories of CECs in WWTPs is pharmaceuticals. A study by N. Liu et al.(2020b) found that 98 pharmaceuticals were detected in the influent and effluent of a WWTP in China. The most frequently detected compounds were carbamazepine, naproxen, sulfamethoxazole, and diclofenac. Another study by Tang et al.(2021) found that 54 pharmaceuticals were detected in the influent and effluent of a WWTP in China. The most frequently detected compounds were sulfamethoxazole, ofloxacin, and carbamazepine. Another study by Muteeb et al.(2023b) examined wastewater samples from three WWTPs in Pakistan and discovered that 23 of the 29 target pharmaceuticals were present, ranging in concentration from ng/L to µg/L. Similarly, a study by J. Chen et al.(2021) examined wastewater samples from a Chinese WWTP and discovered that 12 of the 24 target pharmaceuticals were present, ranging in concentration from ng/L to µg/L. The most frequently detected pharmaceuticals were antibiotics such as sulfamethoxazole, trimethoprim, and norfloxacin. In a study conducted by K'oreje et al.(2018), Several pharmaceutical classes, including antiviral, antibiotic, and anti-inflammatory ones, were found in wastewater stabilization ponds. Trans Nzoia, Kenya, had high levels of paracetamol, ibuprofen up to 1000µg/L, sulfadoxine, sulfamethoxazole, lamivudine, and nevirapine in both influent and effluent. The concentration of doxycycline, amoxicillin, sulfamethoxazole, trimethoprim, ciprofloxacin, and norfloxacin was detected in the range of 0.2 to 21.4µg/L in influent and effluents in selected wastewater(Kairigo et al., 2020)

Another contribution of CECs in WWTPs is personal care products, including cosmetics, fragrances, and sunscreens. These products may include a variety of compounds that can be released into the environment through wastewater discharge and the application of biosolids, such as UV filters, preservatives, and surfactants. A study by Huang et al.(2020b) found that UV filters, such as benzophenone-3 and octyl methoxycinnamate, were the most prevalent personal care products in WWTPs.

Finally, industrial effluents are also a significant contributor to CECs in WWTPs. These effluents can contain a wide range of chemicals, including solvents, dyes, and flame retardants, which can enter the wastewater stream through direct discharge or accidental spills. A study by Dehghani et al.(2022) found that industrial chemicals, such as benzene and toluene, were present in WWTPs at concentrations that exceeded environmental quality standards.

2.8 Fate of CECs in WWTPs

In WWTPs, CECs may undergo a variety of processes, including chemical, biological, and physical changes. These procedures may have an impact on the environmental persistence, bioavailability, and toxicity of CECs. Biodegradation entails a process where microorganisms in the wastewater can metabolize CECs into more harmful compounds(Golovko et al., 2021b). A study by R. Yan et al.(2022) found that the biodegradation of antibiotics in WWTPs varied depending on the type of antibiotic and the wastewater treatment process. They observed that sulfonamides and tetracyclines were more easily biodegraded than macrolides and fluoroquinolones.

Another fate process in WWTPs is sorption, where CECs can attach to particles in the wastewater or biosolids. A study by Yadav et al.(2021a) found that sorption was an important process for removing CECs, particularly for hydrophobic compounds, such as polycyclic aromatic hydrocarbons (PAHs). They observed that sorption onto biosolids was a significant mechanism for removing PAHs from the wastewater.

2.9 Sampling Techniques

Sampling is the process of acquiring a portion of a substance or medium to analyze and understand its different characteristics and properties. In environmental analysis, samples collected should be representative of the whole ecological composition, allowing for detection and quantification. Sampling is a crucial research activity employed to generate physical or chemical data that is representative of a particular volume of substance over a specific period. Different types of sampling techniques are utilized in acquiring ecological samples from the different environmental matrices. These techniques include grab sampling, composite sampling, passive sampling, and continuous sampling. The choice of a sampling technique mainly depends on the type of environmental media, the nature of the contaminants, and the spatial and temporal variability.

2.9.1 Grab Sampling

Grab sampling of wastewater involves the collection of water samples from specific locations (influent, effluent, and ponds) at a specific time, mostly without the use of automated sampling equipment. Grab sampling can either be discrete or duplicate. A discrete grab sample is obtained at a selected location, depth, and time. In contrast, duplicate grab samples are collected periodically by subdividing one sample into two or more identical sub-samples. A snapshot of the concentration in the water at the moment of sampling is given by grab sampling. Since there is no equipment installation required, the procedure is practical and straightforward. Grab sampling has yielded promising results for previous studies. Grab sampling has been suitable for moderate to high pesticide concentrations (Valenzuela et al., 2020). In a study conducted by Augusto et al.(2022), there was no discernible variation in the average concentrations of pollutants between grab sampling and composite sampling, indicating a strong concordance between the two methods. However, it does not consider inter- and intra-day variations(Cristóvão et al., 2021). Since WWTPs' wastewater mass load varies depending on time and day, grab sampling may miss vital components present in the influent or effluent and provide less representative surveillance(Wilson et al., 2022). Grab sampling has been reported to be unsuitable for the concentrations of pollutants that are subjected to fluctuations(Hawker et al., 2022).

2.9.2 Passive Sampling

Passive sampling is a technique utilized in gathering and tracking environmental contaminants in aquatic environments, such as wastewater. It entails setting up devices that gradually accumulate pollutants, allowing the average concentration of pollutants to be measured throughout the sampling period(da Costa Filho et al., 2022). The time-weighted average of pollutant levels that passive sampling provides is a more representative measure than the grab sampling technique.

The fundamental idea behind passive sampling is that pollutants enter the sampler through the diffusion mechanism or permeation from the aqueous phase. The accumulation of pollutants within the sampler is typically proportional to their concentration in the water and the exposure time(Caban et al., 2022). Depending on the type of sampler, contaminants are captured through either adsorption or absorption processes. Adsorption-based samplers are capable of capturing pollutants on the material's surface; however, the effectiveness of the sampler may be affected by the physicalchemical characteristics of the water. Conversely, absorption-based samplers work on the principle of trapping impurities inside the material's internal structure; their efficacy depends on variables like the sorbent's solubility and porosity(Y. Zhang, 2023).

Different types of passive samplers are used, depending on the pollutants that are being tested. Semi-permeable membrane devices, or SPMDs, are used for hydrophobic organic compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and certain pesticides (Dündar, 2020). Pharmaceuticals, insecticides, and personal care products are among the polar organic pollutants that can be effectively monitored with Polar Organic Chemical Integrative Samplers, or POCIS(L. Wang et al., 2020). Metals and inorganic nutrients are the target compounds for Diffusive Gradients in Thin-Films (DGT). These highly sensitive samplers can detect low concentrations that conventional approaches might miss, and they offer an integrated measure of pollutant levels during the sampling period(Sohrabi et al., 2021).

In addition to its ability to provide a time-weighted average concentration, low maintenance requirements during deployment, and high sensitivity to trace pollutants, passive sampling has some drawbacks. For example, accurate calibration is necessary to relate the amount of contaminant accumulated in the sampler to the actual water concentration. The performance of passive samplers can also be impacted by environmental factors such as water flow, temperature, and biofouling(MacKeown et al., 2022). Finally, because passive samplers are typically designed for specific classes of chemicals, they may not be able to capture all pollutants present in wastewater.

2.10 Sample Preparation Techniques

Sample preparation is one of the most crucial steps in every analytical method. It plays a vital role in obtaining reliable, precise, and accurate results. The primary purpose of sample preparation is to transform a sample from a solid, liquid, or gas into a form that an analytical instrument can analyze.

Wastewater sample preparation has made use of a variety of sophisticated sample preparation methods that have been developed, such as solid-phase extraction (SPE), liquid-liquid extraction, and microwave-assisted extraction. The particular needs of the analytical technique to be used in the analysis have always dictated the choice of sample preparation methods.

2.10.1 Solid-Phase Extraction

Solid-phase extraction (SPE) is an extraction technique that utilizes a solid and liquid phase to extract analytes from a liquid sample. In environmental analysis, SPE is employed as a preconcentration step and to clean up samples before their introduction to a chromatographic or any other analytical instrument(Dugheri et al., 2021).

The partitioning of solutes between a sorbent (solid phase) and the sample matrix (liquid phase) is the primary mechanism behind SPE. By sorption onto a solid sorbent, the method enables the concentration and purification of analytes from a solution, as well as the purification of the extract following extraction (Płotka-Wasylka et al., 2016). In general, the process entails loading the sample solution onto an SPE solid phase, washing away undesirable components, and then employing an appropriate solvent to wash off desired analytes(Campíns-Falcó et al., 2006).

Choosing an appropriate sorbent is often essential to attaining optimal results with SPE. The polarity of the analyte and the sample matrix influences the selection of an appropriate sorbent. Oasis HLB cartridges have been used often for both polar and nonpolar compounds because they include lipophilic divinylbenzene units and hydrophilic N-vinylpyrrolidone units, both of which are highly stable across a wide pH range (from 1 to 14). (C. M. M. Almeida, 2021).

In a study conducted by Abbas et al.(2019), SPE displayed a good recovery in the extraction of steroids, pharmaceuticals, and personal care products from wastewater and groundwater samples, in addition to minimizing matrix interferences by reducing natural organic matter and excluding ions, nutrients, or acids. In the utilization of hydrophilic/hydrophobic (Oasis HLB and Supelco ENVI-Carb+) and one composite (Telos 467 C18/ENV) SPE sorbents for extraction, SPE was reported to be effective in the extraction of both neutral and acidified samples(Kolkman et al., 2013). It was discovered that two of the sorbents used in the pharmaceutical extraction process from wastewater samples, weak cation exchange (WCX) and hydrophilic-lipophilic balance (HLB), were appropriate for ultra-trace analysis. Higher analyte trapping capacity and improved accuracy were demonstrated by HLB sorbent, whereas weak cation exchange sorbent performed better in terms of selectivity(Zorita et al., 2008).

2.10.2 Microwave-Assisted Extraction

The process of microwave-assisted extraction (MAE) requires heating polar molecules selectively with microwave energy. This rapid and homogenous heating of a sample allows a short extraction time and a reduction in solvent consumption and solvent waste(Belwal et al., 2020). To achieve an efficient MAE, parameters including: solvent composition, solvent volume, solvent-to-feed ratio, time, power, matrix moisture, and particle size are controlled and optimized. Advanced MAE utilizes a solvent-free microwave extraction mechanism to extract volatile and non-volatile compounds(Nonglait & Gokhale, 2024). When compared to conventional technologies, the application of MAE results in a greater release of chemicals from the matrix of interest while requiring less time for extraction and solvents(Gomez et al., 2020).

2.11 Analytical methods used in detecting CECs present in WWTPs

The analytical method selected and the instrument's sensitivity determine the detection limits of CECs in WWTPs, which usually vary from ng/L to µg/L. For this reason, a variety of analytical methods have been developed, as noted by Shyamalagowri et al.(2023) and Qin et al.(2023). These techniques include gas chromatography (GC), liquid chromatography-mass spectrometry (LC-MS), and high-performance liquid chromatography (HPLC).

SPE, a sample preparation technique utilizing a solid-phase sorbent, enhances sensitivity and selectivity by extracting and purifying target compounds from intricate matrices like wastewater (Badawy et al., 2022; Kaya et al., 2023). Recent developments include passive sampling methods and online solid-phase extraction combined with high-resolution mass spectrometry (online SPE-HRMS), which provide creative approaches to increase productivity and simplify analysis.

HPLC and GC, both separation techniques, play a crucial role in isolating and quantifying individual compounds within mixtures (Kanu, 2021; Silvestro et al., 2013). HPLC excels with polar compounds, while GC is more adept at handling nonpolar ones, necessitating a derivatization step for specific compounds to enhance detectability(Frei, 2013). LC-MS, a powerful analytical method, combines liquid chromatography separation capabilities with mass spectrometry detection sensitivities to identify and quantify different chemicals in complicated mixtures (Kanu, 2021).

Liquid chromatography, especially when coupled with advanced detection techniques, stands out in perfluorinated compound analysis. Mass spectrometry, particularly electrospray, proves highly effective in detecting perfluorinated residues. In a comparative study, different liquid chromatographic systems, including conventional HPLC, ultra-high-performance liquid chromatography (UHPLC), and capillary chromatography (CLC), revealed UHPLC as the most efficient in terms of waste generation, rapidity, and selectivity(Trojanowicz & Koc, 2013).

Kanu. (2021) explored the chromatographic separation of pharmaceutical residues using a UHPLC system with promising results. The study demonstrated good linearity, repeatability, and recovery, with a detection range of 10 to 100 ng/L for various pharmaceuticals, including caffeine, acetaminophen, ciprofloxacin, xylazine, ketoprofen, naproxen, ibuprofen, and diclofenac. Ngumba et al.(2016b) investigated antibiotics and antiretroviral drugs in the Nairobi River Basin, employing solid-phase extraction followed by liquid chromatography-electrospray ionization tandem mass spectrometry (SPE-LC-ESI-MS/MS). The method exhibited excellent linearity, with recoveries ranging from 57.51% to 96.36%. The limit of quantification varied between 8 and 122 ng/L across different samples, providing valuable insights into the occurrence of these compounds in the studied area

2.12 Chemicals of emerging concerns: EU regulations and guidelines

Water quality is a priority due to its increasing demand. This has called for the identification of compounds for regulation and monitoring, due to the adverse effects they impose on the environment. Different organizations, such as the EPA, WHO, and EU, have established policies and guidelines to mitigate and regulate the toxicity effects of various CECs in the environment (Parida et al., 2021b).

The set regulatory standards provide the different compound concentration thresholds in water bodies, beyond which they may pose a risk to the environment with long-term exposure. However, very few compounds have been added to the watch list, with the latest being flame retardants, which are categorized as a priority. 2020 saw the EU add 17-beta-estradiol and nonylphenol to the first watch list due to their potential to affect human hormones and health (EU, 2023).

2.13 Ecotoxicological risk assessment of chemicals of emerging concerns

Several aquatic biotas are chronically or occasionally exposed to chemical substances commonly referred to as chemicals of emerging concern (CEC) in the environment. The mode of action of a chemical can be IA or CA, where IA is oriented on the potency of individual chemicals and CA focuses on the additive effect of concentrations (Kandie, Krauss, Massei, et al., 2020a; Yusuf et al., 2021). The CA concept presupposes that single compounds behave in the same manner and affect the same (Liess et al., 2020; Ogungbemi et al., 2021). When more chemical substances are combined in the aquatic environment, the resulting compounds pose a danger because they can interact with CA, causing negative impacts on human beings and other living organisms (Sousa et al., 2017). However, the IA concept lies under the presupposition that, unlike single substances, compound mixtures interact with several subsystems of an organism and the damage to every subsystem will influence the endpoint separately (Pihlaja, 2024).

There are two ways to evaluate the risk for a particular species: the toxic units (TU) (de CastroCatalà et al., 2016) or the risk quotient (RQ) (Finckh, Beckers et al., 2022). In ERA, the TU is adopted to compare the MEC with the 50% effect concentration on the tested organisms (H. Wang et al., 2021a). In other words, a TU value of more than 1 denotes influence on one-half of the exposed organisms. However, using TU to evaluate risk does not consider the toxicity of a single species and is thus not regarded as a threshold or protective limit. When addressing combination toxicity, it is essential to be able to demonstrate the toxic stress at a particular site by the aggregate of the separate TUs for each site (Finckh et al., 2022a).

2.13.1 Toxic Unit Approach (TU)

The TU approach generally is an ecotoxicological risk assessment approach of diverse CECs concerning algae, crustaceans, and fish. The measured environmental concentration (MEC) is usually divided by the EC50 values, derived from the ECOTOX database for each compound(Moeris, 2020). This type of analysis has been applied before in similar works (CarazoRojas et al., 2018; Finckh et al., 2022a; Kandie et al., 2020b) and allows the evaluation of toxic effects possibly caused by compounds. TU methodology integrates the overall effects of a set of pollutants on the ecosystem, depending on tests of acute or chronic toxicities. For acute toxicity, the L(E)C50 value is used relatively often; for chronic toxicity, the NOEC and LOEC of the chemicals in a mixture are employed. The concept of LC50 means the concentration which has the probability of causing the death of fifty percent of the test organisms; LOEC, on the other hand, is the most minor concentration that gives a negative impact on the test organisms and the highest tested concentration that results in no biological effect is known as NOEC(Moeris, 2020).

The toxic unit (TU) values are calculated by dividing the MEC of a compound by its respective acute effect concentration (EC50 or LC50) for algae, crustaceans, and fish.

$$\mathrm{TU} = \frac{\mathrm{MEC}}{\mathrm{EC50}}$$

The above-derived TU values for each of the compounds are then compared to the developed TU risk thresholds for acute and chronic toxic risks. According to the information, the allowed rate of acute toxic risk for all forms of life has been predetermined as 0.1 TU, and chronic toxic risk thresholds are stated at 0.02 (algae), 0.001 (crustaceans), and 0.01 (fish) (Malaj et al., 2014).

2.13.2 Risk Quotient (RQ)

Risk quotient (RQ) is a deterministic approach that compares toxicity to environmental exposure. In this methodology, the risk quotient (RQ) value is determined by dividing a point estimate of exposure by a point estimate of effects(Gredelj et al., 2018). The RQ value obtained is a screeninglevel approximation that evaluates the potential risk of toxicity posed. The evaluation of RQ is based on ecological, fate, and estimated exposure data of CECs. The environmental concentration is compared to an effect concentration level, such as LC50 (Q. Yan et al., 2014).

Furthermore, the lowest Predicted No Effect Concentration (PNEC) values and the measured environmental concentration (MEC) are used to derive the RQ value. To assess the potential risk associated with the presence of CECs in the aquatic environment, the RQ value calculation is based on the MEC of the detected compounds in the WWTPs' effluent, PNEC for three standard organisms, algae, daphnia, and fish, and the dilution factor (DF)(Gosset et al., 2021).

$$\mathrm{RQ} = \frac{\mathrm{MEC}}{\mathrm{PNEC} \times \mathrm{DF}}$$

PNEC is usually calculated by dividing the LC50 or EC50 value by an appropriate assessment factor (AF)(Gredelj et al., 2018).

$$\mathrm{PNEC} = \frac{\mathrm{EC50}\ \mathit{or}\ \mathrm{LC50}}{\mathrm{AF}}$$

If the RQ is lower than 1, the ecological toxic risk posed by CECs is negligible. In contrast, when the values are equal to or greater than 1, the adverse effects on organisms are probable(Česen et al., 2018).

2.14 Ecotoxicological risks of chemicals of emerging concern

Several studies have confirmed the discharge of CECs in WWTP effluents, including pharmaceuticals, PPCPs, EDCs, and other ECs. These chemical traces are not eliminated during the wastewater treatment, hence posing toxicity to aquatic life. Most CECs are biologically active, have a specific mode of action, and exert acute and chronic effects on aquatic organisms to different extents (Ahmad et al., 2022). H. Wang et al.(2021b) showed that different PPCPs, such as diclofenac, carbamazepine, and triclosan, are toxic to the freshwater snail, *Lymnaea stagnalis*, at the concentrations that exist in the effluents from WWTPs. Due to the presence of these contaminants, water quality in various water sources poses a significant threat to aquatic life.

Another possible impact of CECs in the effluent of WWTP is the increased antibiotic resistance among bacteria. Another study by Bao et al.(2021) revealed that antibiotic-resistant microorganisms and resistance genes were released from WWTPs into the environment. The patterns of antibiotic-resistant bacteria and genes in the effluent of WWTPs were also elevated in this study, which supported the notion of WWTPs' dissemination of antibiotic resistance. (Lorenzo et al., 2018) This notion is supported by the observation that the efficiency of the treatment process increases with the concentration of tetracycline residues in the treated wastewater, which is associated with antibiotic resistance. Also, Hassoun-Kheir et al.(2020) found the presence of antibiotic-resistant gram-negative bacteria in hospital wastewater. A literature review by Yin et al.(2023) discovered that low levels of antibiotics in WWTP effluent boosted antibiotic-resistant bacteria in water bodies, which may pose health risks to humans.

Therefore, in the study under analysis by Golovko et al.(2021a), zebrafish embryos were tested after 144 hours of post-fertilization onset, following the exposure to water samples from various WWTPs. The endpoints used in the study include mortality, malformations, pericardial sac, and yolk-sac edema, spontaneous movement, heart rate, time to hatching, and swimming pattern. It was noted that the number of traumatized embryos was, as a rule, small, while statistically significant toxic actions were determined in certain instances. Specifically, samples collected from the influent of WWTPs 9 and 10 proved to be fatal to all the eggs subjected to this study, consequently dying 24 hours after conception. In another experiment, it was noted that the heart rates of embryos treated with influent and effluent water from WWTP 8 were lower. In contrast, the samples of influent water from WWTP 4 and WWTP 8 delayed the hatching process(Ribeiro et al., 2020). These effects related to hatching time and swimming activity pointed to the total chemical load of the CECs, such as antidepressants, opioids, and stimulants, observed in the samples from WWTP.

In another study, Gosset et al.(2021) examined the CECs' toxicity in water samples from different WWTPs by comparing their concentration to the predicted no effect concentration (PNEC) to determine the possible negative impact on aquatic life, especially the zebrafish embryos. The PNEC values varied among CECs, reaching certain levels like tert-butryn 0.019 ng/L, whereas higher values like irbesartan 704 μg/L were also observed. The herbicide tert-butryn showed a concentration-dependent response in algal growth, with low concentrations showing a 30% growth suppression. Pharmaceuticals, specifically methocarbamol, atorvastatin, and venlafaxine, were evaluated as having a high potential for ecotoxicity among the different pollutants. In contrast, twenty CECs were ranked as having the lowest potential for ecotoxicity. The study and targeted research underlined the fact that, owing to the lack of experimental data and the potential overestimations when using QSAR modeling, several PNEC values have to be optimized. Also, it was revealed that drugs for the nervous system (class N according to the ATC classification) were more ecotoxic than the previous one. In contrast, cardiovascular system drugs (class C according to the ATC classification) were less ecotoxic among the specified pollutants.

CHAPTER THREE: MATERIAL AND METHODS

3.1 Research Design

This research employed an experimental (quantitative) research design. This was done through measurement and analysis of the concentration of these chemicals as well as their removal efficiencies in the treatment plant. Quantitative analysis of CECs is essential for assessing the efficiency of WWTPs in removing these compounds from the wastewater and for evaluating the potential risks associated with their discharge into the environment.

3.2 Reagents and equipment

Analytical-grade solvents (ethyl acetate, methanol, acetonitrile, formic acid, and 7 N ammonia; purity ≥ 98%) were obtained from Sigma Aldrich for the preparation and analysis of water samples. Glass fiber filters (Whatman GF/F, 50 mm) and 6 mL HR-X solid-phase extraction (SPE) cartridges containing 200 mg silica sorbent were also purchased from Sigma Aldrich. Kobian Kenya Limited supplied Nalgene bottles and autosampler vials. All analytical standards were sourced from Sigma Aldrich.

3.3 Study area

This study was conducted in western Kenya, covering Trans Nzoia, Uasin Gishu, and Nandi counties (Table 2, Appendix I). The region lies within the Kenyan highlands and is characterized by a highland equatorial climate, with bimodal rainfall averaging 900–1,200 mm annually and mean temperatures ranging from 14°C to 26°C. The terrain consists predominantly of gently undulating highland plains, with fertile volcanic soils that support extensive agricultural production. Major land uses include subsistence and commercial farming, livestock rearing, agro-industrial processing, and expanding urban settlements.

Four wastewater treatment plants (WWTPs) were selected to represent a range of wastewater sources and socio-economic settings:

- Nzoia Water and Sanitation Company (NZOWASCO) WWTP – Located in Trans Nzoia County, serving Kitale town. Constructed during the colonial period, it has seen minimal upgrades despite increased population and industrial growth. The plant discharges into River Chetoto, a tributary of River Nzoia, which ultimately drains into Lake Victoria.
- Eldoret Water and Sanitation Company Limited (ELDOWAS) WWTP – Situated in Uasin Gishu County, serving Eldoret city. It receives domestic, industrial, hospital, and stormwater runoff and discharges into River Sosiani, an important source for irrigation and domestic use.
- Moi University WWTP – Located in Uasin Gishu County, serving Moi University's main campus, representing an institutional wastewater source with mixed domestic and laboratory inputs.
- D.L. Koisagat WWTP – Located in Nandi County within a tea-growing and processing area. Effluents from this plant may directly affect agricultural soils and nearby aquatic ecosystems.

These sites were chosen to reflect varied wastewater characteristics arising from industrial, urban, institutional, and agricultural activities. Variations in contaminant occurrence and concentrations were expected due to differences in influent composition, population density, economic activities, and wastewater treatment infrastructure.

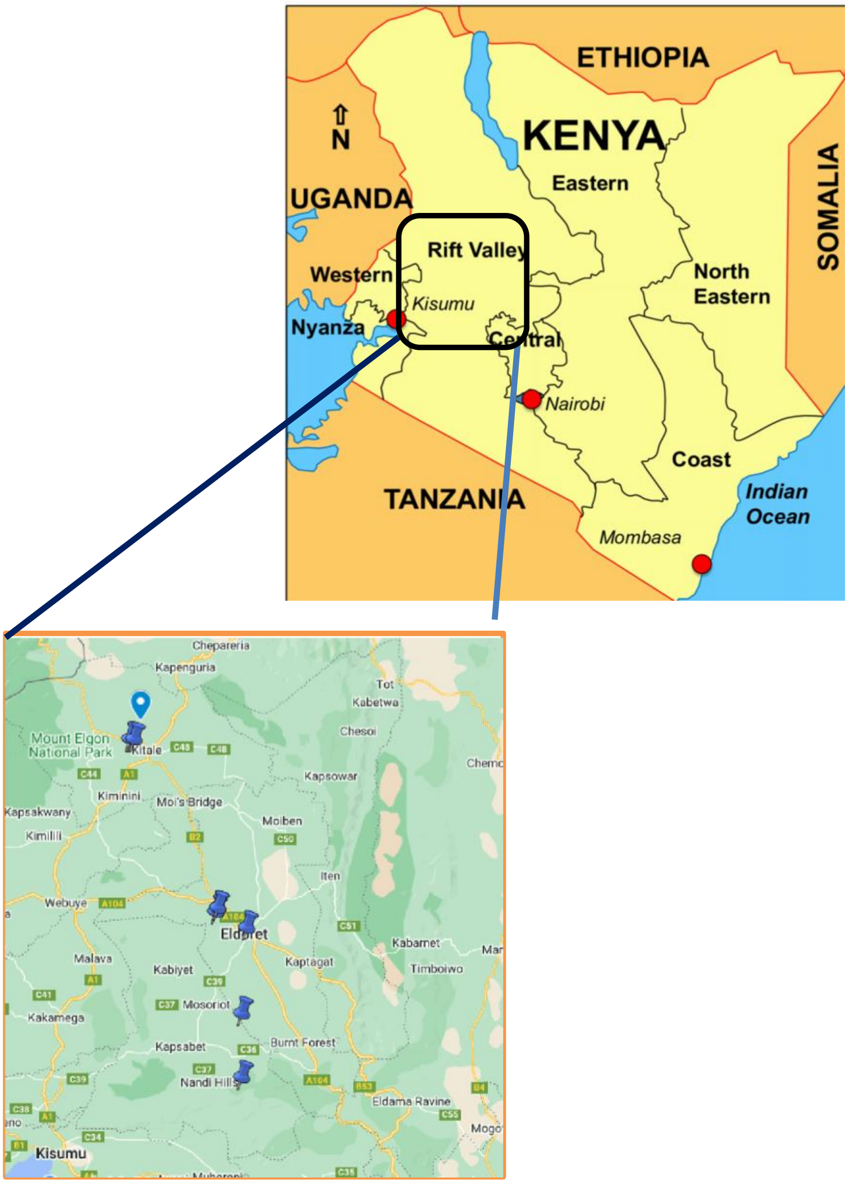

Figure 1: A figure of coordinates of the selected Wastewater Treatment Plants in Western Kenya, across Uasin Gishu, Nandi, and Trans Nzoia Counties in Kenya

3.3.1 Wastewater Treatment Plants Selected.

3.3.1.1 Nzoia Water and Sanitation Company (NZOWASCO)

The Kitale town wastewater treatment facility utilizes lagoon systems explicitly configured for treating domestic wastewater. The design incorporates bar screens, a primary facultative pond, a secondary facultative pond, and a maturation pond. Initially, the influent passes through bar screens to remove large solids. The water then flows into the main facultative pond, where anaerobic bacteria break down organic materials at the pond's lower depths and aerobic bacteria near the surface. During this stage, most suspended solids settle out.

The partially treated wastewater then flows into the secondary facultative pond and, finally, into the maturation pond, which features high algal growth to further polish the water. The North Rift Valley Water Services Board (NZOWASCO) serves a population of approximately 700,000 inhabitants. The effluent is ultimately discharged into the River Chitoto. Unfortunately, specific technical details, such as the dimensions of these treatment units, were not available to the author, as the management of the plant was unable to provide the information.

3.3.1.2 Eldoret Water and Sanitation Company (ELDOWAS)

The Eldoret Wastewater Treatment Plant (**figure 2**) employs a combination of wastewater stabilization ponds and trickling filter technology, with a design capacity of 19,872 m^3/day, serving an estimated population of 500,000 inhabitants. The treatment process begins with the influent passing through a coarse screen to remove large solids, followed by a fine screen. The screened wastewater is then evenly distributed into two rectangular anaerobic ponds, each with an active volume of approximately 33,600 m^3 and an operational residence time of three days.

Subsequently, the water flows into two trickling filters equipped with stainless steel rotary sprinklers and plastic media, where it has a residence time of 3-5 minutes. From the trickling filters, the wastewater is directed into a 2,350 m^3 circular sedimentation pond, which has a residence time of 5-6 hours to facilitate sludge removal. Following sedimentation, the water flows into a secondary pond with an active volume of approximately 67,000 m^3 and a residence time of eight days.

Finally, the treated wastewater is channeled into the maturation pond, which has a total surface area of 29,500 m² and a residence time of about five days before being discharged into the nearby River Sosiani.

Figure 2: A picture showing the ELDOWAS wastewater treatment plant's physical treatment, trickling filters, and secondary pond.

3.3.1.3 Moi University Wastewater Treatment Plant (Moi WWTP).

The Moi University WWTP serves the University fraternity within the campus. The wastewater sources include laboratories, health facilities, staff offices, and student hostels. The WWTP comprised three wastewater stabilization ponds (primary, secondary, and maturation ponds). At the time of the sampling, the maturation pond was not functional. The flow rate in the influent and effluent was low. The treatment plant employed both physical and biological processes. It was dependent on both biodegradation and photodegradation, with the ponds having a large surface area. The normal retention time of each stabilization pond was 12 days, but it was subject to change, depending on the inflow rate. The nearby community utilizes the river, which receives the effluent from this WWTP, as a supply of water for drinking and other household purposes.

3.3.1.4 DL Koisagat WWTP

The DL Koisagat WWTP receives wastewater from the tea processing industry, the households within the estate, and the dispensary in the estate. The WWTP contains two small ponds, a primary and a secondary pond. The flow rate of the WWTP was minimal at the time of sampling. The WWTP primary pond had 12 days of retention time, while the secondary pond had 14 days of retention time. At the time of sampling, the influent from the factory was black and contained tea leaf residuals from the factory. The treatment employs biological processes to treat the wastewater before its discharge. The community that lives along the river receives the effluent from this WWTP and utilizes it for drinking and other household purposes.

3.4 Sample collection and preparation

3.4.1 Sample collection

Before starting the sampling process at each site, physical water quality (odor of the site, color of the flowing water, floating material, nature of the water bed, presence of oil and grease) was checked and recorded in a data sheet.

The sample was collected from the influent and effluent of each WWTP. From the influent and effluent, 500 mL grab samples were taken for Solid-Phase extraction (SPE), and one 125 mL sample for physical-chemical parameters determination in the laboratory. Physical-chemical characteristics such as temperature, flow, dissolved oxygen, pH, turbidity, and total conductivity were monitored in situ throughout the sampling process.

To check for any contamination during the sampling process, trip blanks in 1mL vials were taken to the field and back to the laboratory without opening for each sampling trip and stored in similar conditions as those of the collected samples. Sampling blanks

(1ml LC-MC grade water) were also taken for each sampling site. At each site, the contents were picked and released back into the same vial using a sampling pipette at each sampling site. This helped determine if there was any contamination in the sampling pipette. Every sample was immediately kept at a temperature of less than -4°C in a transportable freezer and brought to the laboratory. They were maintained at -20°C after they entered the lab until the sample was prepared.

3.4.2 Sample preparation

Before sample filtration, a vacuum filtration pump (Rocker chemker 410) with a glass fibre filter (Whatman GF/F 50 mm) was pre-cleaned using 5 mL of ethyl acetate, 5 mL of methanol, and 10 mL of LC-grade water. The filtrate was subjected to solid-phase extraction (SPE) to isolate organic micro-pollutants. The SPE cartridges, which contained 200 mg of HR-X sorbent from Milford, USA, were prepared by conditioning them with 5 mL of ethyl acetate, followed by 5 mL of methanol. They were then rinsed with 10 mL of LC-grade water. Following the conditioning of the cartridges, a total of 350 mL was extracted from each sample (including river samples, trip blanks, and sample blanks) at a flow rate of 5 mL/min. Following the extraction process, the cartridges were dried using the vacuum manifold for 30 minutes and subsequently stored at a temperature of -20 ˚C until further elution and analysis.

Figure 3: A Pictorial of WWTP influent and effluent sample collection (A and B) and preparation (C and D)

3.4.3 Sample elution

The concentrated analytes in the cartridges were extracted using 5 mL of ethyl acetate, then 5 mL of methanol, followed by 4 mL of methanol containing 1.0% formic acid, and finally 4 mL of methanol containing 2.0% 7N ammonia. The extracted analytes were then collected in a 20 mL amber glass vial. The eluate was further concentrated using a nitrogen gas stream with a purity of 99% until it reached a final volume of 1 mL. The samples were filtered using 0.2 µm PTFE syringe filters (Whatman) and transferred into 2 mL amber glass vials. The samples were then dried using a nitrogen stream until the volume reached 200 µL. The sample was subsequently diluted with 350 µL of methanol (enrichment factor (EF-1000)). To achieve complete homogenization before conducting instrumental analysis, the enriched samples were vigorously vortexed for 2 minutes.

3.4.4 Instrumental analysis

The LC-HRMS analysis samples were prepared by combining 50 µL of the enriched sample (EF1000), 15 µL of methanol, 30 µL of water, and 5 µL of an internal standard (1 µg/mL) containing 49 isotope-labeled compounds. To create instrumental blanks, a mixture of methanol and water in a 70:30 ratio was used. Matrix-matched calibration standards were developed using a water sample from Wormsgraben, a pristine stream in the Harz Mountains, Northern Germany. Eleven 1 L aliquots spiked at levels from 0.5 to 1000 ng/L underwent the same solid-phase extraction procedure as the samples to form the calibration standards.

A Thermo Ultimate 3000 LC system was used for the analysis, in conjunction with a Thermo Q Extractive Plus high-resolution mass spectrometer. Separate runs were performed using negative and positive electrospray ionization modes. Chromatographic separation in all modes used a Kinetex Biphenyl LC column (100 x 2.1 mm, 2.6 µm particle size Phenomenex) with an inline filter and a pre-column of the same kind (5x 2.1 mm) at a temperature of 40°C. The positive ion mode used a gradient separation with 0.1% formic acid/methanol and 0.1% formic acid/acetonitrile, whereas the negative mode used 1 mM ammonium formate/methanol and 1 mM ammonium formate/acetonitrile. The HRMS analysis included a complete scan acquisition (m/z 80-1200) at a nominal resolving power of 70,000, as well as six data-independent acquisition scans (m/z 80-182, 178-282, 278-382, 378-482, 478-682, 682-1200) at a nominal resolving power of 35,000.

3.4.5 Data analysis

Peak detection and identification of target chemicals. LC-HRMS raw data were collected using MZmine (Version 2.38 software) (Pluskal et al., 2010) and Ms Convert

(Kandie et al., 2020), with the raw data first converted to mzML format using Proteowizard version 3.0.18265. The discovered target molecules were then validated and quantified using Trace Finder 5.1 (Thermo Scientific). The settings in MZmine and Trace Finder were implemented by Kandie et al (2020). Method detection limits (MDLs) were established using the calibration standards and the parameters stated in USEPA (2011). Graphical depiction and statistical analysis were carried out with R Software version 4.2.1 (Wickham, 2014) and Microsoft Power BI.

3.4.6 Removal efficiencies

The removal efficiency was calculated using the following formula as applied by (Golovko et al., 2021a; Khasawneh & Palaniandy, 2021; Y. Li et al., 2019a)

$$Removal\ efficiency = \frac{([C\_influent]-[C\_effleunt])}{[C\_infleunt]} \times 100 \qquad \text{Equation 1}$$

Where C_Influent represents the concentration of environmentally relevant compounds (ECs) detected in the influent, while C_Influent represents the concentration of ECs detected in the effluent of each wastewater treatment plant (WWTP). The removal efficiency value indicated the degree to which the treatment process has reduced the concentration of the ECs.

3.5 Ecotoxicological Risk Assessment

Ecotoxicological risk assessment (ERA) was performed to evaluate the potential adverse ecological effects on aquatic ecosystems. To assess the potentially toxic effects of CECs on different trophic levels, Toxic Units (TUs) were calculated for each trophic level and detected substance. The TU value was obtained by dividing the MEC (Measured Environmental Concentration) by the LC_{50}/EC_{50} values for each substance. This approach has been utilized in other studies (CarazoRojas et al., 2018; Kandie et al., 2020; Markert et al., 2020), allowing for a comprehensive evaluation of potential toxic effects across the three trophic levels (algae, crustaceans, and fish)

$$TU = \frac{MEC}{\frac{LC50}{EC50}} \qquad \text{Equation 2}$$

3.5.1 Criteria of evaluation

The obtained TUs were then subjected to the criteria proposed by Malaj et al., (2014) by comparing the TUs with the acute and chronic risk threshold values for the standard organisms. The acute toxic risk thresholds for all organisms were TU=0.1, while chronic toxic risk thresholds were set for algae (TU=0.02), Daphnia (TU=0.001), and fish (TU=0.01).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Physical-chemical water quality parameters

DO in influent ranged between 2.24 and 3.47 mg/L, while effluent values ranged from 2.37 to 2.69 mg/L (Table 4.1). DO increased by 20% in ELDOWAS WWTP but decreased by 31% in D.L. Koisagat during wastewater treatment. In all treatment plants, DO levels remained above 2 mg/L, the minimum required for efficient aerobic biological processes. The increase in DO at ELDOWAS suggested adequate aeration, whereas the decrease at D.L. Koisagat indicated possible aeration limitations.

Temperature increased in NZOWASCO (22.8 °C to 29.6 °C) and Moi WWTPs (20.0 °C to 22.2 °C), but decreased in ELDOWAS (21.2 °C to 20.4 °C) and D.L. Koisagat (23.7 °C to 20.5 °C). At ELDOWAS, the temperature change was low throughout treatment, which is beneficial for sustaining optimal biological activity. Effluent temperatures (20.4–29.6 °C) were within the Kenyan standards for effluent discharge (20–35 °C) (WASREB, 2006).

Effluent pH values ranged from 7.45 to 10.3. Most were within the acceptable range (6–9), except Moi WWTP, where the effluent pH was 10.3; 1.3 units above the upper limit, possibly indicating the presence of alkaline substances in the discharge.

Conductivity trends varied among plants. At ELDOWAS, conductivity increased from 758 µS/cm in the influent to 976 µS/cm in the effluent, suggesting the addition of dissolved ions during treatment. Conversely, NZOWASCO showed a decrease from 1267 µS/cm to 992.1 µS/cm, implying effective removal of dissolved ions during treatment.

Table 4.0. Physical-chemical characterization of the selected WWTPs.

Parameters	ELDOWAS Influent	ELDOWAS Effluent	NZOWASCO Influent	NZOWASCO Effluent	Moi Influent	Moi Effluent	D.L. Koisagat influent	D.L. Koisagat effluent
Temperature (°C)	21.2	20.4	22.8	29.6	20.0	22.2	23.7	20.5
Total conductivity(ℳc)	758	976	1267	992.1	NM	150	NM	NM
pH	8.08	7.45	7.63	8.38	7.32	10.3	7.29	8.24
Dissolved Oxygen(mg/l)	2.24	2.69	NM	NM	NM	NM	3.47	2.37

4.2 Occurrence of chemicals of emerging concern in wastewater treatment plants

4.2.1 Detection frequency of CECs in wastewater treatment plants

Among the 695 targeted CECs, 353 chemicals were identified in both influent and effluent samples. The predominant classes encompassed pharmaceuticals (102), pesticides (70), industrial chemicals (72), and biocides (27) (Figure 4). Additionally, stimulants, flame retardants, human and plant metabolites, sweeteners, and the recently incorporated class of CECs, namely ultraviolet radiation screening compounds widely employed in sunscreen products and characterized by high lipophilicity, were also detected (Vasilachi et al., 2021). Notably, Golovko et al.(2021a) reported a similar array of CEC classes, spanning pharmaceuticals, industrial chemicals, and pesticides.

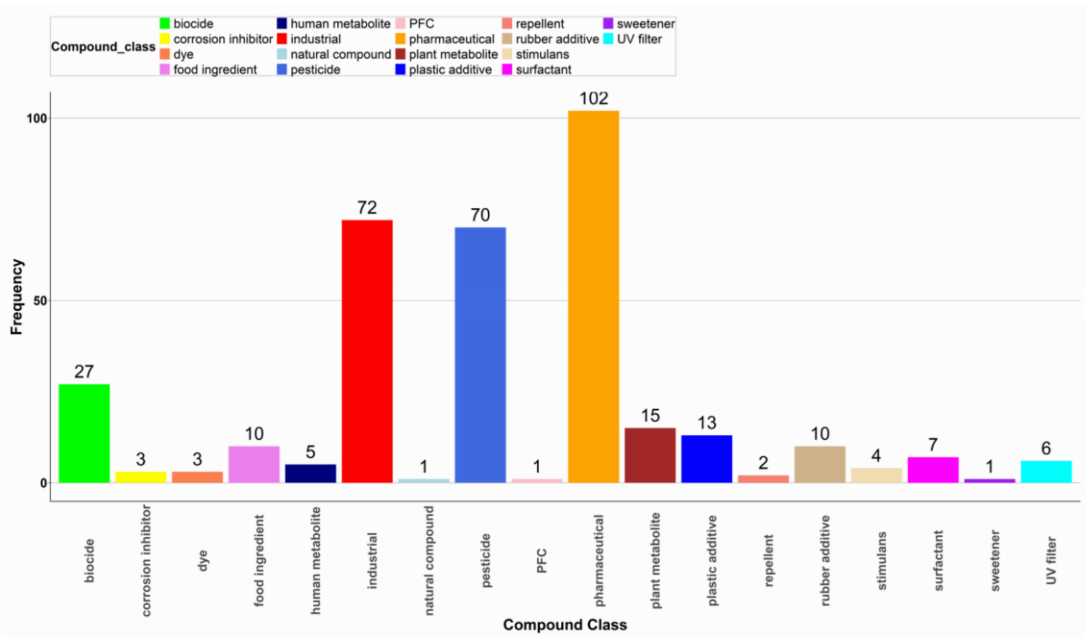

Figure 4: Frequency of the detected compounds per compound class

Regarding the detection frequency of the compounds, 176 compounds exhibited a detection frequency of ≥50%, while 33 of these compounds manifested a detection frequency of 100%. A total of 238 compounds had a detection frequency ≥50% in the influent (Figure 5). The compounds that were detected in half of the sampled sites included: caffeine, abacavir, diuron, TMDD, dichlorvos, diclofenac, carbendazim, 2-oxindole, diazinon, atrazine, amantadine, trimethoprim, imidacloprid-urea, triethylene glycol monobutyl, acetamiprid, and daidzein. Pharmaceuticals were the dominant class, with detection frequencies ranging from 12.5% to 7.7%. Among the pharmaceutical compounds, trimethoprim, amantadine, primidone,2-hydroxycarbamazepine, and bisoprolol registered a 100% detection frequency (Figure 7). The high detection of antibiotic trimethoprim can be attributed to its widespread use in managing bacterial infections and as prophylaxis in HIV-infected adults(Sibanda et al., 2011). Trimethoprim is commonly used in combination with sulfamethoxazole to treat various bacterial infections due to its effectiveness against a broad spectrum of bacteria (K'oreje et al., 2018). Its presence in wastewater influent can be a result of excretion by humans or animals, as well as improper disposal of unused medications, since approximately 60 to 80% of its administered dosage is excreted through urine (Yokoyama et al., 2022). Of the different categories of pharmaceuticals, non-steroidal analgesics/antiinflammatory drugs (NSAIDs) and antibiotics had the highest number of detections. This can be linked to the easy accessibility of over-the-counter NSAIDS, and most are unregulated. Additionally, Enoxolone had an influent detection frequency of 100%. Enoxolone is used in flavoring to mask the bitter taste of drugs, which is why it is used extensively. Primidone, an antiepileptic drug utilized to manage seizures, was observed to have 100% detection frequency in both the influents and effluents. This translates to the rising prevalence of seizure conditions in Kenya(Kariuki et al., 2018).

Among the antiviral drugs, amantadine and abacavir had the highest detection frequency of 100% and 62.5% respectively, while emtricitabine was detected in only one of the sites. The high detection of amantadine can be linked to its vast use in the treatment of viral illnesses such as influenza and hepatitis C. Additionally, it functions as a neurological medication for conditions such as Parkinson's disease and multiple sclerosis (Nannou et al., 2020b). Emtricitabine, used as pre-exposure prophylaxis(PrEP) for HIV prevention, had a low detection frequency due to its limited adoption in Kenya (Molina et al., 2022). Psychiatric drugs were among the class of pharmaceuticals that had the lowest detection frequency, with the highest being lamotrigine (62.5%) and flupentixol (12.5%). The limited detection of the psychiatric drug flupentixol may be attributed to the relatively low incidence of schizophrenia in sub-Saharan Africa, as well as the prescription of low daily doses (maximum 3mg daily), as noted by Mamah et al. (2021).

The UV filters detected included benzophenone-3, benzophenone-4, octabenzone, and phenyl benzimidazole sulfonic acid, with detection frequencies ranging from 12.5% to 75%. High detection of benzophenone-3 can be linked to its wide use as a sunscreen in lotions, conditioners, and cosmetics (Bogunovi´cbogunovi´c et al., 2021).

In the case of pesticides and biocides, these compound classes exhibited high detection frequencies, with most exceeding 80%. Carbendazim, metolachlor, diazinon, dichlorvos, terbuthylazine, diuron, and hexadecyl Pyridium had 100% detection frequency. The presence of pesticides and biocides in

WWTPs is mainly due to their use in households as cleaning agents, pest control, and insect sprays (Powell & Cuthbertson, 2013). The neonicotinoids acetamiprid, imidacloprid, and thiamethoxam exhibited a notably high detection frequency (>50%), likely due to their widespread utilization in Kenya as documented by PCPB (2018).

To the best of our knowledge, industrial chemicals detection in wastewater treatment plants is being reported for the first time in Kenya. 2,2-dihydroxybiphenyl, 2-hydroxyquinoline, quinoline Noxide, 2-oxindole, 4-(4-hydroxyphenyl)-butan-2-one, quinoline N-oxide, TMDD, and triethylene glycol monobutyl ether were detected in all the WWTPs' influents, while bisphenol A and B, isopropylaniline,4-nitrophenol, and pentachlorophenol are among the compounds that were only detected once. Triethylene glycol monobutyl ether is a common solvent utilized in paints, industrial cleaners, soaps, dyes, and disinfectants (Olson et al., 2022). These products are widely used in households and hospitals, resulting in a high detection frequency.

Besides parent compounds, metabolites and transformation products of CECs were also detected at different frequencies. As an example, carbamazepine is extensively metabolized in the liver, resulting in the formation of its metabolites 10,11-Dihydro-10-hydroxycarbamazepine, 10,11Dihydro-10,11-dihydroxycarbamazepine, and 2-hydroxycarbamazepine (Maculewicz et al., 2022), which were detected at 88, 88, and 100% detection frequency, respectively. Other transformation products that were detected included acetyl-sulfamethoxazole, a metabolite of sulfamethoxazole at a detection frequency of 75%, o-desemethylvenlafaxine (12.5%), a metabolite of antidepressant venlafaxine, and 2-hydroxydesethyltertbuthylazine (12.5%), a transformation product of terbuthylazine, the transformation product of imidacloprid, and imidacloprid urea was ubiquitous with a detection frequency of 88% (Figure 7) .

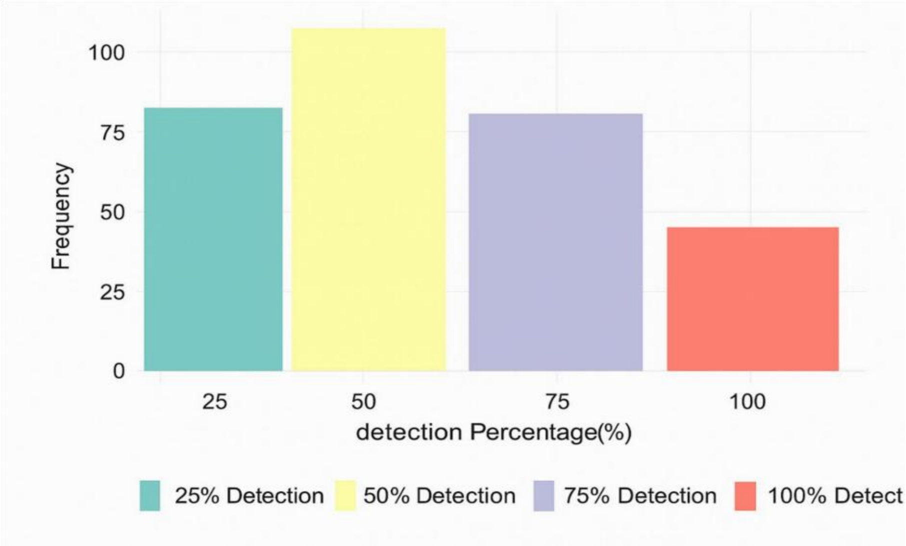

Figure 5: A frequency histogram of compounds in each detection frequency range.

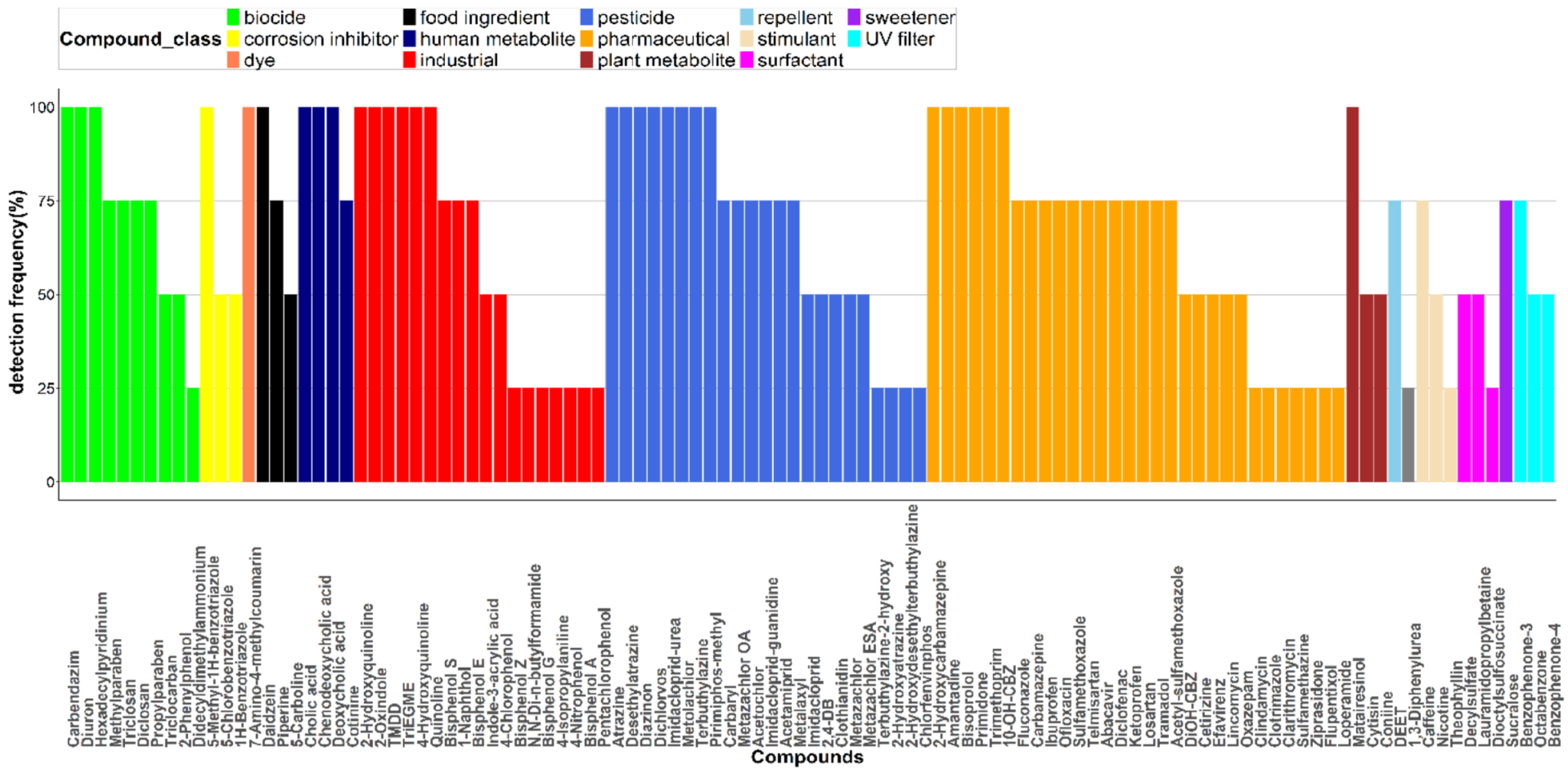

Figure 6: A graph of the detection frequency of CECs in the effluents of the selected wastewater treatment plants in western Kenya. (TMDD): 2,4,7,9-tetramethyl-5-decindiol (DiOH-CBZ): 10,11-dihydro-10,11-dihydroxycarbamazepine (TriEGME): Triethylene Glycol Monomethyl Ether

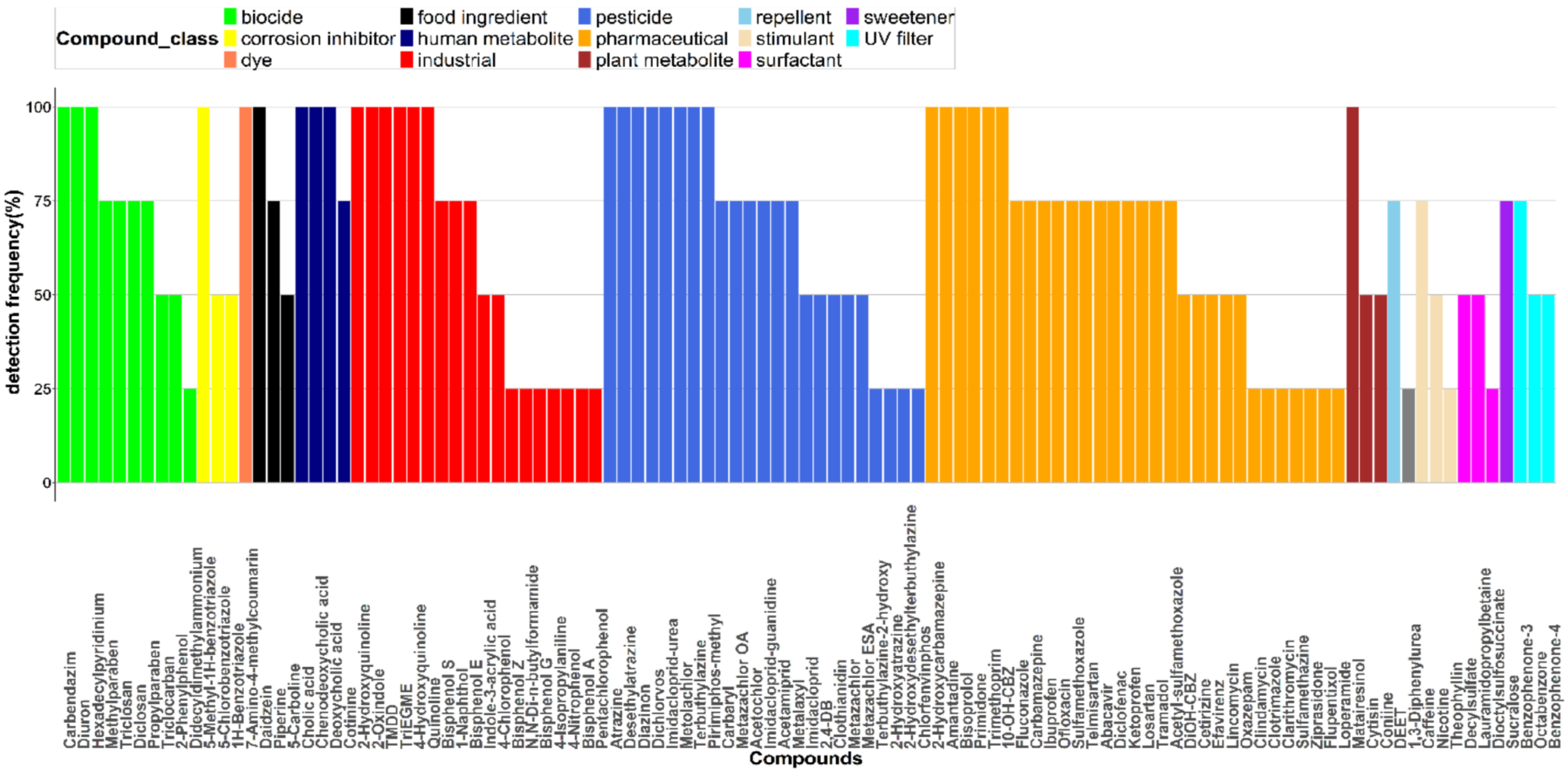

Figure 7: Detection frequencies of CECs in Wastewater treatment plants (WWTPs) influent. (TMDD): 2,4,7,9-tetramethyl-5-decindiol (DiOH-CBZ): 10,11-dihydro-10,11-dihydroxycarbamazepine (TriEGME): Triethylene Glycol Monomethyl Ether (TXIB):2,2,4trimethyl-1,3-pentanediol diisobutyrate.

4.2.2 Concentration of detected CECs in wastewater treatment plants

The total influent concentrations summated for all compounds in the different WWTPs amounted to 1.35 mg/L in NZOWASCO,735.6µg/L in ELDOWAS,864 µg/L in D.L. Koisagat, and 44.7 µg/L in Moi WWTP. Overall, stimulants had the highest influent concentrations (849 µg/L), followed by pharmaceuticals (817 µg/L), industrial chemicals (113 µg/L), and pesticides (20.3 µg/L).

For individual compounds, caffeine registered the highest maximum influent concentration of 830µg/L.

This was followed by deoxycholic acid (471µg/L), cholic acid(411µg/L), Lauramidopropylbetaine(95µg/L),2-oxindole(28µg/L), TMDD (11µg/L), ibuprofen (8.9µg/L), and dichlorvos(3.8µg/L). (Figure 10). The high concentration of caffeine in the influent can be associated with the intensive consumption of caffeinated beverages. Caffeine is the active ingredient in most soft drinks, energy drinks, and pharmaceutical products(Patil, 2012). In 2022, Kenya saw a domestic sale of 41 million kilograms of tea, a product that contains a high percentage of caffeine, proving its high prevalence (Egunjobi & Asatsa, 2022).

For pharmaceuticals, antibiotics, and analgesics/anti-inflammatory drugs (NSAIDs), the highest influent concentrations were observed, reaching up to 8.9µg/L. NSAIDs are easily accessible in overthe-counter and widely consumed pain relievers (K'oreje et al., 2018). Among the NSAIDs, ibuprofen was identified as the pharmaceutical with the highest maximum influent concentration(8.9µg/L), followed by diclofenac(2.6µg/L). This study exhibited alignment with findings from Golovko et al. (2021b), Ashfaq et al. (2017), and Gosset et al. (2021). The application of ibuprofen is associated with pain and inflammation(Cajaraville, 2021). Additionally, ibuprofen has been reported to be among the top ten most consumed drugs in Kenya (19.4%) by Nyabuti et al.(2020) and Waleng & Nomngongo (2022). Ibuprofen is usually taken at a high daily dose (1.2g daily dosage), with its consumption unregulated and easily accessible over the counter (Wydro et al., 2023). High influent concentration was also detected for the antibiotic trimethoprim, with concentrations ranging from 131 ng/L to 5.7µg/L, and fluconazole, an antibiotic drug used in the treatment of fungal infections, with a maximum concentration of 2.5µg/L. Trimethoprim is utilized widely in combination with sulfamethoxazole in the management of a broad spectrum of bacterial infections and opportunistic infections experienced by people living with HIV(Chadwick et al., 2020; Finckh et al., 2022a). Conversely, the concentration of sulfamethoxazole ranged from 37 ng/L to 2.3 µg/L. However, sulfamethoxazole is readily metabolized in the liver to acetyl-sulfamethoxazole, which has registered a higher concentration(45-2.4 ng/l) compared to the parent compound, which explains its low influent concentration compared to trimethoprim, despite being administered at a dosage ratio of 5:1(sulfamethoxazole: trimethoprim)(Thiebault, 2020). At 5.7µg/L, cetirizine, an antihistamine drug used to treat allergic responses, had a high influent concentration. Psychiatric drug carbamazepine, a drug utilized in the management of epilepsy and bipolar disorder, was detected at a concentration between 1ng/L and 311ng/L/L. This can be linked to the low dosage of psychiatric drug prescribed daily (0.1g daily dosage), as well as extensive metabolism of carbamazepine to its primary metabolites, 10,11-dihydro10,11-dihydrocarbamazepine,10,11-dihydro-10-hydrocarbamazepine, and 2-hydroxycarbamazepine, at concentrations (283ng/L-2.2µg/L), (1ng/L-167ng/L), and (1ng/L-8ng/L), respectively. Among the anti-viral drugs, abacavir and emtricitabine were measured at the highest influent concentrations of 511 ng/L and 80 ng/L, respectively.

Human metabolites were among the CECs that had the highest influent concentration. The leading compounds included deoxycholic acid (471µg/L), cholic acid(411µg/L), chenodeoxycholic acid(126µg/L), and 7-oxolithocholic acid(65µg/L). The high influent concentration is a bioindicator for domestic wastewater in WWTPs. Deoxycholic acid (a derivative of cholic acid) is usually employed in the treatment of bile acid synthesis disorders arising from single enzyme defects, as well as an adjunctive treatment for peroxisomal disorders characterized by impaired liver function (Yang et al., 2020).

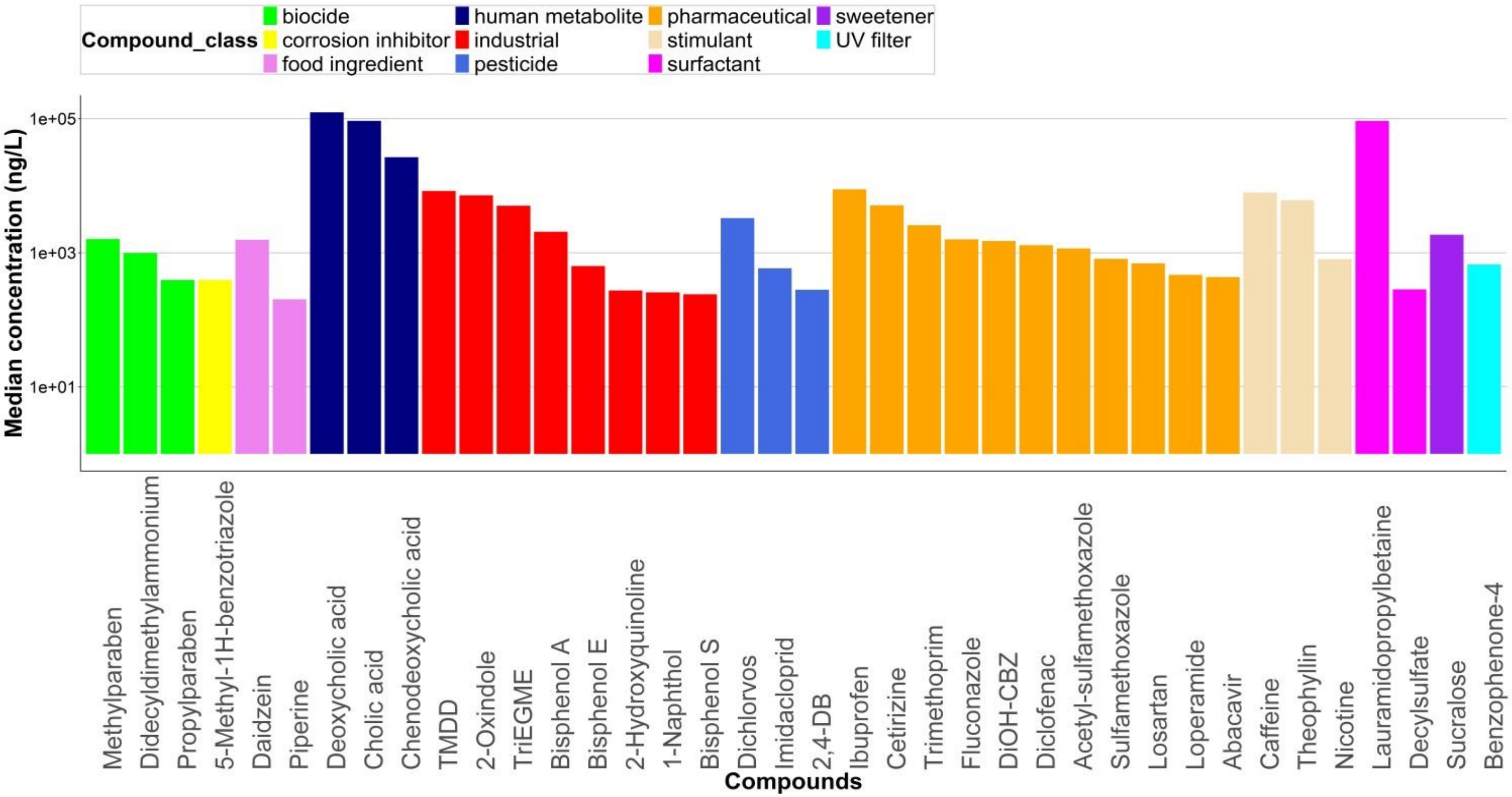

Figure 8: Median concentrations > 200 ng/L of CECs in influent WWTPs samples. TMDD: (2,4,7,9-tetramethyl-5-decindiol); 2,4-DB: (4-(2,4-dichlorophenoxy) butyric acid); (DiOHCBZ):10,11-dihydro-10,11-dihydroxycarbamazepine; (TriEGME): Triethylene Glycol Monomethyl Ether.

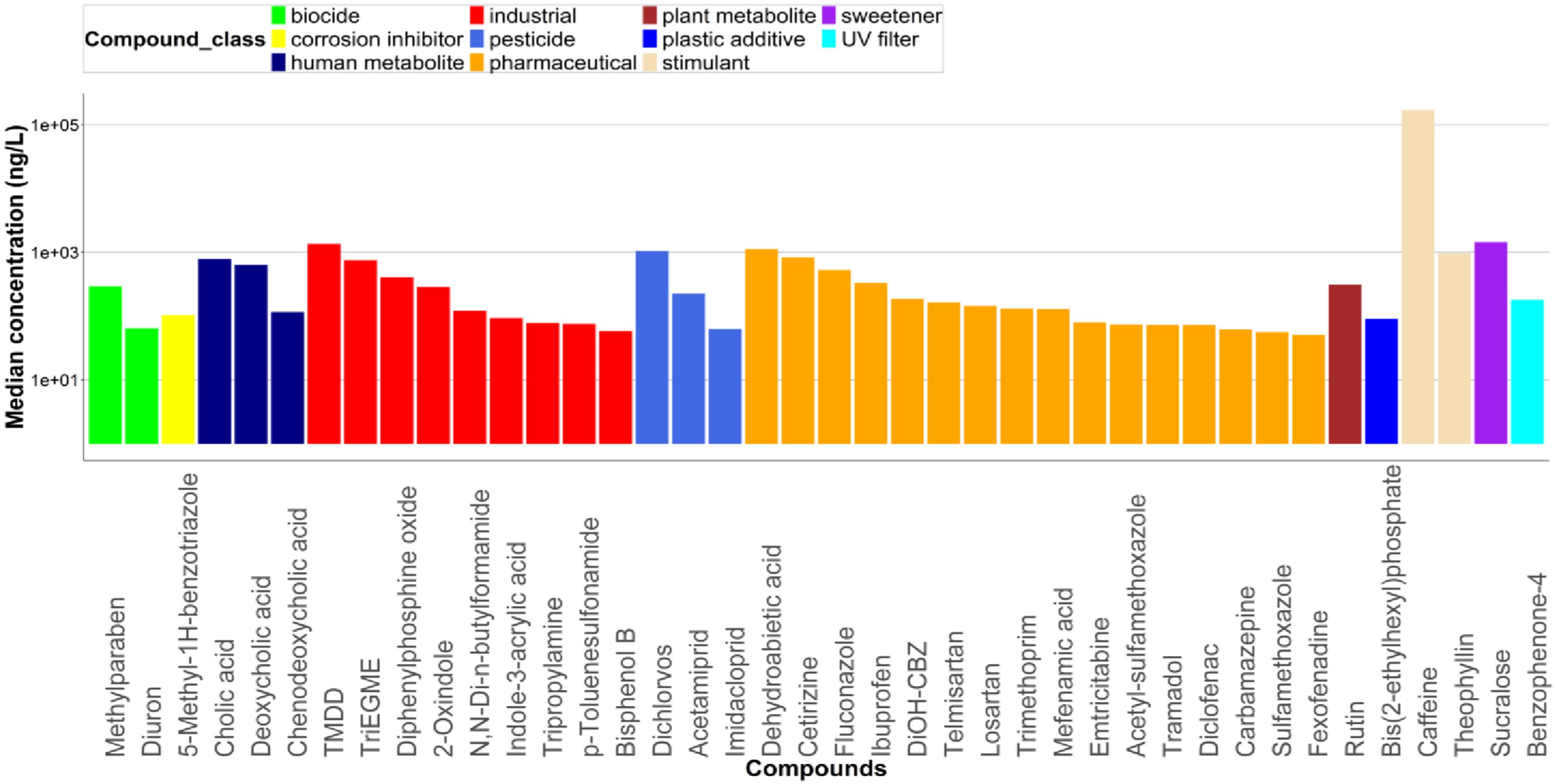

Figure 9: Median concentrations > 200 ng/L of CECs in WWTPs effluent samples. TMDD: (2,4,7,9-tetramethyl-5-decindiol); 2,4-DB: (4-(2,4-dichlorophenoxy) butyric acid); (DiOHCBZ):10,11-dihydro-10,11-dihydroxycarbamazepine; (TriEGME): Triethylene Glycol Monomethyl Ether.

A variation in concentration was measured for pesticides and biocides, with dichlorvos registering the highest influent concentration of 5.6µg/L for pesticides, while methylparaben, a biocide, was measured at 2.1µg/L. Dichlorvos is an organophosphorus pesticide widely used for household pest control(Rao et al., 2017). The detection of dichlorvos in this study surpassed previous reports, emphasizing its significant contribution to the concentration of CECs in wastewater in Kenya. Dichlorvos is still widely used in households to control pests in Kenya, despite its ban by the EPA due to carcinogenicity and liver damage potential, contributing to its presence in wastewater (APVMA, 2008). Other compounds that had a high concentration included didecyldimethylammonium(948.6ng/L), imidacloprid (787.4 ng/L), carbaryl (686.9 ng/L), propylparaben (584 ng/L), and 2,4-DB (518ng/L). Parabens, including methylparaben and propylparaben, are known synthetic preservatives utilized in cosmetics, personal care products, and pharmaceuticals(Nowak et al., 2021). Imidacloprid, a neonicotinoid pesticide, has been banned by the European Commission due to its harmful effects on honey bees and wild pollinators (Schmidt et al., 2022). The findings regarding the presence of pesticides and biocides in this investigation largely align with earlier studies, specifically Carazo-Rojas et al.(2018) and Loos et al.(2013), which documented the presence of diuron, propanil, tertbutryn, and carbendazim, which were likewise identified in our research, albeit at reduced concentration levels. Contrarily, the study conducted by Kandie et al. (2020c) noted higher concentrations of carbendazim, while Loos et al. (2013) observed elevated levels of atrazine compared to our study outcomes.

Similarly, industrial chemicals were measured, with the highest maximum concentration being registered for 2-oxindole (28.3 µg/L). This was followed by TMDD (10.7µg/L), triethylene glycol monobutyl ether(5.5µg/L), bisphenol

A(2.1µg/L), bisphenol S(1.4µg/l), and 2-hydroxyquinoline(1.3µg/L). Overall, industrial chemicals had the predominant number of compounds, which had a concentration greater than 1.0µg/L. Bisphenol A is widely produced and used among the bisphenol analogs, with its production estimated at more than 5 million per year. It is mostly used as a raw material in the production of polycarbonate plastics, epoxy resins, paper coatings, and powder paints, and thus it is ubiquitous in wastewater(Lee et al., 2015). 2-hydroxyquinoline is a transformation product of quinoline, which is mostly used in the manufacture of dyes. TMDD, a non-ionic surfactant, serves as an adjuvant in various industries, including paint manufacturing, printing inks, adhesives, paper production, pesticides, coatings, defoamers, dispersants, and polymerization reactions(Roegner et al., 2023). Its high water solubility, chemical stability, and wide continuous use make it highly mobile and persistent in wastewater influents(García-Galán et al., 2021).

In the effluents, substances with the highest concentrations were caffeine (170 µg/L), sucralose(3.1µg/L), cetirizine(2.3µg/L), TMDD(1.6µg/L), fluconazole(1.2µg/L), triethylene glycol monobutyl ether (1.2µg/L), and dichlorvos(1.1µg/L). Further identified compounds with effluent concentration ≥200ng/L included 2-oxindole(industrial), theophylline, ibuprofen (NSAID), 10,11dihydro-10,11-dihydroxycarbamazepine, acetamiprid, diuron, carbamazepine, telmisartan, methylparaben, losartan, and dipenylphosphine oxide (Figure 11). TMDD demonstrated a notable presence in effluent samples, likely attributed to its high-water solubility (1.7 g/L at 20 °C) as reported by Schwanen & Schwarzbauer (2022), coupled with a low octanol/water partition coefficient (Log Kow

2.8), which limits its adsorption onto particulate matter according to Guedez & Püttmann (2011b). According to earlier studies, TMDD persists throughout wastewater treatment procedures and is partially released into the environment at quantities between <MDL and 5.8 µg/L, as reported by Blum et al. (2017)and Guedez & Püttmann. (2011b). Sucralose's high water solubility and chemical and biological stability have been linked to its enhanced concentration in effluent, as demonstrated by Dietrich et al.(2021). The high concentration of fluconazole up to 1239ng/L registered in effluents can be associated with the slow microbial and photo transformation of azole fungicides in WWTP treatment processes.

Among the metabolites, a high concentration of 10,11-dihydro-10,11-dihydroxycarbamazepine, a metabolite of carbamazepine used in the treatment of epilepsy and bipolar disorder, was detected at 715 ng/L, alongside the metabolite of sulfamethoxazole (acetylsulfamethoxazole, 74 ng/L), typically excreted in urine, as reported by Göbel et al. (2004). The parent compound, sulfamethoxazole, had an average effluent concentration of 156ng/L, which can be related to the enzymatic deacetylation of acetyl-sulfamethoxazole to its parent compound. Considering the conjugates revert to the parent compounds throughout the wastewater treatment process, the concentration of carbamazepine in the effluent was greater than that of its metabolites, 10,11-dihydro10,11-dihydroxy carbamazepine, and 10,11-dihydro-10-hydroxycarbamazepine. Fipronil was among the biocides that registered the lowest effluent concentration of 2ng/L. The low concentration of fipronil can be linked to its easy oxidation, hydrolysis, and photolysis to form fipronil sulfide and fipronil desulfinyl(Qu et al., 2016).

Corrosion inhibitors 5-chlorobenzotriazole and 5-methylbenzotriazole had a substantial effluent concentration of 113 ng/L and 107 ng/L, respectively. Due to their poor sorption propensity and fair water solubility, benzotriazole compounds are only partly removed in WWTPs, leaving significant percentages in the effluents(Callahan, 2010).

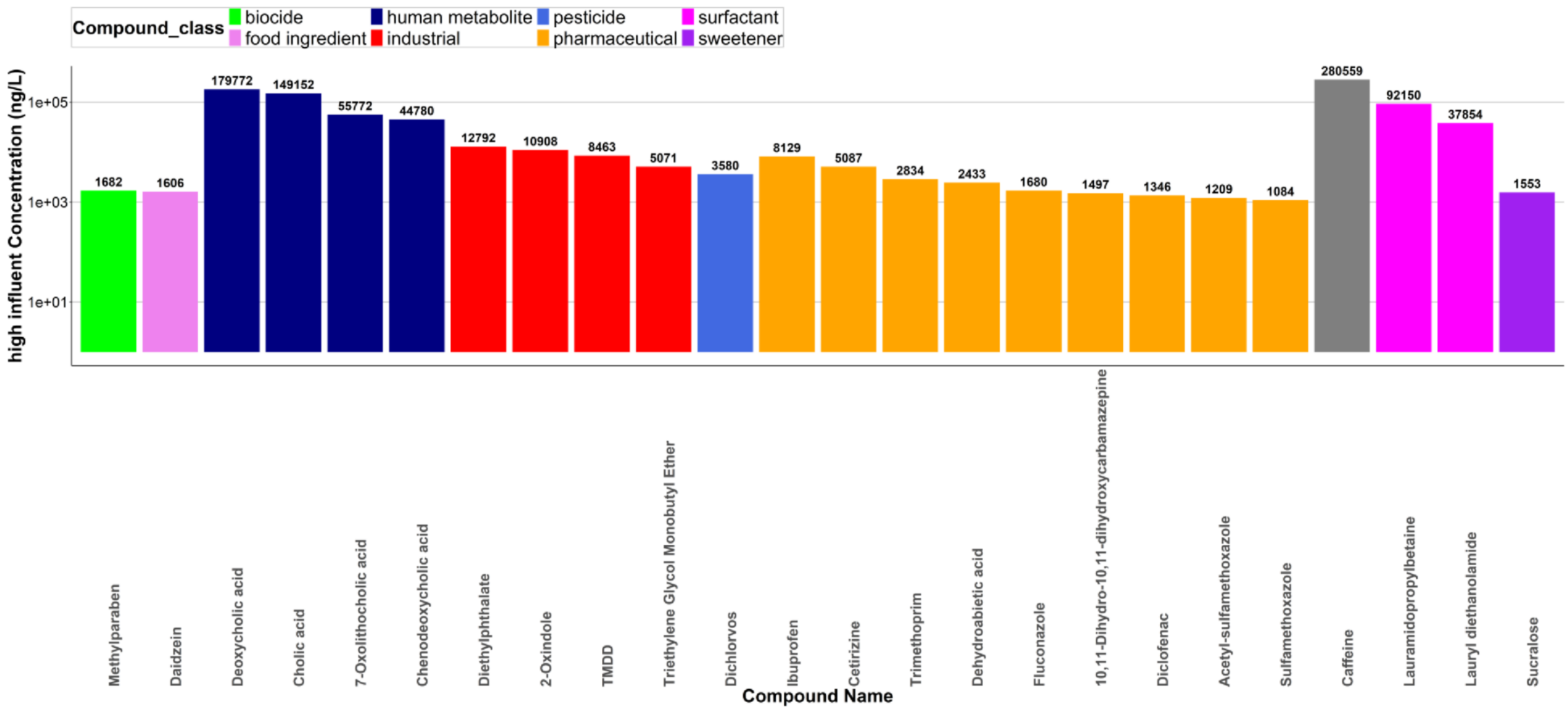

Figure 10: Highest influent concentrations of CECs in WWTPs samples. TMDD: (2,4,7,9-tetramethyl-5-decindiol)

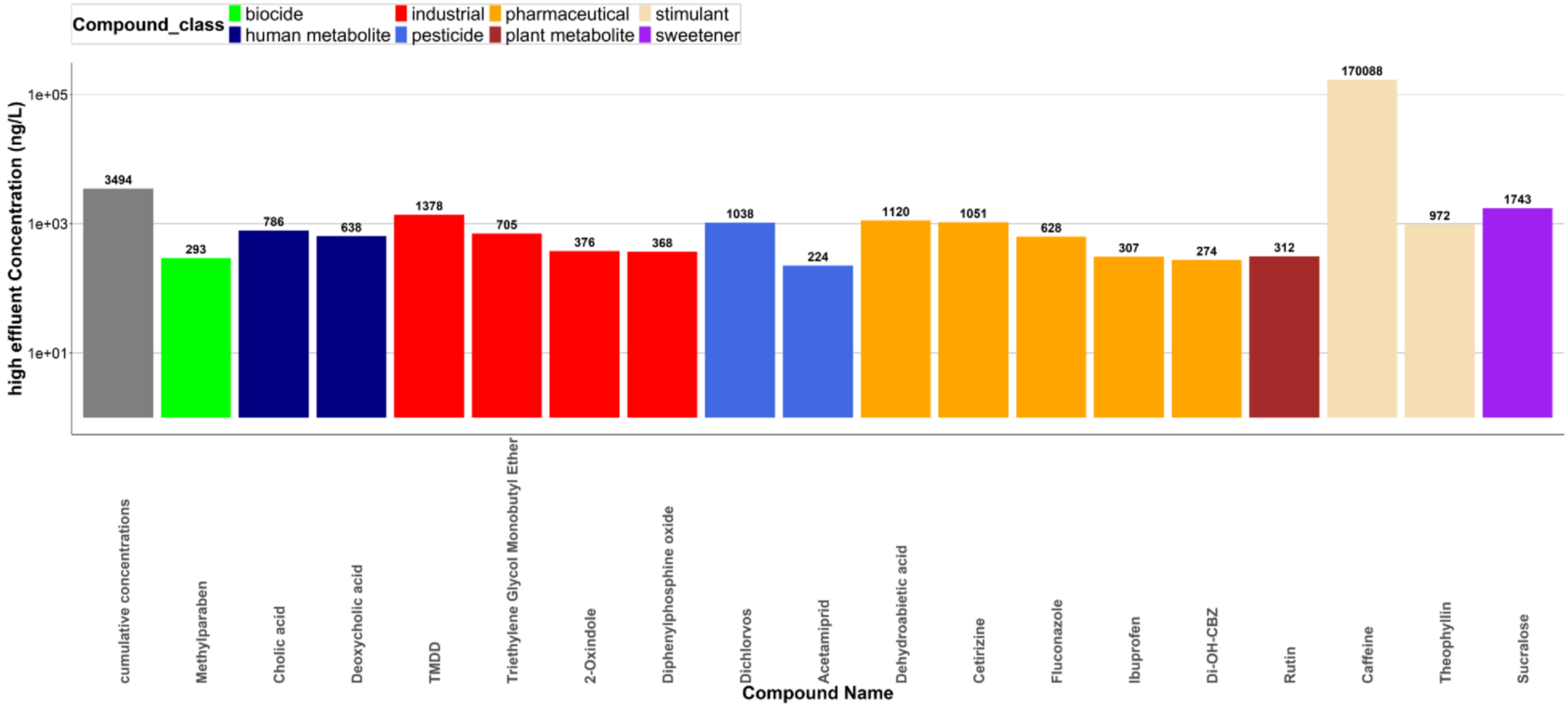

Figure 11: Highest effluent concentrations of CECs in WWTPs samples. TMDD: (2,4,7,9-tetramethyl-5-decindiol)

4.3 Removal efficiencies of chemicals of emerging concerns in wastewater treatment plants

Based on the concentrations of each chemical in the influent and effluent, a comparison study was conducted to assess the removal effectiveness of each detected compound in the selected wastewater treatment plants (WWTPs). The study revealed variability in removal efficiency percentages among the 353 identified compounds, spanning from -2056% to >99%. Although most compounds had removal efficiency of more than 50%, the precise proportion varied throughout WWTPs, probably due to variations in plant architecture, technologies used, and treatment methods. Based on the removal efficiency for each compound and WWTP, a categorization was undertaken, classifying compounds into two groups: those with positive removal and those with negative removal. Overall, 286 compounds had an average positive removal, while 67 compounds registered a negative removal efficiency. Among the 286 compounds that had positive removal, 121 of the compounds had an average removal efficiency of >50%. These compounds included 4-hydroxyquinoline, mateiresinol, quinoline, scopoletin, 2-hydroxyquinoline, trimethoprim, indole-3-carboxyaldehyde, triethylene glycol monobutyl ether, caffeine, diclosan, acetyl-sulfamethoxazole, ibuprofen, and diclofenac **(figure 12)**. The removal efficiency of most of these compounds is highly determined by the flow rate of concentration entering the WWTPs, chemical structure, solubility, physicochemical properties such as pKa, charge, and Log K_{ow}, and their biodegradative capabilities. Pharmaceuticals with higher K_{ow} values, such as diclofenac, have much lower pKa values than the pH of wastewater and are highly dissociated, resulting in their good removal (90-100%). Diclofenac exhibited a removal efficiency ranging from 90% in ELDOWAS to 100% in NZOWASCO. These results align with the 94% removal efficiency observed in WSPs by Khasawneh & Palaniandy

(2021), due to enhanced biodegradation in facultative ponds with better aeration and advanced microbial communities. Additionally, trickling filters showed a removal efficiency of 74-85%, which is consistent with the findings of this study. Diclofenac also undergoes rapid photodegradation when exposed to sunlight. Overall, quinoline and its products were well removed across all the WWTPs. Quinoline undergoes aerobic biodegradation, photolysis, and adsorption during treatment processes. During aerobic biodegradation, quinoline acts as the sole carbon and nitrogen source for microbial metabolism, through hydroxylation and oxidation, resulting in the formation of metabolites such as 4-hydroxyquinoline,2-hydroxyquinoline, and quinoline-N-oxides. Additionally, the adsorption of quinoline from the liquid phase to the solid phase accelerates the removal of quinoline from the wastewater. Subsequently, the metabolites of quinoline are readily biodegradable and undergo photolysis. Additionally, activated sludge is usually utilized as an electron donor, which accelerates biodegradation. Caffeine displayed an average removal efficiency of 70%, within the reported range of 70-100% (Golovko et al., 2021a). Caffeine is usually removed through microbial biodegradation, resulting in the formation of the primary metabolite theophylline. Caffeine has been demonstrated to be readily biodegradable with an average removal efficiency of between 88 % and 100%, similar to that observed in this study. Despite its reasonable removal rate, the detected concentration was higher than 1µg/L in treated effluent. This high rate of caffeine removal may therefore be associated with stimulants' propensity to biodegradation and sorption. This finding is corroborated by the study's excellent removal rate of nicotine (100%) (Z. Li et al., 2016).

Among the pharmaceuticals, ibuprofen showed a good removal efficiency ranging from 96-100%. Ibuprofen has been reported to have a good removal efficiency achieved through biodegradation by heterotrophic non-ammonia-oxidizing microorganisms(Roh et al., 2009). Sulfamethoxazole demonstrated a higher removal efficiency in wastewater stabilization ponds (WSPs) at 98%, compared to 81% in ELDOWAS, consistent with Nas et al. (2021), who reported over 72% removal in WSPs.

This efficiency is linked to the high solid retention time (SRT) and hydraulic retention time (HRT) in WSPs, which allow sufficient time for biological degradation and settling of suspended solids (BEGEDE, 2023). Acetyl-sulfamethoxazole gathered a removal efficiency of >94% across all the WWTPs. The good removal can be linked to it reverting to the parent compound after enzymatic cleavage. Ofloxacin, a quinolone drug, had an average removal of 91% and 81% in NZOWASCO and ELDOWAS, respectively. Quinolones have been reported to adsorb efficiently into sewage sludge, supported by their higher sorption constant by Zou et al. (2022), thus explaining their higher removal. The migration and transformation of quinolone antibiotics are usually a result of the synergistic effects of sludge adsorption, biodegradation, and photolysis. Triclosan and the paraben compound family (butylparaben, ethylparaben, methylparaben, and propylparaben) exhibited good average removal efficiencies (>50%) across all WWTPs (Krzeminski et al., 2019b). Azole pesticides have been reported to have stronger adsorption, and higher concentrations have been detected in sludge (Iancu et al., 2024). This explains the good removal of tebuconazole (55%). Triclosan exhibited a removal efficiency ranging from 67% to 100% across all treatment plants (Figure 12). Its high removal can be attributed to biosorption and biodegradation processes, facilitated by its high adsorption capacity and octanolwater partitioning coefficient (Kow), which promotes its adsorption onto sludge and sediments. Ahmed et al. (2017a) reported over 80% removal efficiency of triclosan in wastewater stabilization ponds and lagoons, which utilize biosorption and biodegradation processes. Among the compounds that registered positive removals, some compounds were removed but not entirely, with most gathering an average removal efficiency ranging from 2 – 40 %. These compounds included: telmisartan (4%), carbendazim (13%), naproxen (8%), DEET (24%), and fluconazole (41%). Naproxen is usually transformed under co-metabolism conditions with glucose naproxen phenol monooxygenase, and naphthalene dioxygenase through hydroxylation(Peng et al., 2024). However, naproxen has low biodegradation compared to other NSAIDs and is thus partially removed during treatment. Carbendazim's low removal efficiency of 13% found in this study can be explained by its photodegradation stability and its low sorption coefficient (Kd<500 $LKgSS^{-1}$). According to studies, biological degradation methods such as activated sludge have not been able to remove carbendazim from the liquid phase (Kocaman, 2019).

Subsequently, carbendazim degrades slowly by direct photolysis.

Sucralose was reluctantly removed across the WWTPs, with an average removal efficiency of 3%. Sucralose is highly water soluble; thus, it adsorbs less to sludge. Additionally, due to its high chemical and biological stability, sucralose is poorly degraded by microorganisms as well as through photodegradation(Sang et al., 2014). Ozonation, an advanced treatment process that has been utilized in the elimination of persistent organic matter, has been reported to potentially abate only about 31% of sucralose, confirming its chemical and biological stability(Y. Yang et al., 2021). DEET showed a removal efficiency of 30% in ELDOWAS and 20% in NZOWASCO. The presence of trickling filters in ELDOWAS enhanced its removal through the formation of biofilms by aerobic bacteria, which promote degradation and biotransformation (Ren et al., 2021).

Despite a good percentage of the compounds registering a positive removal efficiency, 67 compounds had a negative removal efficiency. These compounds include; acetamiprid (-6%), clotrimazole (-7%), 2-hydroxyatrazine (-8%), terbuthylazine (-8%), tertbutylazine-2-hydroxy (-25%), diuron(-44%), desisopylatrazine (-50%), imidacloprid-urea (-54%), Metolachlor OA (-61%), imidaclopridguanidine(-91%), atrazine (-75%), amantadine (-78%), carbamazepine (-162%), gabapentin-lactam (173%), primidone(-156%), bisphenol Z(-969%), and desethylatrazine (-166%). Carbamazepine (162%), amantadine (-78%), and primidone (-156%) had the highest negative removal among their respective pharmaceutical classes (Figure 12), consistent with previous studies (Golovko et al., 2021a). It has been shown that carbamazepine has a low or negative removal efficiency (-76% to 23%) and is challenging to remove using activated sludge, lagoons, and artificial wetlands (Wagner et al., 2023).

This is due to its resistance to biodegradation, low distribution coefficient between water and sludge (Kd = 1.2 L/KgSS), which is lower than the required 500 L/KgSS for efficient sorption into sludge (Y. Zhang et al., 2008). Interestingly, 10,11-dihydro-10,11-dihydroxycarbamazepine and 10,11-dihydro10-hydroxycarbamazepine, two of its metabolites, are biologically deconjugated, which contributes to the rise in its concentration (He et al., 2019), resulting in a better average removal of 66% and 88% respectively.

For biocides, diuron exhibited poor removal (-145% to -1.1%) across all WWTPs, consistent with global variation in removal efficiencies reported in previous studies(Ferreiro et al., 2021). Additionally, the increase in effluent concentration of propiconazole is due to desorption from the sludge. Atrazine, a widely used pesticide, has been reported to have low but positive removal efficiency ranging from 0 to 45%

(Campo et al., 2013). However, in the present study, atrazine and its metabolites exhibited average negative removal efficiencies, which included atrazine (-75%), desethylatrazine (-166%),2hydroxyatrazine (-8%), and desisopropylatrazine (-50%) (Figure 12). In the case of industrial chemicals, Diphenyl phosphine oxide (-253%), N, N-di-n-butyl formamide (-323%), 4-cumylphenol (50%), bisphenol Z (-969%), and 4-(4-hydroxyphenyl)-butan-2-one (-80%) had the poorest removal efficiencies.

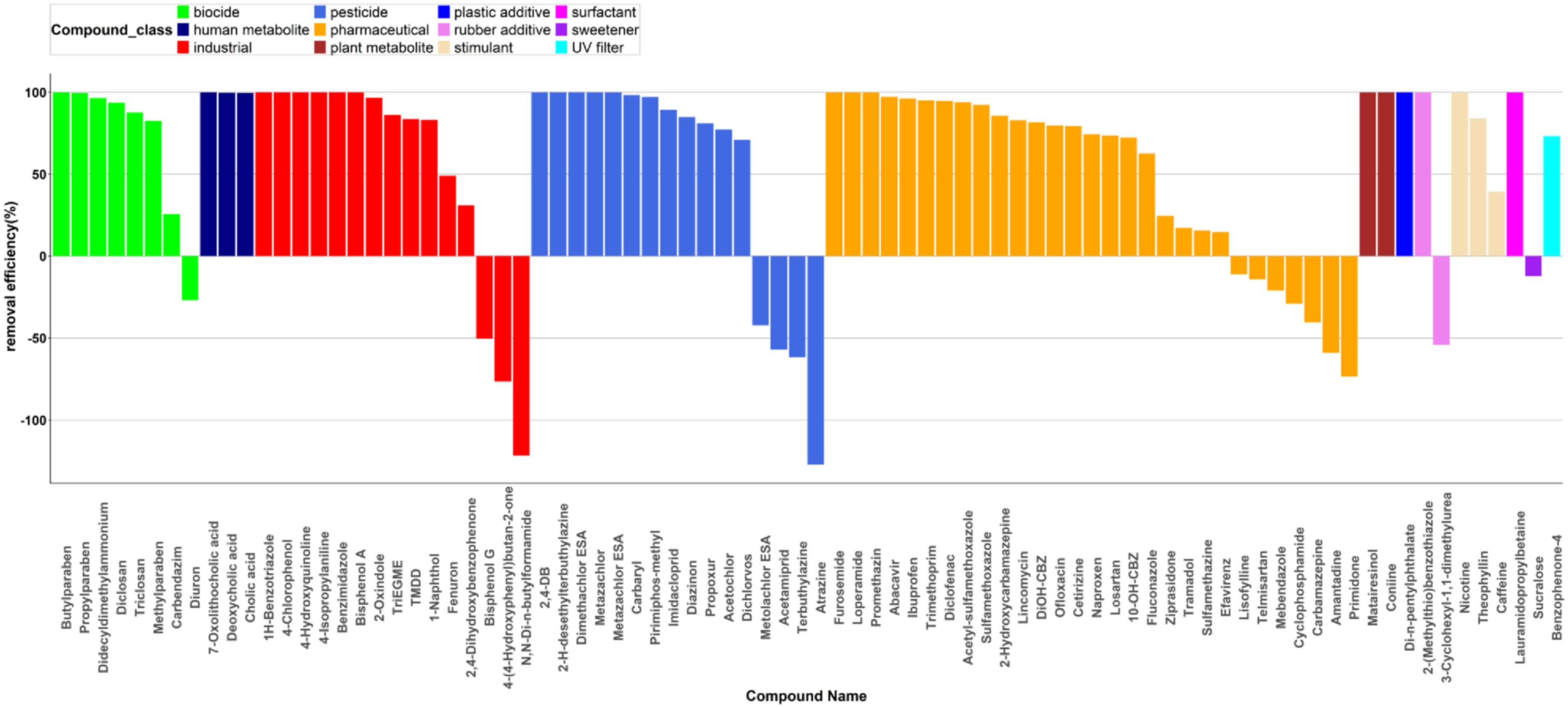

Figure 12: Removal efficiencies of CECs in selected WWTPs

As demonstrated in this study, the removal efficiencies of distinct CECs are determined mainly by their physicochemical characteristics and the treatment strategies used by the various WWTPs(Rout et al., 2021b). Generally, the best methods for removing chemicals of emerging concern (CECs) are based on biological processes, such as trickling filters, activated sludge, and WSPs (Dhangar & Kumar, 2020). However, these methods have some disadvantages, including the adaptation of microorganisms to the environment and the requirement for long hydraulic retention times to achieve effective biological degradation.

NZOWASCO recorded the most chemicals (131) with elimination >99% on individual WWTPs, followed by ELDOWAS (110) and Moi WWTP (60). D.L. Koisagat had the fewest compounds (9). Overall, each WWTP demonstrated a percentage decrease in the cumulative concentration of Contaminants of Emerging Concern (ΣCECs) exceeding 85%, with NZOWASCO exhibiting the most substantial concentration reduction at 98.75%. The variations in removal efficiency and concentration reductions highlight the importance of unique treatment methods and operational factors on the efficacy of individual WWTPs in minimizing the presence of emerging pollutants. (Richardson & Ternes, 2021)(figure 7). NZOWASCO employs wastewater stabilization ponds (WSPs) and lagoons, which use biological processes in the removal of CECs. These biological processes have been reported to be more effective in the removal of CECs compared to physical and chemical treatment techniques (Cecconet et al., 2017). In WSPs, biodegradation is the most efficient method. Because facultative ponds include both aerobic and anaerobic bacteria, there is improved aeration, and biodegradation occurs (Kadri et al., 2021). Modernized primary, secondary, and maturation ponds, anaerobic procedures, physical and mechanical techniques, activated sludge, trickling filters, and other treatment processes are responsible for the effective removal of CECs shown in ELDOWAS. Additionally, ELDOWAS had a higher solid retention time (SRT) and a hydraulic retention time (HRT) of 21 days, which contributed to a positive removal of CECs(Dubey et al., 2022). Trickling filters employed in ELDOWAS have been reported to have a better removal of CECs, such as ibuprofen (70-80%) and diclofenac (74-85%), which was also displayed in ELDOWAS WWTP (Ahmed et al., 2017b). Both ELDOWAS and NZOWASCO had maturation ponds, which contributed greatly to the removal of most of the CECS. Direct photodegradation, a mechanism utilized in the removal of CECs, has been reported to occur highly in maturation ponds, with almost 20% of CECs, such as sulfamethoxazole (48%) and trimethoprim (18%), removal accounted for by photodegradation (Otieno et al., 2020). Several key operational factors can impact the biological elimination of CECs residues during wastewater treatment. The presence and size of anoxic and anaerobic zones, suspended solids (SS) levels, hydraulic retention time (HRT), sludge retention time (SRT), food/microorganism ratio (F/M ratio), mixed liquor suspended solids (MLSS), pH levels, temperature of the influent sewage, and the design of the treatment plant are some of these factors (Zambello, 2016). Generally, there was poor removal of antibiotics in D.L. Koisagat WWTP, with compounds such as climbazole, sulfadimethoxine, and ofloxacin registering negative removal efficiencies. This is a result of the fact that the effectiveness of antibiotic removal depends on both the treatment procedures being used and the physicochemical and structural properties of the antibiotics. The three primary processes that remove antibiotics are hydrolysis, biodegradation, and adsorption (Q. Yang et al., 2021). However, D.L.Koisagat WWTP lacks these processes, instead employing primary and volatilization techniques that are ineffective in removing antibiotics due to their non-volatile characteristics (Hazra et al., 2022).

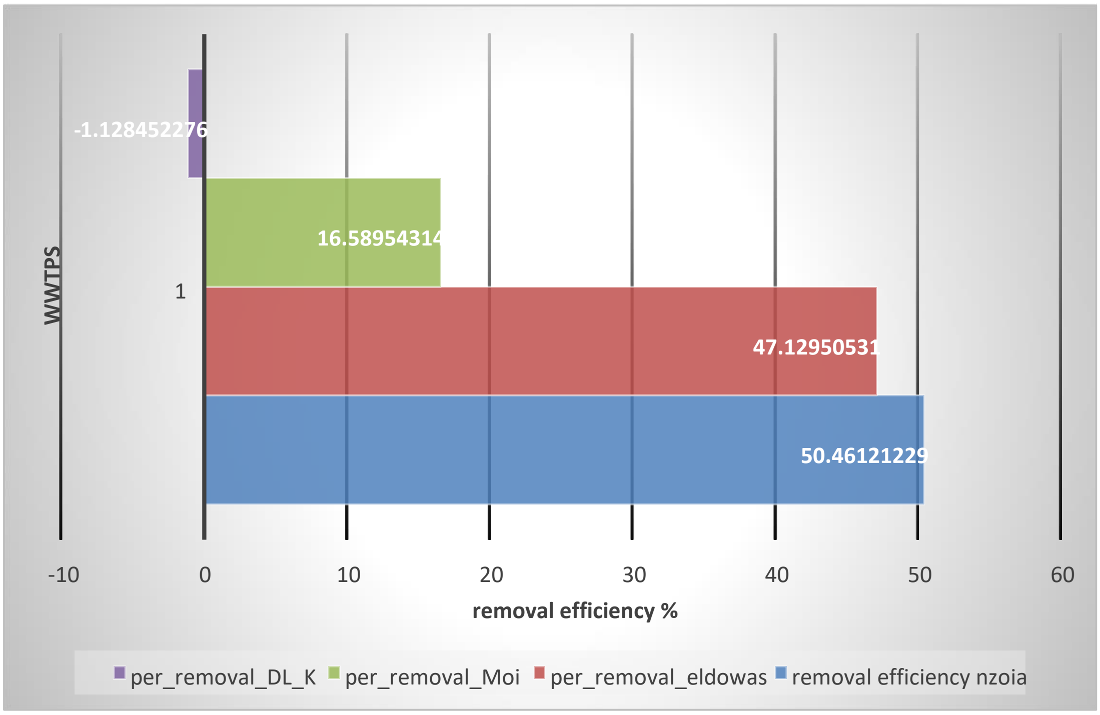

Figure 13. Average removal efficiency of the selected WWTPs.

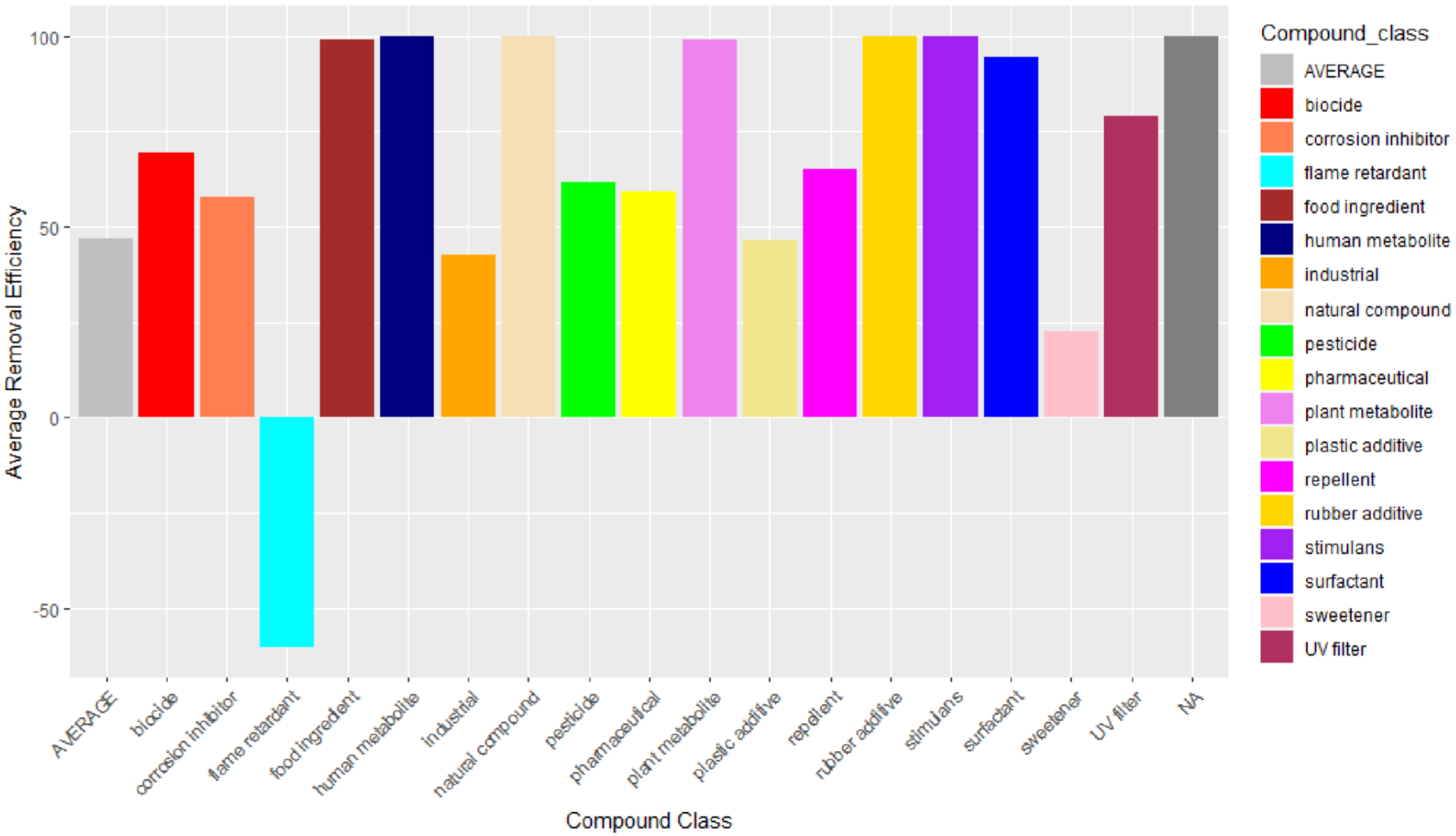

Figure 14.Average removal efficiency in ELDOWAS by compound class

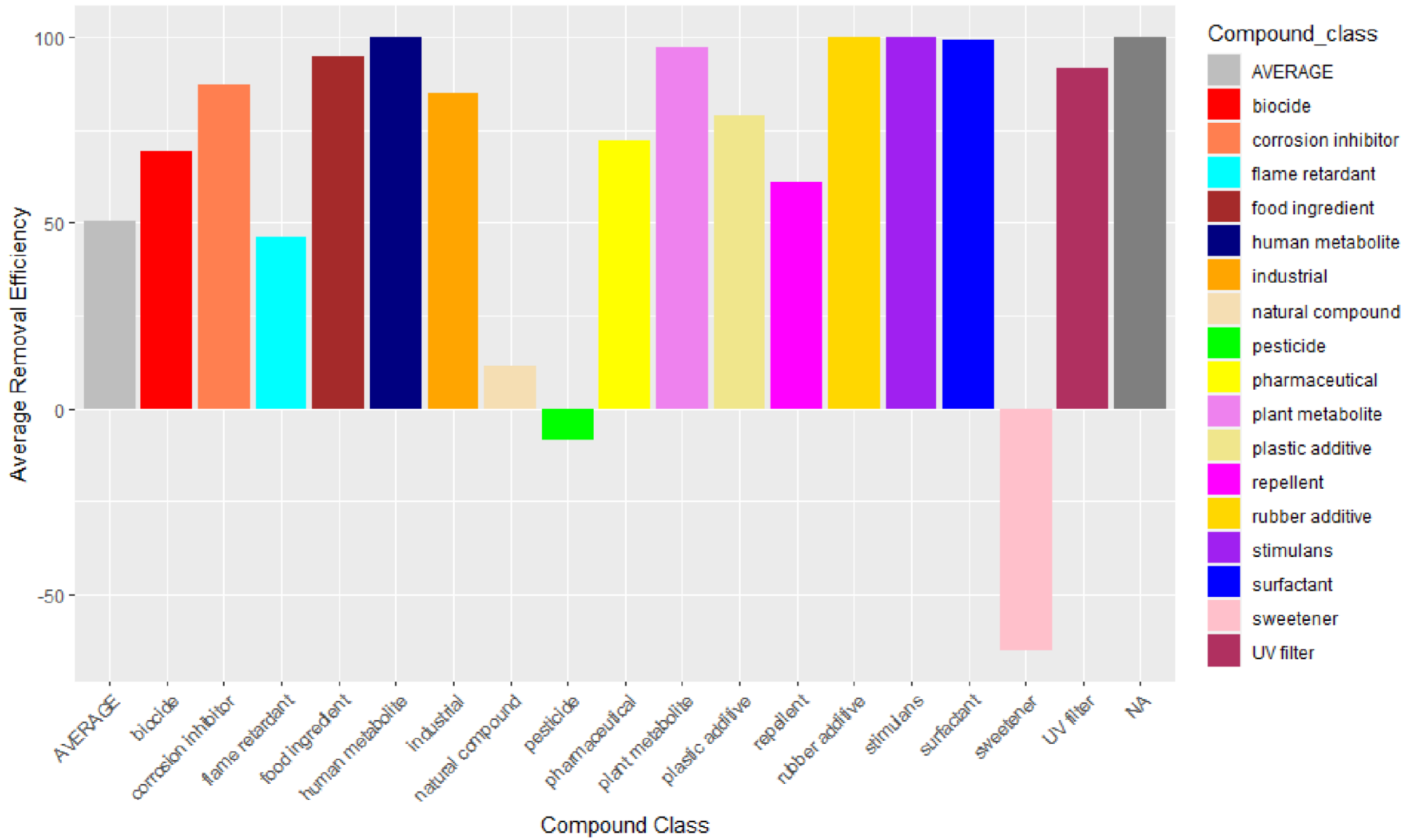

Figure 15: Average removal efficiency in NZOWASCO by compound class

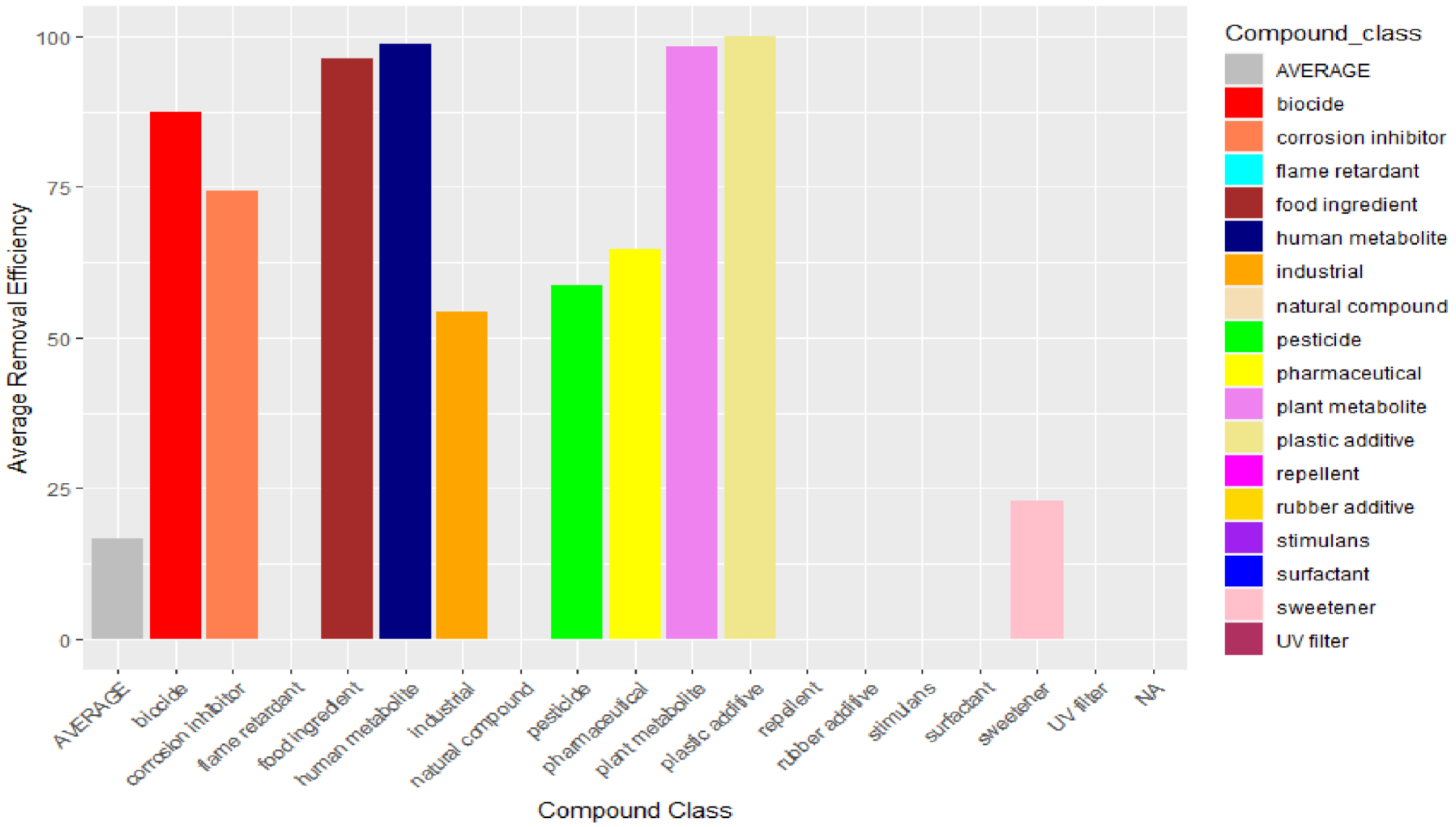

Figure 16: Average removal efficiency in Moi by compound class

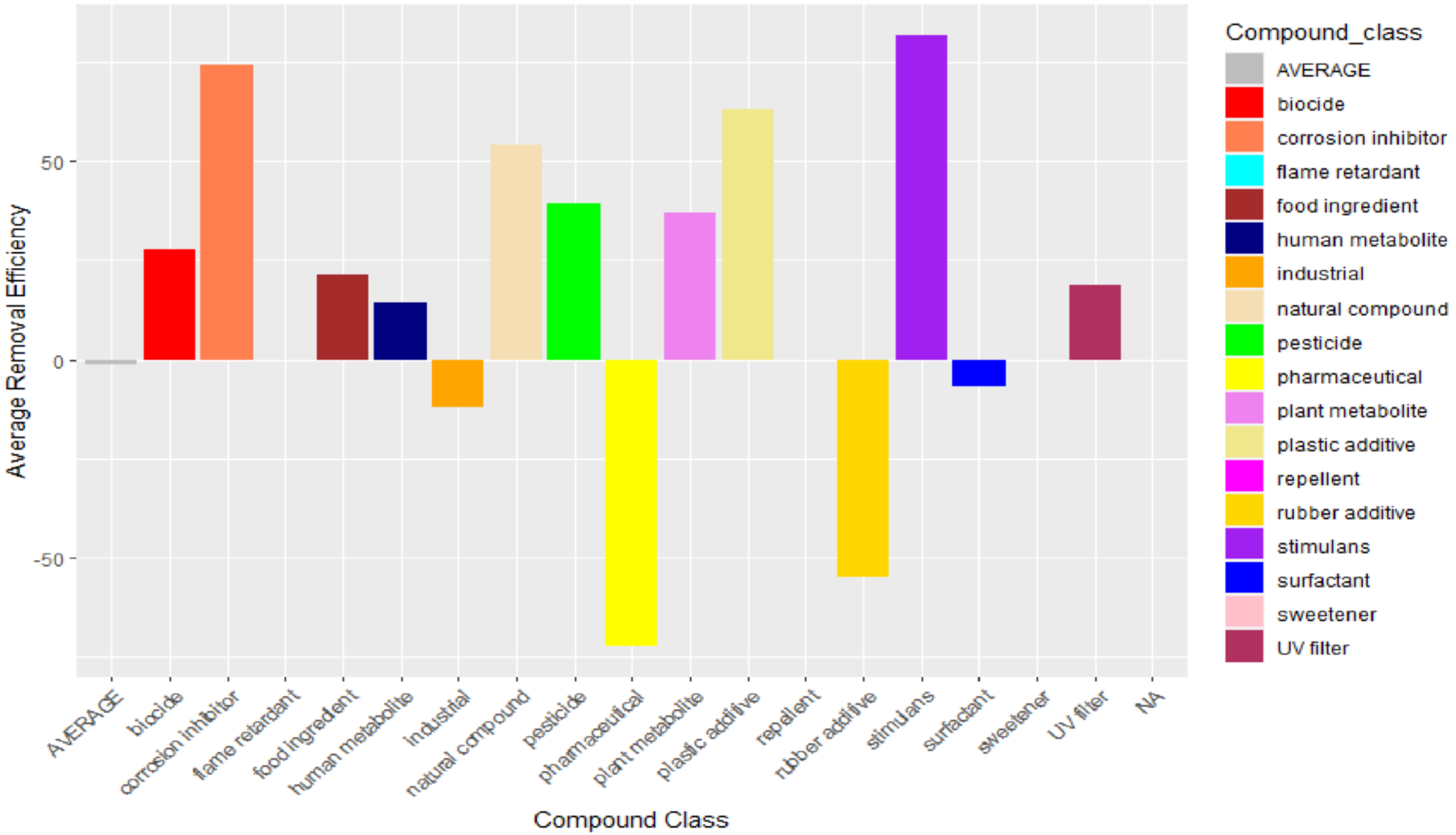

Figure 17. Average removal efficiency in D.L. Koisagat by compound class

Other factors, such as physicochemical characteristics and WWTP treatment methods, may also have a role in the effluent concentration exceeding the influent concentration. Sampling variation is one such factor, as the grab sampling method employed collected influent and effluent samples on the same day, providing only a snapshot of micropollutant concentrations at that specific time. This approach did not account for the retention duration of wastewater throughout treatment procedures before discharge, hence it was insufficient for estimating effective removal efficiency (Campo et al., 2013).

Sample preparation for LC/MS analysis involves filtration using vacuum filtration, which may overlook micropollutants adsorbed in the non-aqueous phase, undermining influent load assessments (Campo et al., 2013). Additionally, the dilution of wastewater during treatment processes, where water is added, can lead to the appearance of low-level compounds having seemingly higher concentrations in the effluent, even if their actual concentrations have not increased (Gajewska et al., 2024).

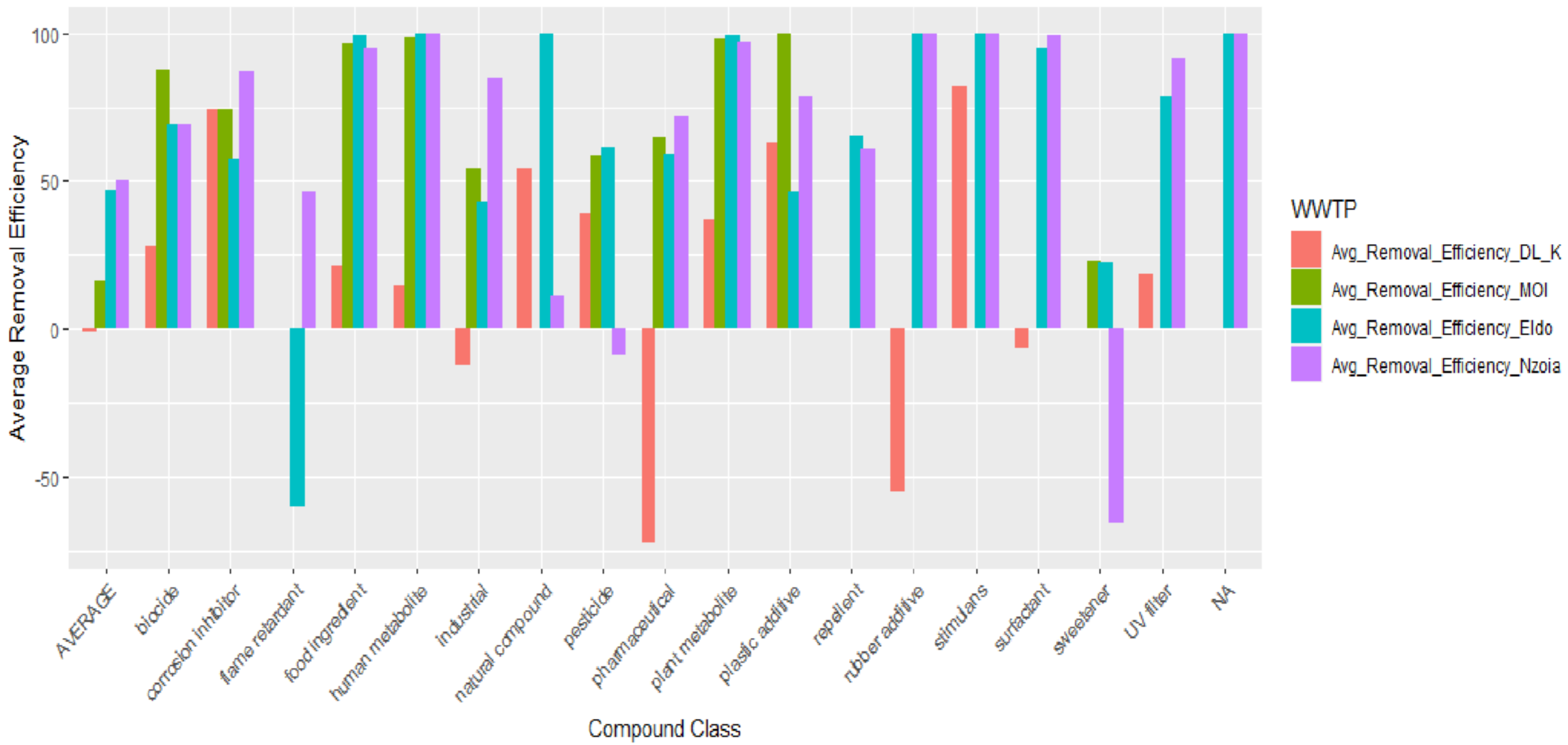

Figure 18: Comparison of the removal efficiencies of CEC classes across the selected WWTPs

4.4 Ecotoxicological Risk Assessment

4.4.1 Crustacean toxicity

Crustaceans had the highest potential risk up to TU_{sum} =9.6, with all four WWTPs exhibiting both chronic (TU>0.001) and acute toxic risk (TU>0.1). All the TU_{sums} of the selected WWTPs were greater than 1. The exceedance of both chronic and acute toxicity thresholds indicated a high sensitivity of invertebrates towards CECs. Overall, 19 of the compounds detected exhibited chronic toxicity, while 3 of these exerted acute toxicity for crustaceans. Only three compounds had TU values >1, displaying their concentrations exceeding the EC guidelines; these compounds included diazinon, dichlorvos, and acetamiprid. Diazinon and dichlorvos were the primary risk drivers of toxicity for crustaceans, with TU values ranging from 1.73 to 5.54 and 3.95 to 4.65, respectively. This result agrees with previous studies, which reported diazinon as a significant risk driver for crustaceans. The compounds whose maximum TU value exceeded the chronic toxicity risk thresholds included diazinon (Tumax=5.54), dichlorvos (TU max=4.65), imidacloprid (Tumax=1.01), acetamiprid (Tumax=0.039), primiphosmethyl (TU max=0.01), and terbuthylazine (Tumax=0.01). Other compounds included didecyldimethylammonium, fipronil, diclofenac, diuron, and carbendazim. Dichlorvos demonstrated a significantly higher TU (4.7) in crustaceans compared to its toxic effects on algae (0.7), underscoring the substantial risk posed by dichlorvos to invertebrates. Diazinon's high level of hazardous risk can be attributed to its vigorous biological activity and non-detectability at extremely low doses (Kandie et al., 2020). Both diazinon and dichlorvos are organophosphorus insecticides whose primary mode of toxicity is the inhibition of cholinesterase present in the nervous system(Todd et al., 2020). Diazinon readily undergoes oxidative desulfuration to diazoxon, which has a higher anticholinesterase activity, with its activity being 4,000 times greater than that of the parent diazinon(Epa, 2003). Invertebrates have been reported to be more sensitive to organophosphorus, especially diazinon, due to their less efficient detoxification of diazoxon. Other compounds that have been reported to cause chronic toxicity to crustaceans include fipronil sulfone and primiphos-methyl (Kandie et al., 2020b), which agrees with the present study. (Girón-Pérez et al., 2022). Diuron has been documented to exhibit both acute and chronic toxicity towards crustaceans, resulting in alterations in feeding patterns and the induction of oxidative stress upon exposure to *Tigriopus japonicus*, as reported by (Yun et al., 2023).

Among the selected WWTPs, ELDOWAS exhibited the highest acute toxicity risk for crustaceans, with a TUsum of 9.6. NZOWASCO followed with a TUsum of 6.5, D.L. Koisagat TUsum = 4.9, while Moi WWTPs had the least toxicity risk for crustaceans (TUsum=4.5) **(figure 20) across** all the WWTPs, pesticides and biocides predominated as the risk drivers for crustaceans. Pesticides and biocides are designed to target pest organisms and weeds. They exert their toxic effects through different modes of action, such as acetylcholinesterase inhibition, nicotinic acetylcholine receptor competitive modulation through binding to receptors, triggering a biochemical reaction, and preventing normal reactions from occurring(Warne & Reichelt-Brushett, 2023).

In the NZOWASCO WWTP, the evaluation of toxicity risk to crustaceans revealed TU values ranging from 0.0004 to 4.7 for the leading drivers. Dichlorvos (4.7), along with diazinon (1.73) and acetamiprid (0.1), exhibited the highest toxic risk to crustaceans, surpassing both acute and chronic toxic risk thresholds. Imidacloprid (0.04), a pesticide, terbuthylazine (0.01), carbendazim (0.003), and atrazine

(0.001) displayed potential chronic toxic effects on crustaceans. In ELDOWAS WWTP, diazinon (TU=5.5) had the highest toxicity, followed by dichlorvos (TU=4.0), acetamiprid (TU=0.04), imidacloprid (TU=0.04), didecyldimethyl ammonium (TU=0.007), fipronil (TU=0.006), and 3,5,6trichloro-2-pyridinol (TU=0.005) (Figure **21)**. Diclofenac was the only pharmaceutical among the leading drivers that contributed to chronic toxic impact, consistent with its known high risk, as highlighted by studies such as (Egli et al., 2021) and (Ashfaq et al., 2019). Diclofenac has been found to cause oxidative stress on crustaceans and impede their growth (Parolini, 2020; Russo et al., 2023).

In the Moi WWTP, 10 drivers of toxicity risk for crustaceans revealed significant contributors with Dichlorvos (TU=4.3), primiphos-methyl (TU=0.01), and didecyldimethyl ammonium (TU=0.009) being the only compounds that exceeded the chronic toxic risk threshold (TU>0.001). Two industrial compounds, TMDD (TU=0.0001) and bisphenol G (TU=0.0004), contributed to overall toxicity risk but were absent in ELDOWAS and NZOWASCO, emphasizing the role of industrial compounds in environmental degradation. In the D.L. Koisagat WWTP, the assessment of the top compounds contributing significantly to crustacean toxicity highlighted several notable drivers, including dichlorvos (TU=4.4), diazinon (TU=0.11), primiphos-methyl (TU=0.01), caffeine (TU=0.002), dehydroabietic acid (TU=0.001), hexadecyl pyridinium (TU=0.0007), 1-naphthol (TU=0.0004), and carbaryl (TU=0.0006). Dichlorvos and diazinon emerged as major drivers, surpassing both acute and chronic toxic risk thresholds. Dichlorvos exhibited a higher TU value in D.L. Koisagat WWTP (TU=4.4) compared to ELDOWAS (TU=4.0) and Moi WWTP (4.3), indicating a higher risk for crustaceans in D.L. Koisagat WWTP but a similar impact to NZOWASCO.

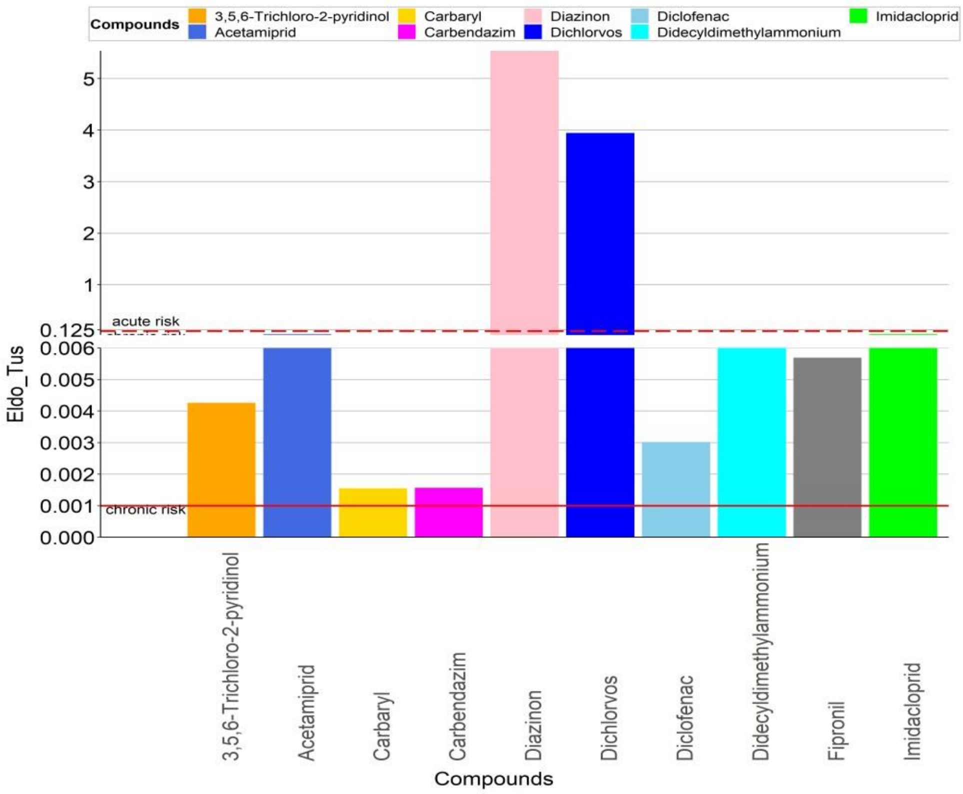

Figure 19: A graph TUS of risk driver for Crustaceans toxicity in ELDOWASWWTP

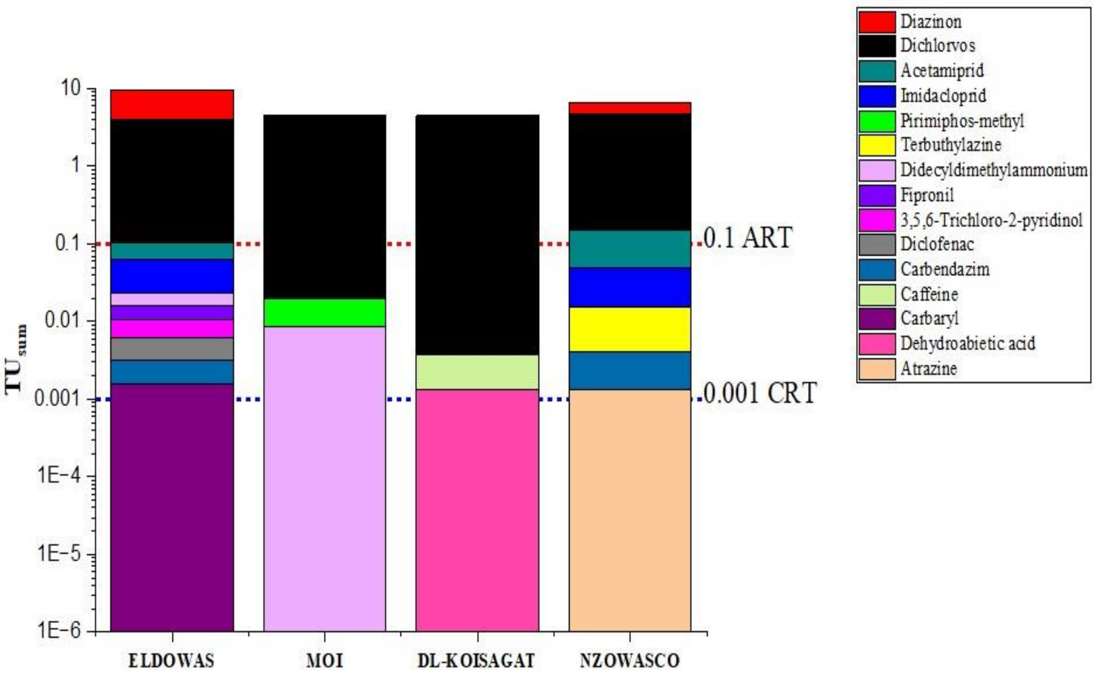

Figure 20: Compounds driving the risk toxicity among crustaceans in the four selected WWTPs

4.4.2 Algae toxicity

Algae exhibited the second-highest potential toxicity risk imposed by the CECs detected in the WWTP effluents. Five of the identified chemicals had TUs that exceeded both chronic and acute toxicity risk limits, while 13 chemicals constituted a chronic toxicity risk for algae. The primary drivers of toxicity risk for algae were predominantly pesticides and biocides, including dichlorvos (TU_{max}=0.08), diuron (TU_{max}=0.07), terbuthylazine (TU_{max}=0.03), ametryn (TU_{max}=0.02), and atrazine (TU_{max}=0.013). The other compounds that were driving chronic toxicity across the WWTPs included didecyldimethylammonium (TU_{max}=0.009), clarithromycin (TU_{max}=0.006), triclosan (TU_{max}=0.006), and metachlor (TU_{max}=0.003). It has previously been observed that diuron and terbuthylazine are highly toxic to algae, inhibiting photosynthesis by causing blockage of electron transport, driving production and damage to the conventional photosystem II(Thomas et al., 2020). Algae are sensitive to dichlorvos, with its exposure decreasing cell density in microalgae, affecting their population growth(Thomas et al., 2020)

Among the selected WWTPs, NZOWASCO exhibited the highest toxicity risk towards algae, with a TUsum of 0.21, followed by ELDOWAS (TUsum=0.13). At the same time, Moi WWTP and D.L. Koisagat WWTP registered the least toxicity towards algae with cumulative $TU_{\_sums}$ of 0.009 and 0.007, respectively. All the cumulative $TU_{\_sums}$ for all the WWTPs exceeded the toxicity impacts imposed by individual compounds, displaying a substantial toxicity effect of environmental mixtures towards algae**(figure:19)**. In the NZOWASCO WWTP), the primary risk drivers to algae were dichlorvos (TU=0.07), Diuron (TU=0.067), terbuthylazine (TU=0.03), and ametryn (TU=0.02). Notably, all of these major drivers exceeded the chronic toxic risk threshold set at TU=0.02 (Figure 18). In ELDOWAS, the TUs of dichlorvos (0.06) and diuron

(0.04) closely reflected those found in NZOWASCO, indicating that these compounds are key contributors to algal toxicity. Their respective Toxic Units (TUs) exceeded the chronic toxic risk threshold but remained below the acute toxic risk threshold. Additionally, atrazine (TU=0.002), ametryn (TU=0.0023), terbuthylazine (TU=0.002), and tertbutryn (TU=0.001) were detected, albeit with low TUs falling below both acute and chronic toxic risk thresholds. Notably, the TUs for these compounds were lower than those observed in NZOWASCO for the corresponding substances. Notably, dichlorvos was the sole compound in Moi WWTP that exceeded the chronic toxic risk threshold. All compounds within this WWTP remained below the acute toxic threshold. Among the other prominent drivers were didecyldimethyl ammonium (0.009), a biocide, triclosan (0.004), also a biocide, and acetochlor (0.001), a pesticide. Three compounds, hexadecyl pyridinium (0.0007), TMDD (0.0002), and sulfamethoxazole (0.00001), a pharmaceutical, in Moi WWTP were among the leading 10 contributors to overall toxicity. In the D.L. Koisagat WWTP, dichlorvos (0.07) and diuron (0.002) emerged as the primary contributors to toxic risk. Notably, only dichlorvos surpassed the chronic toxic threshold, with the remaining compounds registering values below the chronic and acute toxic risk thresholds. Distinguishing D.L. Koisagat WWTP from the other three WWTPs were three new compound drivers, namely caffeine (0.0005), a stimulant, dehydroabietic acid (0.0001), and bisphenol B (0.00002).

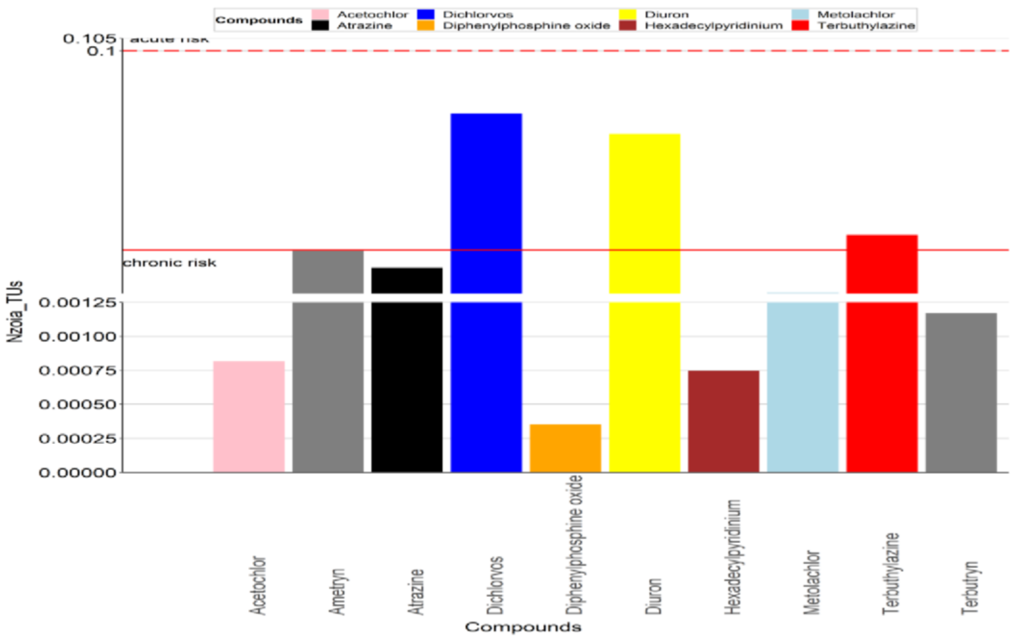

Figure 21: A graph of TUs of risk drivers in NZOWASCO WWTP

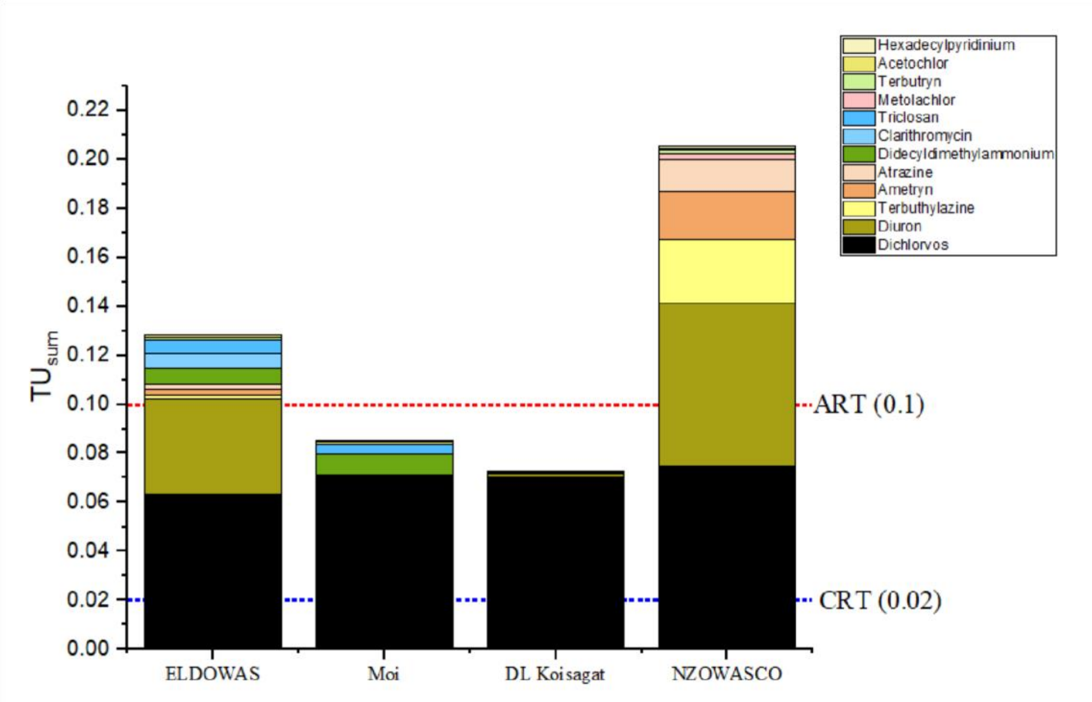

Figure 22: A graph of TUsum for Algae toxicity in the selected WWTPs.

4.4.3 Fish toxicity

Fish had the least potential toxicity risk from CECs among the organisms, with TU_sums up to 0.017, with none of the TU_sums exceeding the acute toxicity risk threshold (TU<0.1). However, all the WWTPs TU_sums exceeded the chronic toxicity risk threshold, except D.L. Koisagat WWTP, whose TU sum was 0.009, less than 0.01**.** The primary risk drivers for fish were didecyldimethylammonium with a maximum TU of 0.009, carbendazim with a maximum TU of 0.006, and dichlorvos (Tumax=0.002). The predominant compounds in all the WWTPs included dichlorvos with TUs ranging from 4.58×10^{-3} to 5.39×10^{-3}, carbendazim from 1.827×10^{-4} to 6.16×10^{-3}, and diazinon from 1.58×10^{-5} to 1.03×10^{-3}. Despite didecyldimethylammonium having a maximum TU of 0.009, it was below the acute and chronic risk thresholds. The fungicide carbendazim has previously been reported to drive risk for fish(Kandie et al., 2020). A controlled experiment of carbendazim on zebrafish embryos displayed developmental anomalies (Fan et al., 2021). Additionally, carbendazim has been listed as a priority substance according to WFD(Finckh et al., 2022a). Didecyldimethyl ammonium has been reported to cause short-term toxicity effects to fish. This was displayed by the induced genotoxicity in rainbow trout and malformations in zebrafish embryos(Cruz et al., 2021). Fish have an extremely high level of genetic diversity, and thus are less sensitive to pollutants(Hamilton et al., 2016). As a result, the majority of CECs have minimal fish toxicity since there are very few compounds in use that are intended to harm fish (Finckh et al., 2022b). Therefore, despite the top primary drivers being dominated by biocides and pesticides, the overall toxicity risk impact was still low. However, this study's assessment did not cover potential risks caused by steroids, which possibly have long-term effects on fish endocrine metabolism (M. Zhang et al., 2022).

Among the four WWTPs, ELDOWAS had the highest potential risk for fish with a TU sum of 0.017.NZOWASCO followed with a TUsum of 0.014, Moi WWTP (TUsum=0.015), while D.L. Koisagat WWTP had the list of potential risks with a TUsum of 0.009 **(figure 23).** In NZOWASCO, the primary drivers of toxicity effects for fish were identified as carbendazim, a biocide (TU=0.006), dichlorvos (TU=0.005), and hexadecyl pyridinium (TU=0.0007). Notably, the Toxicity Unit (TU) values for these leading chemicals were lower than the acute and chronic toxic risk thresholds for fish, indicating a lesser toxicity impact on fish in receiving water systems. Furthermore, the industrial chemical diphenylphosphine, insecticides diazinon and atrazine, biocide diuron, and medicines fluconazole and telmisartan all contributed significantly to the overall hazardous risk for fish in NZOWASCO.

In ELDOWAS, didecyldimethyl ammonium (TU=0.007), dichlorvos (0.005), carbendazim (0.004), and diazinon (TU=0.001) emerged as the leading drivers of toxicity for fish. Significantly, none of the Toxicity Unit (TU) values for these chemicals in the ELDOWAS effluent surpassed either the chronic or acute toxic risk criteria. **(Figure 22)**. Additionally, biocide hexadecyl pyridinium, didecyldimethyl ammonium metabolite hexadecyl trimethyl ammonium, pharmaceutical telmisartan, biocide diuron, and industrial compound TMDD were identified as contributors to the overall toxic risk for fish in

ELDOWAS. Didecyldimethyl ammonium, which is abundant in ELDOWAS, was lacking in NZOWASCO and contributed significantly to toxicity in ELDOWAS. Classified as a quaternary compound used as a disinfectant, didecyldimethyl ammonium has been reported to have moderate toxicity for fish (Lewis et al., 2016).

In Moi WWTP, didecyldimethylammonium emerged as the leading toxic driver for fish, with a TU value of 0.009, comparable to ELDOWAS but with a higher magnitude. Other contributors to the overall toxicity for fish in Moi WWTP included dichlorvos (TU=0.005), hexadecylpyridinium (TU=0.0007), carbendazim (TU=0.0001), TMDD (TU=0.00007), bisphenol G (TU=0.00007), and fluconazole (TU=0.00005). All Toxicity Unit (TU) values obtained for compounds in Moi WWTP effluent were below both acute and chronic toxic risk thresholds, indicating very low individual toxic impact for fish. Notably, only fluconazole, a pharmaceutical, and bisphenol G, an industrial compound, were unique drivers present in this WWTP. TMDD, hexadecylpyridinium, and dichlorvos, which were identified as algae toxicity drivers in Moi WWTP, had lower TU values for fish compared to algae and crustacean toxicity.

In D.L. Koisagat WWTP, dichlorvos (TU=0.005), dehydroabietic acid (TU=0.002), and caffeine (TU=0.001) stood out as the primary drivers of toxicity risk. Despite their predominance, none of the detected compounds posed chronic or acute toxic risk at an individual level due to their low TU values, all falling below the acute (TU<0.1) and chronic toxic risk thresholds (TU<0.01). D.L. Koisagat WWTP revealed new drivers for fish, such as caffeine and 1-naphthol, which exhibited considerable toxic impacts unique to this WWTP effluent.

An experiment performed through exposure of freshwater fish to caffeine concentrations revealed a decrease in their hepatosomatic index(Bikker, 2023). An in vitro investigation employing HepG2 cells for 1-naphthol demonstrated oxidative stress toxicity, which led to an increase in reactive oxygen species generation and a change in the expression of antioxidant enzymes (Shetty & Udupi, 2022).

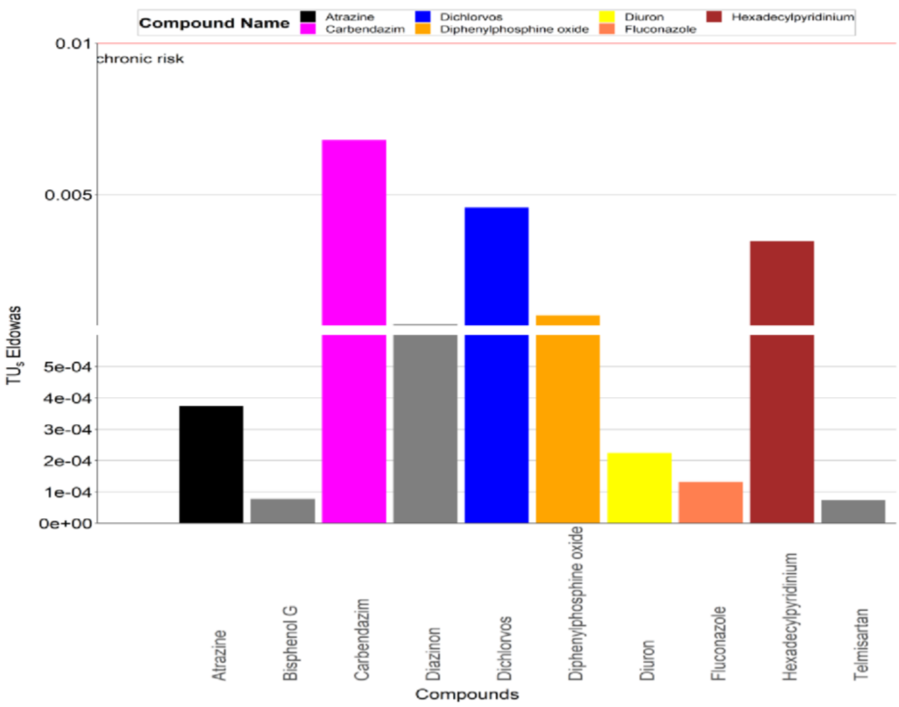

Figure 23: A graph TUS of fish drivers for fish toxicity in ELDOWAS WWTP

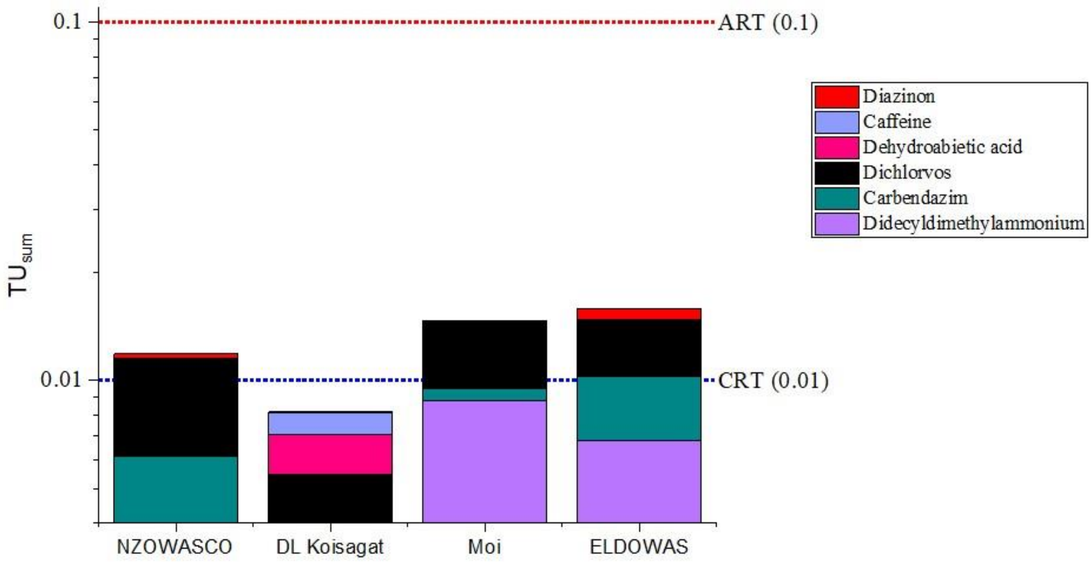

Figure 24: A graph of TUsum for fish toxicity in the selected WWTPs

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study provides a comprehensive identification of CECs in WWTPs, an analysis of the contribution of WWTPs to the introduction of CECs into the aquatic environment, and a risk assessment of CECs in WWTP effluents for three common organisms: fish, crustaceans, and algae in western Kenya. Three hundred and fifty-three (353) chemicals were found in the influent and effluent of the four wastewater treatment plants (WWTPs) that were selected. The chemicals' average concentrations ranged from ng/L to µg/L. The compound classes that registered a high detection frequency of>50% included pharmaceuticals, pesticides, biocides, and industrial chemicals. Two hundred and fifty-one (251) of the identified compounds were present in over 50% of the influent samples, including TMDD, 2-oxindole, dichlorvos, atrazine, diazinon, diuron, carbendazim, and trimethoprim, all of which were consistently detected across all wastewater treatment plants (WWTPs). Antibiotics and NSAIDs had the highest frequency of detection of up to 100% and 88% respectively, due to high consumption and ease of accessibility. Compounds such as carbendazim, diuron, TMDD, diazinon, trimethoprim, cetirizine, 2oxindole, triethylene glycol monobutyl ether, and terbuthylazine showed high detection (100%) of CECs in the WWTP effluents. Individual compound concentrations up to 830 µg/L were reported for caffeine. The effluent wastewater also included high concentrations of caffeine (170 µg/L), closely followed by cetirizine (827 ng/L), dichlorvos (1.1 µg/L), TMDD (1.4 µg/L), and sucralose (3.5 µg/L). Due to different plant designs, technologies, and treatment methods, the identified compounds' removal efficiencies varied, ranging from negative removal to positive removal. Ibuprofen, trimethoprim, and sulfamethoxazole were among the compounds that were eliminated (<99%), while other compounds like 10,11-

Dihydro-10,11-dihydroxycarbamazepine and cetirizine exhibited recalcitrance, indicating resistance to removal processes.

According to risk assessment, crustaceans had the highest potential toxicity risk (TUsum) up to 9.6, followed by fish (0.017) and algae (0.21). Dichlorvos (TU 4.6) and diazinon (TU 5.5), both of which were over the acute risk threshold (TU 0.1), were the sources of potential risk in crustaceans. The potential toxicity risk of fish was lower than that of the CRT (0.01), primarily due to the low TU values of didecyldimethylammonium (TU up to 0.009), carbendazim (TU up to 0.006), and dichlorvos (TU up to 0.005). The potential risk for algae was driven by dichlorvos (TU up to 0.07), diuron (TU up to 0.07), and terbuthylazine (TU up to 0.03). Out of all the WWTPs that were investigated, ELDOWAS wastewater presented the most significant risk of toxicity to crustaceans. According to this study, WWTPs act as a source and sink of CECs in the environment, with these compounds posing different potential toxicity to aquatic organisms and higher trophic levels in the environment.

5.2 Recommendation

This study provides critical insights into the occurrence, removal efficiency, and ecological implications of contaminants of emerging concern (CECs) in wastewater treatment plants (WWTPs). To address identified gaps and strengthen management strategies, future research should incorporate the analysis of transformation products formed during treatment, as these may be more toxic than the parent compounds and significantly influence risk assessments.

Strengthening policy and regulatory frameworks is equally important, with national and county-level regulations explicitly setting permissible limits for CECs in wastewater effluents and establishing robust enforcement mechanisms. Continuous monitoring of effluents discharged into receiving water systems is recommended to ensure compliance and track contaminant trends over time. Furthermore, spatial monitoring downstream of WWTPs should be undertaken to map dispersion and dilution patterns. In contrast, comparative spatial analyses of plants employing different removal techniques would help identify the most effective treatment configurations for various classes of CECs. The detection, occurrence, bioaccumulation, and biomagnification of CECs in various aquatic organisms should be integrated to determine their potential impacts across aquatic food webs.

A shift from single-compound assessments to mixture-based approaches is needed to account for synergistic and cumulative effects of multiple pollutants. In addition, extending sampling strategies beyond single-day grab samples to continuous or time-integrated methods will capture fluctuations in contaminant inflow, treatment performance, and effluent concentrations. Advancing wastewater-based epidemiology studies can offer valuable insights into population-level consumption patterns and related public health risks. Given the growing use of treated wastewater in agriculture, further studies should evaluate CEC accumulation in irrigated crops to address potential food safety concerns. Finally, promoting advanced treatment technologies such as advanced oxidation processes, membrane filtration, and activated carbon systems can enhance CEC removal efficiencies and minimize environmental impacts. Implementing these measures will not only enrich future research but also support evidencebased policymaking, improve treatment plant performance, and safeguard aquatic ecosystems and public health.

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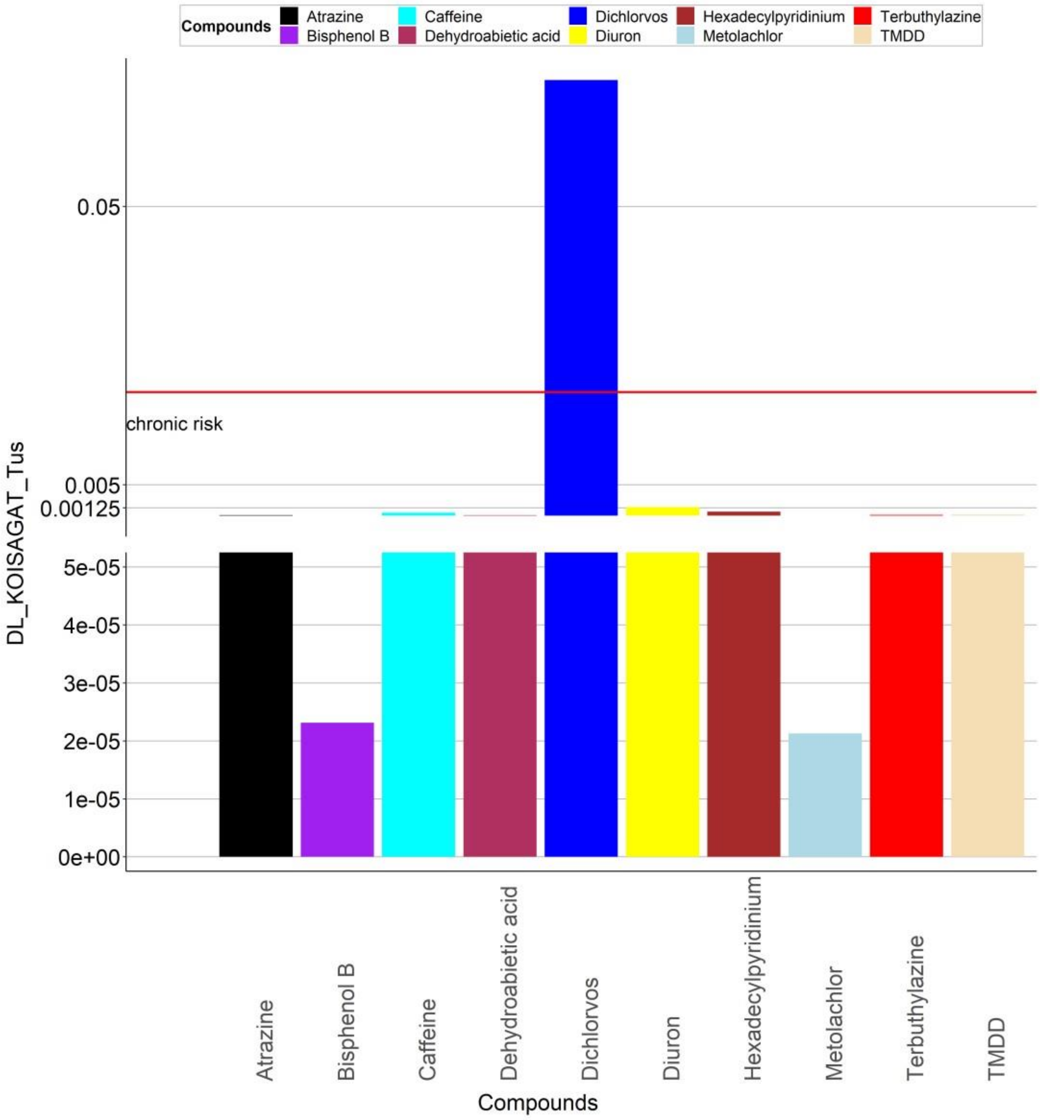
APPENDICES

Appendix I.

Table 1: SITES COORDINATES

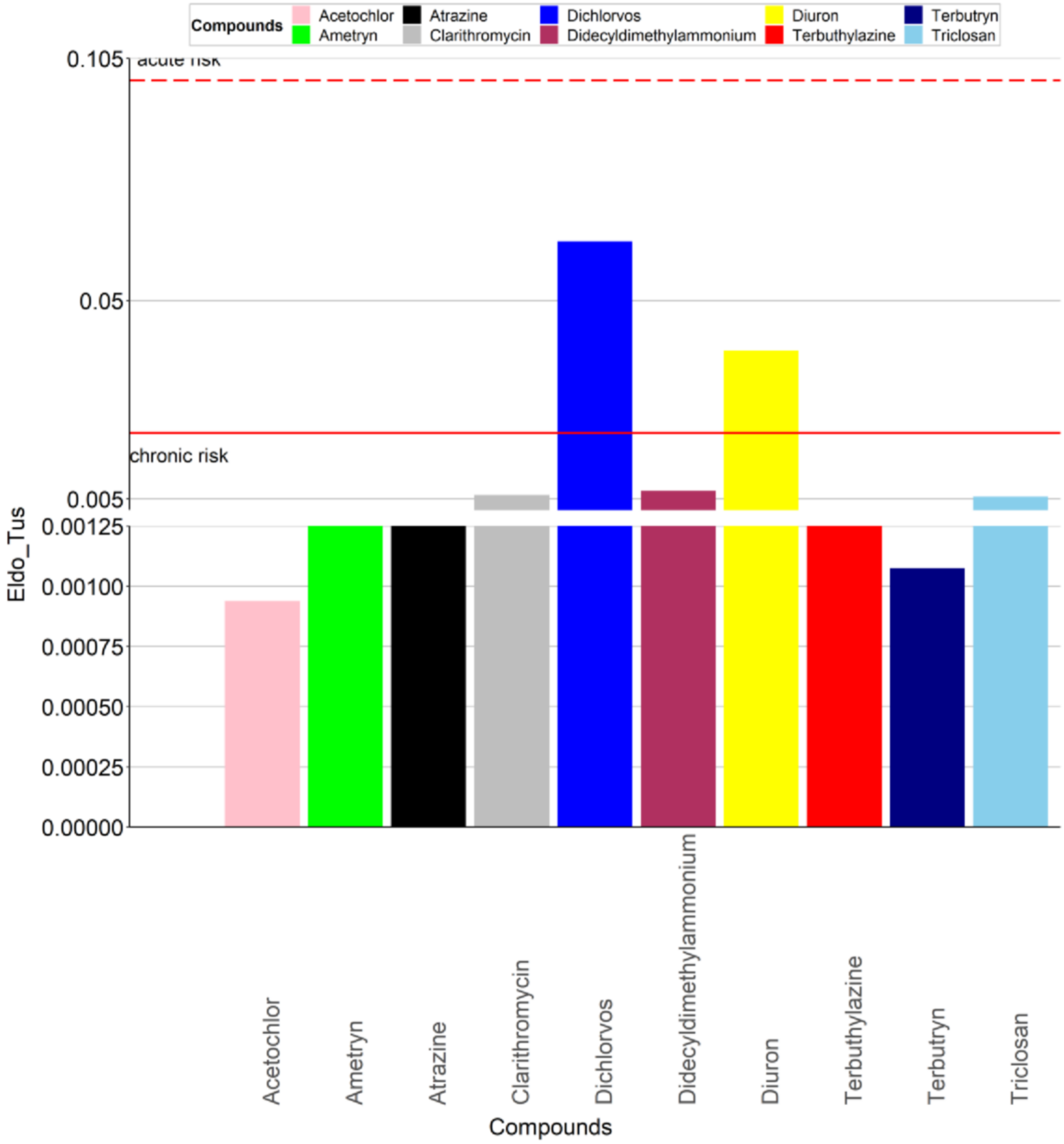
Site name	**Latitude**	**Longitude**
ELDOWAS	0º32’30.42	0º12’35.17
NZOWASCO	1.016984S	34.973157
MOI University WWTP	0.26109	35.27936
DL Koisagat WWTP	0.51130	35.164279
RIVER CHITOTO UP	1.1015934	34.984519
R. CHITOTO DN	1.06496	34.971557
R. SOSIAN UP	0.495878	35.29355
R. SOSIAN MID	0.55114	35.21318
R. SOSIAN DN	0.55266	35.21297

Appendix II.

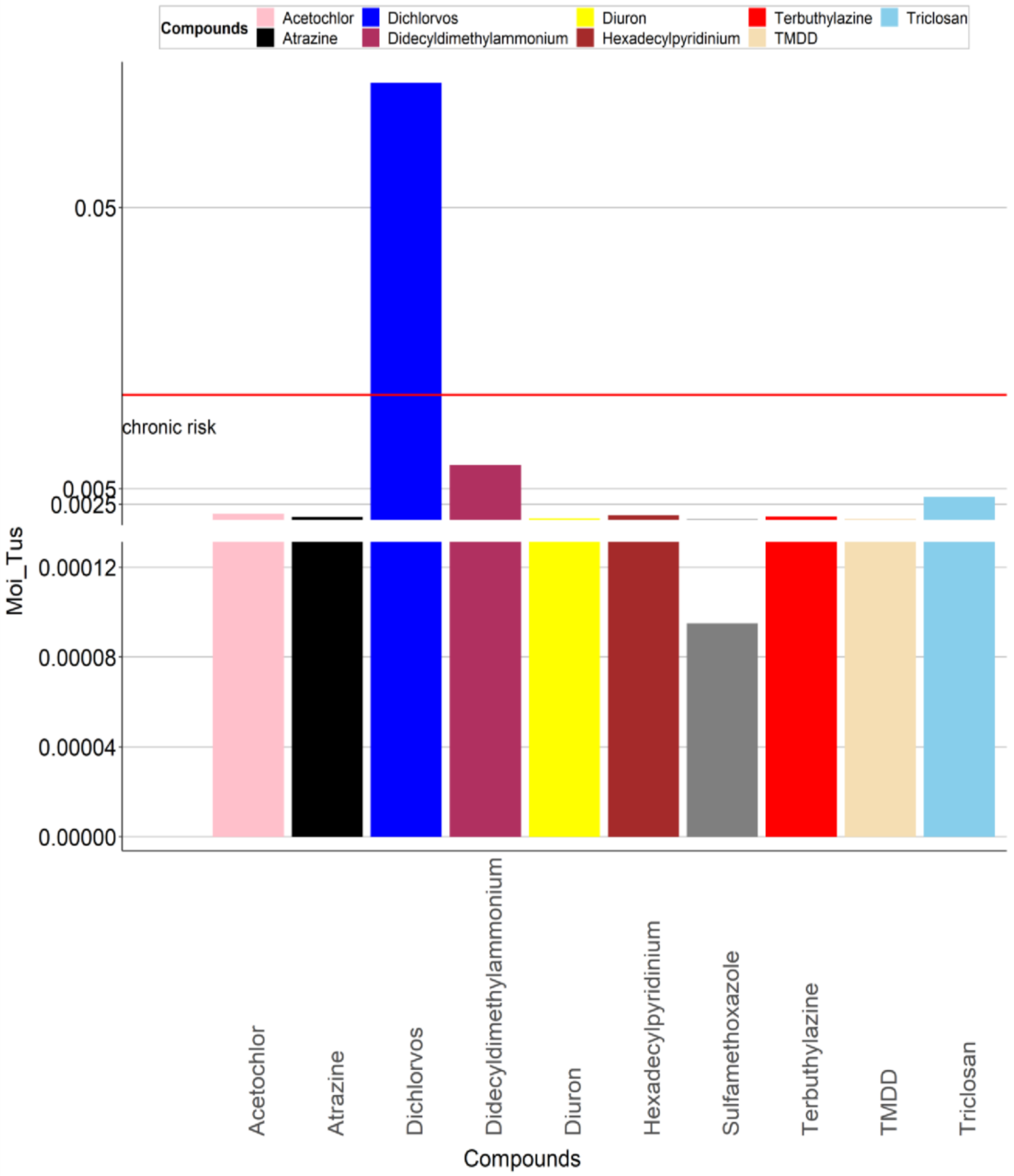


Compounds
Atrazine
Bisphenol B
Caffeine
Dehydroabietic acid
Dichlorvos
Diuron
Hexadecylpyridinium
Metolachlor
Terbuthylazine
TMDD
DL_KOISAGAT_Tus
0.05
chronic risk
0.005
0.00125
5e-05
4e-05
3e-05
2e-05
1e-05
0e+00
Compounds

Appendix III



Appendix IV


Compounds
Acetochlor
Atrazine
Dichlorvos
Didecyldimethylammonium
Diuron
Hexadecylpyridinium
Terbuthylazine
TMDD
Triclosan
0.05
chronic risk
0.005
0.0025
0.00012
0.00008
0.00004
0.00000
Moi_Tus
Acetochlor
Atrazine
Dichlorvos
Didecyldimethylammonium
Diuron
Hexadecylpyridinium
Sulfamethoxazole
Terbuthylazine
TMDD
Triclosan
Compounds

Appendix V

SR682

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