The removal of endocrine disrupting compounds by wastewater treatment plants

Report to the

WATER RESEARCH COMMISION

by

MAA Coetzee, MNB Momba, GM Kibambe, KT Thobela, T Kgositau, P Mahlangu

Tshwane University of Technology

WRC Report No. 2474/1/18
ISBN 978-0-6392-0117-7

JUNE 2018

Obtainable from

Water Research Commission

Private Bag X03

Gezina, 0031

orders@wrc.org.za or download from www.wrc.org.za

The publication of this report emanates from a project entitled *The removal of endocrine disrupting compounds by wastewater treatment plants* (WRC Project No. K5/2474)

Printed in the Republic of South Africa

EXECUTIVE SUMMARY

Globally, wastewater treatment plants (WWTPs) are increasingly being monitored for their efficiency in removing endocrine disrupting compounds (EDCs) from wastewater. Results to date have revealed inconsistencies in the removal of the same compounds when using different treatment technologies. It has become evident that the design and operating conditions of WWTPs also have an influence on EDC removal efficiency. In South Africa, there is a lack of information about the fate of EDCs during wastewater treatment using different commonly used technologies.

The objectives of this study were to:

- Screen the EDCs in wastewater influent and monitor various stages of three selected types of wastewater treatment plants for target EDCs.
- Determine and compare EDC removal efficiencies using a mass balance approach
- Set up laboratory-scale activated sludge and anaerobic digestion reactors and determine how efficient they are at removing selected EDCs during steady state operation.

Three WWTPs, Daspoort and Zeekoegat WWTPs (both in the City of Tshwane Metropolitan Municipality, Gauteng) and Phola WWTP (Emalahleni Local Municipality, Mpumalanga) were used as case studies. The three WWTPs had different treatment technologies and operating conditions. Daspoort activated sludge plant uses the 3-stage Phoredox configuration and operates at a sludge age of 26 days. Furthermore, they internally recycle the primary sludge in the primary settling tank to enhance volatile fatty acid production for enhanced biological phosphate removal in the subsequent activated sludge process. The Zeekoegat WWTP uses a variety of configurations, with the Johannesburg configuration in operation, at a sludge age of 35–45 days, during the study. Phola WWTP utilises a pond enhanced treatment and operation (PETRO) process, in combination with trickling filters.

The raw influent wastewaters entering Daspoort, Zeekoegat and Phola WWTPs were sampled to perform the initial screening for the presence of EDCs, with a total of 44 compounds identified. Following screening of the three influents, the identified EDCs were classified into five groups: oestrogens, perfluorinated chemicals (PFCs), pharmaceuticals, pesticides, personal care products and plasticisers, with most of the EDCs falling into three groups: oestrogens, PFCs and pharmaceuticals. Selected EDCs from these three groups were monitored over the study period and these included:

- Oestrogens: estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2)
- Perfluorinated chemicals: perfluorobutanoic acid (PFBA), perfluorodecanoic acid (PFDA), perfluorooctanoic acid (PFOA), perfluorohexanoic acid (PFHxA), perfluoro-1-octanesulfonate (PFOS), perfluoro-n-pentanoic acid (PFPeA) and perfluoro-1-hexanesulfonate (PFHxS)
- Pharmaceuticals: nalidixic acid, bezafibrate, acetaminophen, carbamazepine, stavudine and lamivudine.

The removal of the EDC groups, during wastewater treatment, by each treatment technology, comprising of various unit processes, has been mapped as given in Table 1.

Table 1: The EDC groups' influent daily mass loads, percentage removal and effluent daily mass loads for the three case study WWTPs

WWTP	EDC group	Influent daily mass load (g/d±SD)	PS + AS (%)	AP + BF (%)	Effluent daily mass load (g/d±SD)
Zeekoegat	PFCs	11 ± 3	84	-	1.8 ± 0.1
	Oestrogens	15 ± 8	47	-	8 ± 5
	Pharmaceuticals	628 ± 101	78	-	140± 33
Daspoort	PFCs	24 ± 7	75	-	6 ± 1
	Oestrogens	6 ± 3	33	-	4± 3
	Pharmaceuticals	300 ± 61	56	-	131 ± 38
Phola	PFCs	0.4 ± 0.1	-	75	0.1 ± 0.02
	Oestrogens	1.3 ± 0.7	-	61	0.5 ± 0.3
	Pharmaceuticals	81 ± 23	-	88	10 ± 1

PS – Primary Settling; **AS** – Activated Sludge; **AP** – Anaerobic Pond; **BF** – Biofilter; **SD** – Standard Deviation

The pharmaceuticals were identified as the main contributors to the influent EDC groups' daily mass loads at the three WWTPs, with influent loads of 628 g/d, 300 g/d, and 81 g/d for Zeekoegat, Daspoort and Phola WWTPs respectively. The mass loads of the oestrogens were the second highest in the influents to Zeekoegat (15 g/d) and Phola (1.3 g/d) WWTPs, while the influent daily mass load of PFCs at Daspoort (24 g/d) was higher than the daily mass load of oestrogens (6 g/d). In general, the influent daily mass loads followed the order pharmaceuticals (628 g/d_Z; 300 g/d_D; 81 g/d_{PH}) > oestrogens (6 g/d_D; 15 g/d_Z; 1.3 g/d_{PH}) > PFCs (24 g/d_D; 11 g/d_Z; 0.4 g/d_{PH}), with PFC influent daily mass load at Daspoort an exception.

Daily mass loads discharged into the receiving water bodies were also calculated and it was found that significant amounts of pharmaceuticals and oestrogens entered the environment daily. The total amount of pharmaceuticals discharged daily at Zeekoegat WWTP was estimated as 140 g/d, while 131 g/d were discharged from Daspoort WWTP and 10 g/d from Phola WWTP. The effluent daily mass loads for the sum of all the oestrogens in the effluents discharged from Zeekoegat, Daspoort and Phola WWTPs were found to be 8 g/d, 6 g/d and 0.5 g/d, respectively. The effluent daily mass loads of PFCs discharged from Zeekoegat, Daspoort and Phola WWTPs were 1.8 g/d, 6.5 g/d and 0.1 g/d, respectively. In general, it was estimated that the EDC groups were discharged from the three WWTPs into the receiving water resources in a daily mass load of the order; pharmaceuticals (131 g/d_D; 140 g/d_Z; 10 g/d_{PH}) > oestrogens (4 g/d_D; 8 g/d_Z; 0.5 g/d_{PH}) > PFCs (6 g/d_D; 1.8 g/d_Z; 0.1 g/d_{PH}), with the PFC effluent daily mass load at Daspoort also an exception.

Laboratory-scale activated sludge and anaerobic digestion reactors were run to evaluate the removal of EDCs under controlled conditions, using representative EDCs selected from the three groups that had been monitored at the full-scale WWTPs. A three-stage activated sludge laboratory-scale reactor was operated at a sludge age of 30 days, with air supplied by means of a diffuser, at a flow rate of 320 L/h. The average mixed liquor suspended solids (MLSS) concentration was 2 800 mg/L. The mass load into the reactor system was 154 µg/d, 77 µg/d and 38 µg/d for pharmaceuticals, PFCs and oestrogens, respectively. The observed average effluent mass loads for pharmaceuticals, PFCs and oestrogens were 17, 12 and 0.05 µg/d, representing removal efficiencies of 89, 84 and 100%, respectively. The improved removal of EDC groups in laboratory-scale studies indicates the importance of control and optimisation of operational parameters (e.g. sludge age and oxygen dosage), easily achievable under controlled conditions and quite transferrable to full-scale WWTPs to achieve better EDC removal.

The laboratory-scale anaerobic digesters were operated using waste activated sludge (WAS), primary settling tank sludge (PS) and a mixture of WAS and PS (WAS/PS) sludge, digested for 20 days at 36°C.

The concentrations of PFCs, before and after digestion, were 327 g/ton and 142 g/ton (WAS), 606 g/ton and 219 g/ton (PS), 402 g/ton and 224 g/ton (mixed sludge). The concentrations of pharmaceuticals were 1089 g/ton and 668 g/ton (WAS), 1 110 g/ton and 627 g/ton (PS), 882 g/ton and 465 g/ton (mixed sludge). The concentrations of oestrogens were 2.2 g/ton and 2.3 g/ton (WAS), 1.8 g/ton and 0.1 g/ton (PS), 2.3 g/ton and 0.5 g/ton (mixed sludge). The results represented removal efficiencies of 39, 44 and 44% from mixed sludges, which are commonly used in anaerobic digestion, for pharmaceuticals, PFCs and oestrogens respectively (Table 2).

Table 2: The mass loads, grams per ton, and percentage removal of the three groups of EDCs before and after anaerobic digestion

Sludge type		PFCs	Oestrogen	Pharmaceuticals
Waste activated sludge (WAS)	**Before digestion, g/ton**	327	2.2	1089
	After digestion, g/ton	142	2.3	668
	% removed	**57**	**0**	**39**
Primary sludge (PS)	**Before digestion, g/ton**	606	1.8	1110
	After digestion, g/ton	219	0.1	627
	% removed	**64**	**93**	**44**
Mixed PS/WAS	**Before digestion, g/ton**	402	2.3	882
	After digestion, g/ton	224	0.5	465
	% removed	**44**	**78**	**47**

Digested sludge is generally used in agriculture as a soil conditioner and fertiliser. Guidelines set by the Department of Water and Sanitation for the application of sludge for agricultural purposes state that the application rate should not exceed the plant nutrient requirements, with a maximum application rate of 10-ton dry sludge/hectare/ year. Using the maximum application rate and the mass of EDCs still present in the digested mixed sludge (PS and WAS mixture commonly co-digested in anaerobic digesters) it can be estimated that 7.0 kg/hectare/year pharmaceuticals, 2.3 kg/hectare/year PFCs and 1.3 kg/hectare/year oestrogens could be applied to agricultural land. In this regard, the studied EDC groups have significant potential to accumulate in the soil and leach into the water resources where their presence could pose environmental and human health risks.

ACKNOWLEDGEMENTS

Funding from the Water Research Commission for this project is greatly acknowledged.

The authors would like to thank the Reference Group of this WRC Project for the assistance and constructive discussions during the duration of the project:

Dr JN Zvimba (Water Research Commission – Chairman)

Mr J Topkin (ERWAT)

Dr P Hlabela (Council for Scientific and Industrial Research)

Prof L Petrik (University of the Western Cape)

Mr C Swartz (CSWUE)

Prof T Msagati (University of South Africa)

D Ntsowe (City of Tshwane)

Dr N Ngube (Randwater)

Dr P Welz (Cape Peninsula University of Technology)

K Esterhuyze (City of Tshwane)

The research team would also like to thank the following institutions and persons:

- Personnel from the City of Tshwane (Daspoort, Zeekoegat wastewater treatment plants) and Emalahleni Local Municipality (Phola wastewater treatment plant) for their cooperation and assistance throughout the project
- Dr Y Naude from the Department of Chemistry, University of Pretoria, for conducting the GC×GC-TOFMS analysis
- Dr Peter Dazo, Department Environment, Water and Earth Science at Tshwane University of Technology, for assisting with the LC/MS/MS analysis.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

BNR	Biological nutrient removal
COD	Chemical oxygen demand
DDT	1,1,1-trichloro-2,2-di(chlorophenyl)ethane
E1	Estrone
E2	17β-estradiol
E3	Estrol
EDC	Endocrine disrupting compound
EE2	17α-ethinylestradiol
GC	Gas chromatography
H	Henry's law constant
HCl	Hydrochloric acid
HRT	Hydraulic retention time
K_{OW}	Octanol/water partition coefficient,
K_{OC}	Organic carbon to water partition coefficient
LC/MS/MS	Liquid chromatography-tandem mass spectrometry
MLSS	Mixed liquor suspended solids
NP	Nonylphenol
NP1EO	Mono- ethoxylated nonylphenol
NP2EO	Di-ethoxylated nonylphenol
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated bisphenols
PFBA	Perfluorobutanoic acid
PFBS	Perfluoro-1-butanesulfonate
PFC	Perfluorinated compound
PFDA	Perfluorodecanoic acid
PFDoS	Perfluoro-1-dodecanesulfonate
PFDS	Perfluoro-1-decanesulfonate
PFHpS	Perfluoro-1-heptanesulfonate
PFHxA	Perfluorohexanoic acid

PFHxDA	Perflyohexadecanoic acid
PFHxS	Perfluoro-1-hexanesulfonate
PFNA	perfluorononanoic
PFNS	Perfluorononanesulfonate
PFOA	Perfluorooctanoic acid
PFODA	Perfluorooctadecanoic acid
PFOS	Perfluorooctane sulfonate
PFPeA	Perfluoro-n-pentanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUdA	perfluorohexadecanoic acid
PST	Primary settling tank
SPE	Solid phase extraction
SRT	Sludge retention time
SST(s)	Secondary settling tank(s)
TS	Total solids
UAE	Ultrasonic assisted extraction
VFA	Volatile fatty acid
VS	Volatile solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

Chapter 1
INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

For the past two decades, there has been great concern about the presence of organic contaminants, in very low concentrations ranging from nanograms to micrograms per litre, in water sources. Some of these chemical compounds have endocrine disrupting characteristics. Endocrine disrupting compounds (EDCs) can mimic or inhibit the endocrine systems of humans and animals (Chang et al., 2009). Therefore, they pose serious health and environmental risks if discharged into the environment.

The oestrogenicity in the aquatic environment is largely due to the presence of EDCs in effluents from sewage treatment works (Lofrano, 2012). Many of these EDCs pass through wastewater treatment systems and are discharged continuously into the environment, mainly into surface waters. Oestrogenic activities have been detected in water sources throughout the world, including South Africa. Therefore, it has become increasingly important to understand the presence of EDCs in wastewater, their potential discharge from treatment plants, and their fate and behaviour during wastewater treatment. So far, research into the removal of EDCs from wastewater by typical wastewater treatment plants (WWTPs) can be classified into the following types:

- Removal of EDCs from different types of WWTPs, by measuring EDCs in the influent and effluent of plants. In this regard, researchers have determined the EDCs found in wastewater and the extent of their removal from the effluent.
- Removal by sludge treatment processes, mainly focussing on aerobic and anaerobic digestion.
- Comparison of the removal efficiencies of different types of WWTPs for selected EDCs.
- Fate of selected EDCs through simultaneous analysis of the liquid and sludge streams.
- Laboratory-scale or pilot-scale processes to evaluate removal under controlled conditions.

The majority of the initial investigations focused on the removal of different types of EDCs from WWTPs by monitoring the influent and effluent from the plants. Several reviews summarise research carried out globally on EDC removal. For instance, Heberer (2002) reviewed data from several global studies and categorised pharmaceutical compounds according to their use, reporting more than 80 different pharmaceutically active compounds detected in effluents from WWTPs. Auriol et al. (2006) reported concentrations of oestrogens and alkylphenols and their ethoxylates in effluents from WWTPs from different countries, while Liu et al. (2009) listed examples of different EDCs in influent and effluent from different WWTPs, as reported by different researchers.

However, most research, focussing only on the EDC concentrations in influent and effluent from WWTPs, has failed to provide an understanding of the fate of the different EDCs in wastewater treatment. Therefore, recently, researchers have shifted their focus to the determination of mechanisms for removing different EDCs, by monitoring both the water and sludge streams in WWTPs. In this regard, different wastewater treatment processes have been evaluated and comparisons can now be made between the removal efficiencies of different wastewater treatment technologies.

The aim of this study is to establish the removal of EDCs by WWTPs within the South African context, through screening of effluents, and monitoring of EDCs to understand the EDC removal efficiencies of full-scale WWTPs. The investigation mainly focused on commonly used treatment technologies. These include activated sludge systems, biological filtration systems and aerobic and anaerobic digestion. Waste stabilisation pond systems are also included. These are mainly used in rural areas.

1.2 WHAT ARE ENDOCRINE DISRUPTING COMPOUNDS?

The endocrine system controls several other systems in the human body. The system consists of endocrine glands which can be found in different parts of the body, and are named the hypothalamus, pituitary, thyroid, parathyroid, pineal body, gonads and adrenal glands (Birkett & Lester, 2003). Chemicals in the endocrine glands (called hormones) are released into the bloodstream, and travel to targeted organs in the body where they attach to specific protein molecules, named “receptors”, to initiate a specific response. Hormones which are not attached to a receptor will be inactivated by organs like the liver and kidneys, so that no “free hormones” exist in the body for long time periods (Birkett & Lester, 2003). These hormones control growth rate, reproduction, metabolic rate, salt/water balance and blood glucose levels in the body (Rushton, 2004).

Although specific hormones attach themselves to very specific receptor sites, these receptor sites also bind with other chemicals, such as EDCs, thereby interfering with the hormone–receptor interaction and changing the response of the endocrine system. The different mechanisms of interaction between EDCs and hormones are summarised as follows (Birkett & Lester, 2003):

- Mimicking the hormone by binding and activating a response, although different from the natural response
- Binding with the receptor and blocking the response so that nothing happens
- Stimulating the formation of more hormones, either natural or foreign
- Accelerating the breakdown and destruction of hormones, leading to the depletion of specific hormones in the body
- Interfering with enzymes which destroy hormones in the body
- Destroying hormones by altering the chemical structure of a hormone so that it cannot find the receptor

Most trace organic compounds display oestrogenic activity and therefore interfere with the reproduction systems of animals and humans (Hamid & Eskicioglu, 2012). However, interference with the reproduction system is not the only concern, as other hormone functions can also be altered.

Research indicates the thyroid hormone can also be affected. The thyroid hormone has many essential roles in human physiology. For instance, it controls normal brain development, metabolism, and many other important aspects of normal adult physiology. EDCs can change the function of the thyroid gland or interfere with the ability of the thyroid hormone to function properly (Kabir et al., 2015). Thyroid disruptors may include polychlorinated bisphenols (PCBs), bisphenol A (4,4’-isopropylidenedipheno), perchlorate, polychlorinated dibenzofurans, flame retardants and dioxins (Kabir et al., 2015).

1.3 ENVIRONMENTAL AND HEALTH CONCERNS ASSOCIATED WITH THE PRESENCE OF EDCs IN WATER.

Human health depends on a well-functioning endocrine system to regulate the release of hormones that are essential for functions such as metabolism, growth and development, sleep and mood. Endocrine disruptors can alter the functions of this hormonal system, increasing the risk of adverse health effects (Kabir et al., 2015). EDCs contribute to the development of non-descended testes in young males, breast cancer in women, prostate cancer in men, developmental effects on the nervous system in children, attention deficit/hyperactivity in children, and thyroid cancer (Bergman et al., 2012). However, there is no strong scientific evidence which directly relates the occurrence of the above mentioned defects and diseases in humans to the presence of EDCs in drinking water (Hamid & Eskicioglu, 2012). Nevertheless, the increasing rate of incidences of these diseases over the past decades is alarming (Bergman et al., 2012). On the other hand, adverse

effects on wild life and aquatic animals are undeniable. Table 1.1 lists common EDCs which were found in animal tissue by Bergman et al. (2012). Hamid and Eskicioglu (2012) have also reported evidence of defects in the reproduction systems of several fish species and turtles.

In South Africa, similar observations were made by Barnhoorn et al. (2010). The authors found intersex in indigenous fish species in the Luvuvhu River in the Limpopo Province, which they attributed to the presence of DDT in these waters. Kruger et al. (2013) reported feminised male catfish in the Rietvlei and Marais dams in Gauteng. They have also analysed mesenteric fat from the sampled fish and found high levels of nonylphenol, indicating bio-accumulation due to long-term exposure.

Table 1.1 Common EDCs found in animal tissue (Bergman et al., 2012)

Common EDCs	Exposed wildlife
Benzotriazole, UV stabilisers, parabens, triclosan, organophosphorous	In tissues of wildlife living near outfalls of sewage treatment works
Triclosan	Aquatic organisms that include algae, invertebrates, fish and dolphins
Pharmaceuticals like anti-epileptic carbamazepine and the active ingredients of several antidepressants (fluoxetine, sertraline, venlafaxine, citalopram, norfluoxetine, diphenhydramine, diltiazem)	In muscle or liver tissue of wild fish or fish caged downstream of wastewater outfalls
Human contraceptives (EE2 and levonorgestrel)	In fish muscle and plasma

EDCs have been detected in water resources globally (Heberer, 2002; Liu et al., 2009; Aneck-Hahn et al., 2008; Pool, 2008). Furthermore, EDCs have been found in drinking water. Benotti et al. (2009) have screened drinking water in the US for the presence of pharmaceuticals and other EDCs and found that a number of these compounds were present, in concentrations below 10 ng/L.

1.4 TYPES OF EDCs

Numerous compounds have endocrine disrupting characteristics. They can be natural or synthesised compounds. In modern times, we can barely live without these chemicals as they generally tend to increase the quality of human life. Therefore, more and more organic compounds are manufactured annually. Currently, more than 38 000 pharmaceutical compounds are identified as endocrine disrupting and more than 87 000 new chemicals have not been tested yet (Tijani et al., 2013).

EDCs belong to different classes of organic compounds and can be classified as follows (Birkett & Lester, 2003):

- Steroid compounds (oestrogens)
- Surfactants (nonylphenol)
- Pesticides (DDT, dieldrin)
- Poly aromatic compounds (PAH, PCBs, brominated flame retardants)

- Organic oxygen compounds (phthalates, bisphenol A)

A more convenient way to classify EDCs is according to their use. Chang et al. (2009) identified various classes of EDCs, namely, steroid (oestrogen), pharmaceutical, personal care products and industrial chemicals. Each of these classes can be sub-divided according to application. Figure 1.1 indicates the classification of pharmaceutical compounds and personal care products (Caliman & Gavrilescu, 2009).

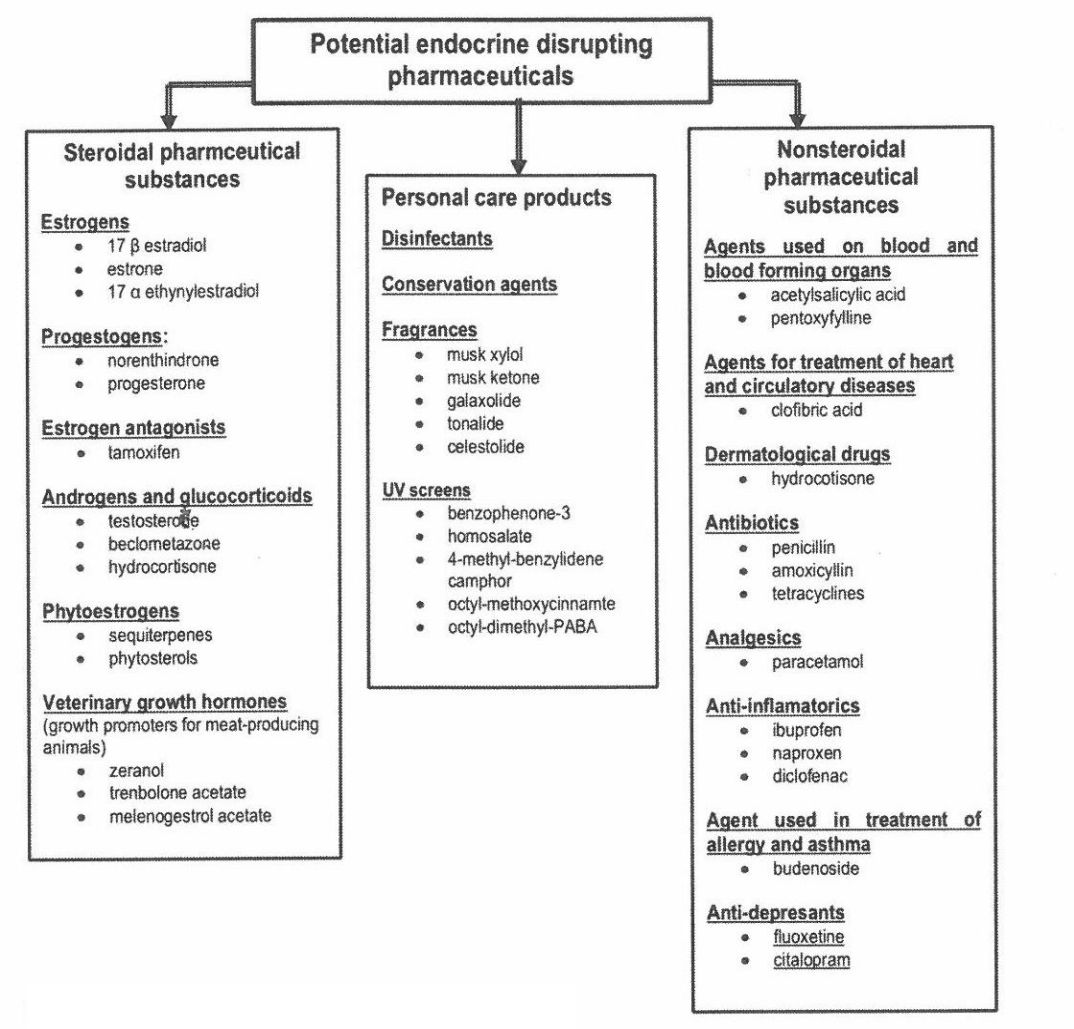
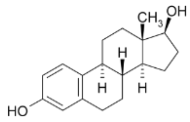
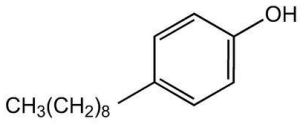
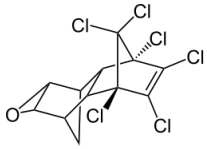
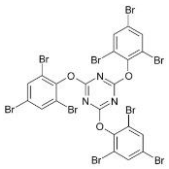
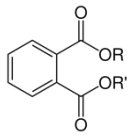


Figure 1.1: Different classes of pharmaceutical and personal care products. (Caliman and Gavrilescu, 2009).

The oestrogen hormones are the foremost oestrogenic compounds found in effluent from domestic WWTPs (Muller et al., 2010) and play a major role towards the feminisation of male fish. Typical concentrations of oestrogens reported in raw wastewater vary from 0.02–0.25 µg/L (Verlicchi et al., 2012). Table 1.2 lists types of EDCs, classified according to chemical structure.

Table 1.2: **Different types of EDCs, classified according to chemical structure (Birkett & Lester, 2003; Chembase.en 2017)**

Type of organic compound	Example	Structure
Steroid compounds	Estradiol	
Surfactants	4-n-nonylphenol	
Pesticides	Dieldrin	
Poly aromatic compounds	1,2-bis(tetrabromophthalimido) ethane	
Organic oxygen compounds	Phthalates	
Perfluorinated compounds	Perfluorooctane sulfonate	

Oestrogens can be classified according to their origin (Hamid & Eskicioglu, 2012):

- Natural oestrogenic hormones: estrone (E1) 17β-estradiol (E2), estriol (E3)
- Synthetic hormones: 17α-ethinylestradiol (EE2)
- Phyto- and mycoestrogens: daidzein

Oestrogen hormones are excreted from the body, via urine, in a conjugated form (Hamid & Eskicioglu, 2012), which implies that they are biologically inactive and more soluble in water than the deconjugated form. However, these conjugated hormones are readily deconjugated in a WWTP (Hamid & Eskicioglu, 2012) which renders them biodegradable.

Perfluorinated compounds (PFCs) are another group of emerging compounds of concern. PFCs are organic compounds in which the hydrocarbons of the compound are substituted with fluorine (Stahl et al., 2011). Table 1.2 shows the chemical structure of perfluorooctane sulfonate (PFOS), an eight-carbon compound, which is used as a stain repellent and fabric protector (Chembase.en, 2017). PFC compounds are commonly used to make products water resistant, for packaging of foodstuffs and in firefighting materials (Sciences, 2016).

PFCs are a concern because they are persistent and breakdown very slowly (NIH, 2016). PFCs are frequently detected in water resources and are also detected in wild animals and in human blood and breast milk samples (Stahl et al., 2011). Research indicates that these compounds also have endocrine disrupting characteristics. Kjeldsen and Bonefeld-Jorgensen (2013) found that perfluoro-1-hexanesulfonate (PFHxS), PFOS and perfluorooctanoic acid (PFOA) significantly affect the oestrogen receptors, and PFHxS, PFOS, PFOA, perfluorononanoic (PFNA) and perfluorodecanoic acid (PFDA) have an effect on androgen receptors, and furthermore, the effect is directly related to the concentrations of these compounds.

1.5 REMOVAL OF EDCs BY DOMESTIC WASTEWATER TREATMENT PLANTS

Domestic WWTPs are primarily designed for nutrient removal in the form of organic carbon, nitrogen, and occasionally phosphorous, and are therefore not optimised for the removal of EDCs. Furthermore, EDC compounds belong to different classes of organic compounds and have a variety of functional groups which can make them less biodegradable. Knowledge of the physicochemical properties of the different compounds can aid in determining their fate in wastewater treatment processes (Sawyer et al., 1994).

1.5.1 Mechanisms for removal of EDCs from wastewater

EDCs can be removed from wastewater via the following mechanisms (Birkett & Lester, 2003; Sawyer et al., 1994):

- Adsorption onto suspended solids, fats and oils
- Biological degradation
- Chemical transformations: hydrolysis, photolysis, and oxidation and reduction reactions
- Volatilisation

The fate of EDCs in wastewater is determined by the physicochemical properties of the different organic compounds (Birkett & Lester, 2003, Sawyer et al., 1994). The most important properties are the octanol/water partition coefficient (K_{OW}), Henry's law constant (H), the organic carbon to water partition coefficient (K_{OC}), and the solubility of the organic compound in water. Partitioning describes the distribution of an organic compound between the different phases, water and solid or water and gas (Eckenfelder et al., 2009). By using the coefficients and constants of a specific organic compound, the possible removal mechanism can be predicted.

1.5.1.1 Adsorption onto solid particles

The octanol/water partition coefficient (K_{OW}) and organic carbon to water partition coefficient (K_{OC}) can be used to predict if an organic compound will adsorb onto a solid particle, such as suspended and settleable solids present in raw sewage, or the biomass in the biological process (Sawyer et al., 1994).

The octanol/water partition coefficient (K_{OW})

K_{OW} is the concentration ratio of the organic compound partitioned between the organic liquid and water (Birkett & Lester, 2003) at equilibrium. The K_{OW} coefficient is related to the hydrophobic and lipophilicity characteristic of an organic compound and can be used to predict if an organic compound will adsorb onto solid particles. Log K_{OW} is indirectly proportional to the solubility of an organic compound (Birkett & Lester, 2003). Hydrophobic organics have a large log K_{OW} value (> 4) and will adsorb onto solid particles; hydrophilic compounds have low log K_{OW} values (< 2.5) and tend to stay in solution.

Organic carbon to water partition coefficient (K_{OC})

K_{OC} is defined as the ratio between the concentration of the organic compound's organic carbon (mg/g) and the concentration in water (mg/L) (Birkett & Lester, 2003). Compounds with high log K_{OC} values will adsorb onto solids particles, while compounds with small values will remain in solution.

1.5.1.2 Volatilisation

Henry's constant (H) is used to predict the distribution of a compound between the liquid and the gas phase and can be defined as follows (Sawyer et al., 1994):

$$H = \frac{Concentration\ of\ the\ compound\ in\ gas}{Concentration\ of\ the\ compound\ in\ water.}$$

H is indirectly related to temperature; thus, at higher temperatures the H value becomes smaller and higher concentrations of a compound will be in the gas phase. If an organic compound has a H value greater than 10^{-3} atm-m^3/mole, it can be effectively removed by an air stripping process (Sawyer et al., 1994).

Figure 1.2 (from Eckenfelder et al., 2009) illustrates the relationship between the Henry's law constant and the octanol water partition coefficient (K_{OW}) values of a compound, vapour pressure and the likelihood of finding the compound in the liquid phase, vapour phase or adsorbed onto solid particles. Compounds which have H values < 10^{-4} atm-m^3/mole and K_{OW} values < 10^4 tend to stay in solution (like phenolic compounds and acrylamides), while pesticides, which usually have K_{OW} values > 10^4 and low H values tend to adsorb onto solid particles. Conversely, compounds like benzene can easily be removed from water by aeration as their H value is larger than 10^{-2} atm-m^3/mol.

1.5.1.3 Chemical transformations

The most common chemical transformation which takes place in water is hydrolysis. During hydrolysis, water acts as a nucleophile, attacks the organic molecules and substitutes some of the molecules with OH^- molecules (Sawyer et al., 1994). The oxidation state of the organic molecule does not change during this reaction. Examples of organic compounds that can hydrolyse are: halogenated aromatic organics, esters, carbamates and amides (Sawyer et al., 1994).

The three main oestrogens found in the female body are estrone (E1), 17 β-estradiol (E2) and estriol (E3) (Kikandi, 2008). In order to be excreted in urine, the oestrogens must be rendered water soluble; this is achieved by conjugation to glucuronide or sulphate (Puri, 2014). The oestrogens are inactive in the conjugated form, but in wastewater they deconjugate and become active. The deconjugation of the oestrogens results in an increase in the concentrations of some of the oestrogens during the treatment process (Lee et al., 2004).

Another type of chemical transformation which could play a role in the removal of EDCs is photochemical oxidation. Photochemical reactions take place where light energy is absorbed by an organic molecule to form a transient excited state with different chemical and physical properties. In this excited state the organic compound converts into another compound and simultaneously releases energy (Sawyer et al., 1994). Photochemical reactions could play an important role in waste stabilisation pond systems where light energy is an essential element in the treatment process.

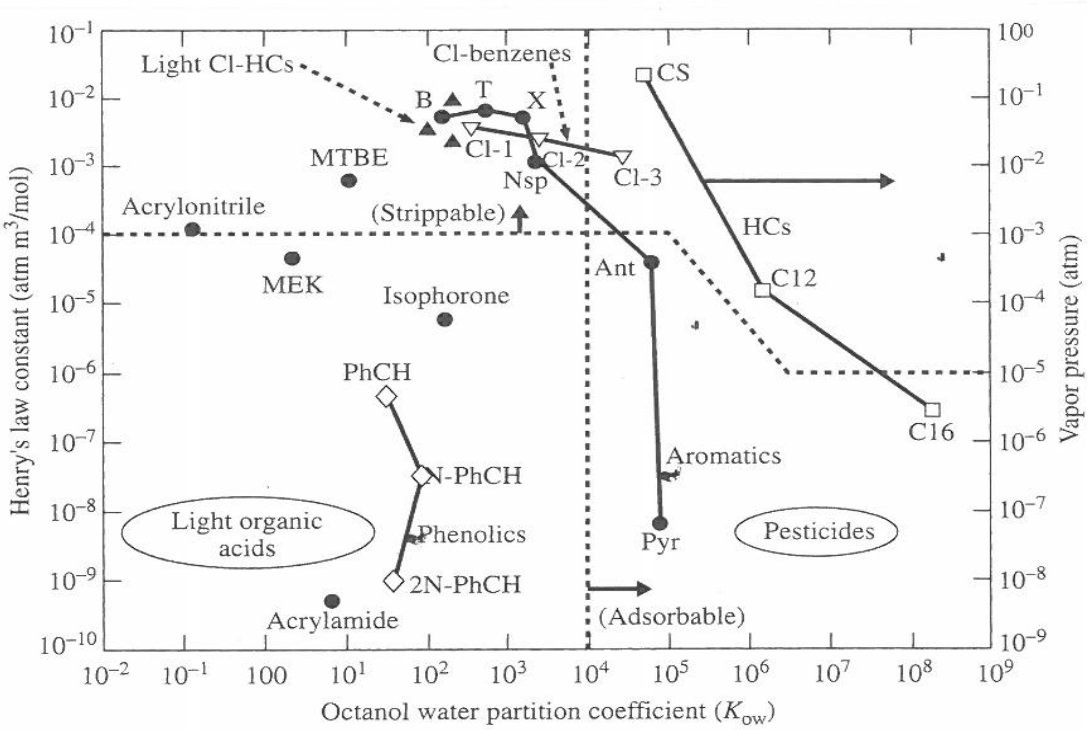


Figure 1.2: The relationship between Henry's constant, the octanol partition coefficient and vapour pressure (Eckenfelder et al., 2009).

1.5.1.4 Biodegradation by microorganisms

EDCs which are not volatilised could be degraded by the microorganisms present in the treatment process. Bacteria utilise two mechanisms for biodegradation of organic matter (Henze & Van Loosdrecht, 2008), namely, direct uptake by diffusion into the cell, or adsorption onto the cell wall. The adsorbed particles can be liquefied by extra-cellular enzymes after it diffuses through the cell wall. However, adsorbed compounds take much longer to be utilised. The biodegradability of organic compounds is affected by their chemical structure. The following criteria (Birkett & Lester, 2003) can be used to predict the biodegradability of organic compounds:

- Highly branched hydrocarbon chains are less biodegradable compared to linear chain hydrocarbons
- Substituents on an organic molecule, like halogen, methoxy, sulfonates and nitro groups make it less biodegradable
- High molecular mass limits active transport through the cell wall
- Environmental factors affect the growth rate of microorganisms and, therefore, the EDC removal rate. Important factors are pH, temperature, dissolved oxygen, light and the presence of nutrients and trace elements.

Co-metabolism is another pathway that microorganisms can employ to biodegrade an organic compound. Co-metabolism transformations (Birkett & Lester, 2003) take place when a micro-

organism breaks down a compound without using it as a carbon or energy source but only to support growth.

Some organic compounds, such as lipophilic compounds (with high K_{OW} values), can be accumulated in microorganisms. Once accumulated, these compounds become part of the food chain (Birkett & Lester, 2003).

1.5.2 Typical concentrations of EDCs in domestic wastewater

Concentrations of EDCs in domestic wastewater vary significantly. Liu et al. (2009) and Wang et al. (2016) have compiled typical concentration ranges reported by different researchers and these are given in Table 1.3.

Table 1.3: Physicochemical properties and typical concentrations of selected EDCs. (Liu et al., 2009; Wang et al., 2016)

Compound	Main category	Water solubility mg/L	LogK_{OW}	Typical concentration range in wastewater ng/L	
				Influent	Effluent
Estrone (E1)	Natural oestrogen	30	3.13	4–510	1–147
17β-estradiol (E2)	Natural oestrogen	3.6	4.01	n.d–161.6	n.d–158
Estriol (E3)	Natural oestrogen	441	2.45	2–660	n.d–275
17α-ethinylestradiol (EE2)	Synthetic oestrogen	116	3.67	n.d–14.4	n.d–178
Testosterone	Natural androgen	30	3.32	n.d–143	n.d–21
Progesterone	Natural androgen	8.84	3.87	n.d–14	0.31–0.37
Daidzein	Phytoestrogen	-	-	75–120	n.d–22
BPA	Industrial chemical	120	3.32	88–5620	6–4090
Nonylphenol	Industrial chemical	3000	3.28	240–41 207	n.d–90 043
Octylphenol	Industrial chemical	5	1.4	n.d–13 000	n.d–1 300
PFOS	Industrial chemical			3.5–31	2.7–53
PFHxA	Industrial chemical			2.7–10	2.7–3.2
PFOA	Industrial chemical			2.8–29	3.3–11

1.5.3 Fate of EDCs in wastewater treatment plants

Domestic wastewater is typically treated in a series of different unit processes. These processes are broadly classified as preliminary, primary, secondary and tertiary (Metcalf and Eddy Inc., 2004).

The purpose of the different unit processes is as follows (Metcalf and Eddy Inc., 2004):

- Preliminary treatment – removal of screenings and grit
- Primary treatment – removal of suspended and settleable solids
- Secondary treatment – biodegradation of organic compounds, nitrogen and phosphorous in biological processes such as activated sludge and biological filters
- Tertiary treatment – disinfection

Sludge is generated during primary and secondary treatment. In an activated sludge process, waste activated sludge (WAS) is removed to maintain a specified sludge age and mixed liquor suspended solid (MLSS) concentration in the aeration tank (Metcalf and Eddy Inc., 2004). In a biological filter, the biofilm will become detached and will be removed in the humus tank (Grady et al., 1999). The sludge from the various sources is usually unstable and needs to be treated before final disposal. Common stabilisation processes used are aerobic and anaerobic digestion.

1.5.3.1 Primary treatment

Most WWTPs have a primary treatment step where settleable solids are removed. EDCs can be removed during primary treatment by adsorption onto the primary sludge, which will mean that they will be discharged with the sludge, or by attachment to fats, oils and grease which are skimmed from the surface of the primary settling tank (Hamid & Eskicioglu, 2012). The important factors to consider for the prediction of EDC removal during primary treatment are the physicochemical properties of the compound and the characteristics of the wastewater (Koh et al., 2008; Hamid & Eskicioglu, 2012). Khanal et al. (2006) have proposed a model to predict the removal during primary treatment, based on the hydrophobic character of the compound, the retention time and the settling characteristics of the wastewater (Hamid & Eskicioglu, 2012). The application of the model predicts very little removal of EDCs in a primary settling tank. However, results from different investigations indicate that removal efficiencies for the same compounds were not consistent, and this is given in Table 1.4.

The removal efficiencies of PFCs during primary sedimentation is not well documented. The K_{OW} values for PFC compounds are also not readily available (Stasinakis et al., 2013). However, Stasinakis et al. (2013) found PFDA and perfluorohexadecanoic acid (PFUdA) in sludge samples which indicates that some PFCs could be removed by adsorption on primary sludge.

Table 1.4: Percentage removal of EDCs from primary settling tanks, as reported by different workers

Compounds	Removal efficiency %	Reference
Nonylphenol (NP)	5.5	(Bertanza et al., 2011)
Bisphenol A (BPA)	6.4	
Mono- ethoxylated nonylphenol (NP1EO)	2.4	
Di-ethoxylated nonylphenol (NP2EO)	5.4	
oestrone (E1)	0–2.5	(Ifelebuegu, 2011)
17 β-estradiol (E2)	15–31	
17β-ethinyestradiol (EE2)	14–17	
nonylphenol (NP)	4–12	
E1	6.4	(Zhang et al., 2011)
E2	6.4	
EE2	4.0	
E3	14.2	
Diethylstrilbestrol (DES)	0	
Bisphenol A (BPA)	34.1	
Nonylphenol (NP)	46.1	
Octylphenol (OP)	7.5	

1.5.3.2 Secondary treatment

Secondary treatment processes involve biological treatment followed by sedimentation. During biological treatment, three main nutrients are removed via the following processes (Metcalf and Eddy Inc., 2004):

- Carbon oxidation by heterotrophic bacteria for the removal of organic compounds, commonly measured as COD (chemical oxygen demand)
- Nitrification and denitrification for the removal of nitrogen in the form of biodegradable organic nitrogen, ammonium and nitrate
- Enhanced phosphorous removal by phosphate accumulating organisms

Conventional activated sludge processes usually only remove COD and, if the sludge age is long enough, also ammonium. If removal of nitrogen and phosphorous is required, a biological nutrient removal (BNR) process is utilised (Henze & Van Loosdrecht, 2008).

Two types of secondary processes are distinguished, namely, suspended growth processes like activated sludge and attached growth processes like biological filters (Grady et al., 1999).

Removal of EDCs from activated sludge processes

Activated sludge processes are commonly used and most investigations into EDCs have focused around these processes. Various reviews report the removal efficiencies for different EDCs from activated sludge processes (Castiglioni et al., 2006; Ko et al., 2007; Komesli et al., 2015; Mnif et al., 2010). Some EDCs, such as natural and synthetic oestrogens, can be removed in activated sludge plants by a combination of sorption and biodegradation processes (Racz & Goel, 2010).

However, Verlicchi et al. (2012) went one step further and interpreted data collected by researchers from 244 conventional activated sludge plants and 20 membrane biological reactors. The authors classified the results according to compounds in the different therapeutic classes in relation to sludge retention time (SRT), percentage removal by biological transformation and percentage adsorption onto sludge. The findings are given in Table 1.5. The following conclusions could be drawn from these findings:

- The majority of the compounds are biodegraded and, with longer SRTs, more compounds can be biodegraded
- The removal mechanism varies, even for compounds in the same therapeutic class, and some compounds are not removed at all

Researchers also found that nitrification processes enhance the removal of oestrogen compounds (Hamid & Eskicioglu, 2012). Ammonia-oxidising bacteria co-metabolise oestrogens during degradation of ammonium (Ren et al., 2007). Thus, factors which have an influence on nitrification will also influence the efficiency of oestrogen removal. However, the most important factors to consider are SRT and organic loading rates (Ren et al., 2007). How efficient ammonium-oxidising bacteria are at biodegrading triclosan, bisphenol A and ibuprofen was examined by Roh et al. (2009). They found that *Nitrosomonas europaea* degraded triclosan and bisphenol A, but not ibuprofen.

The effect of adding an anaerobic zone to an activated sludge process to include biological phosphorous removal has been investigated by Nie et al. (2012). They evaluated a three-stage process designed for nitrogen and phosphorous removal, for the removal of the oestrogens E1, E2, EE2, E3, bisphenol A (BPA) and 4-nonylphenol (NP) and found 75% removal of E1, and more than 90% removal for the rest of the compounds during summer time. As the results are similar to

those obtained by other researchers it seems as if phosphate-accumulating organisms do not affect EDC removal (Hamid & Eskicioglu, 2012).

Locally, an activated sludge plant in Pietermaritzburg (South Africa) was evaluated for the removal of E1, E2, E3, EE2, testosterone and progesterone. They found 98% and 96% removal efficiencies for progesterone and testosterone, respectively, while the percentage removal for the hormones E1, E2 and E3 varied between 72 and 100%. The percentage removal for EE2 was 90% (Manickum & John, 2014).

In the aeration zone of an activated sludge process, conditions are favourable to strip volatile compounds from the liquid. However, literature suggests that most EDCs have very low H values; therefore volatilisation is not an important removal mechanism (Hamid & Eskicioglu, 2012).

Removal of EDCs by biological filtration

Most researchers found that biological filtration is less efficient than activated sludge processes for the removal of EDCs, as reported by Hamid and Eskicioglu (2012) and Verlicchi et al. (2012).

Table 1.5: EDCs – Removal efficiencies in activated sludge processes (Verlicchi et al., 2012).

Therapeutic class	Biodegradation (> 60%)	Sorption onto sludge (>60%)	Present in the effluent (> 60%)
Analgesics and anti-inflammatories	Ibuprofen Ketoprofen Mefenanic acid Naproxen		Diclofanec Indomethacin
Antibiotics	Clarithromycin (SRT > 50 d) Sulfapyridine Trimethoprim (SRT > 50 d)	Ciprofloxacin Enrofloxacin Lomefloxacin Norfloxacin Ofloxacin	Azithromycin Chloramphenicol Erythromycin Metronidazole Roxithromycin
Antidiabetics			Glibenclamide
Antihypertensives	Enalapril	Hydrochlorothiazide	
Beta-blockers	Atenolol		Metoprolol Nadolol Sotalol Timolol
Diuretics	Furosemide (SRT > 16 d)		
Lipid regulators	Bezafibrate (SRT > 16 d) Gemfibrozil (SRT > 16 d) Pravastatin (SRT > 16 d)	Fenofibrate	
Psychiatric drugs	Fluoxetine	Diazepam (SRT > 16 d)	Carbamazepine Lorazepam
Receptor antagonists	Cimetidine (SRT > 16 d) Ranitidine (SRT > 16 d)		Famotidine
Hormones	Estradiol Estrone Ethinylestradiol		
Beta-agonists			Salbutamol
Contrast agents	Iopromide (SRT 30 d)		

The work done by Kasprzyk-Hordern et al. (2009), as given in Table 1.6, further illustrates the point. The authors evaluated a biological filter and an activated sludge plant for their efficiency at removal of a selection of EDCs.

Similar results were obtained for oestrogens. Ifelebuegu (2011) found the percentage removal for the hormones E1, E2 and EE2 from a biological filter system to be 51.8%, 62.3%, and 8.4% compared to removal from an activated sludge process of 53.8%, 89.9%, and 40.0%, respectively.

No evidence was found in literature where researchers related the removal efficiencies in biological filters to design features and the operating conditions of the filter. For instance, the organic loading rate could influence the ability of a biological filter to achieve nitrification, which was reported to assist in the removal of EDCs.

Microorganisms attach to the media in biological filters to form a biofilm. When the biofilm becomes too thick, it sloughs from the attached filter media and is removed in the humus tank. The inner part of the biofilm could become anaerobic when it is very thick, while the outer layers will stay aerobic. Furthermore, biofilms have variable sludge ages (Grady et al., 1999). Therefore,

analysing sludge from humus tanks could be useful in determining the fate of EDCs in biological filters.

Table 1.6: Comparison of removal efficiencies of a biological filter and an activated sludge plant (Kasprzyk-Hordern et al., 2009)

Compound	% Removal	
	Biological filter	Activated sludge plant
Antibacterial drug		
Trimethoprim	47	70
Erythromycin-H2O	14	72
Metronidazole	59	38
Anti-inflammatory/analgesic		
Paracetamol	94	99
Ibuprofen	84	94
Diclofenac	0	31
Ketoprofen		
Naproxen	56	86
Aspirin	96	99
Salicylic acid	97	99
Mefenamic acid		
Codeine	49	61
Tramadol	0	42
Anti-epileptic		
Carbamazepine	0	13
Gabapentin	0	86
Beta-adrenoceptor blocking drug		
Propranolol	52	57
Metoprolol	8	56
Atenolol	78	85
Lipid-regulating agent		
Bezafibrate	45	71
H2-receptor antagonist		
Ranitidine		92
Cimetidine	26	79
Sulfasalazine	0	0
Sulfapyridine	70	91
5-Aminosalicylic acid	0	94
Diuretic		
Furosemide	21	77
Calcium channel blocker		
Diltiazem	65	77
Antidepressant		
Amitriptyline	84	96

Removal of EDCs by waste stabilisation ponds

Two research articles describing the removal of oestrogens, bisphenol A and 4-nonylphenol from wastewater stabilisation pond systems have been identified in literature. Pessoa et al. (2014) evaluated three different stabilisation pond systems and observed variable removal efficiencies in

the different configurations for E1, E2 and EE2 (Table 1.7). Plants 1 and 2 both had one facultative pond and two maturation ponds while Plant 3 only had a facultative pond. The authors suggested that the operational and design conditions might have directly affected the treatment performance of the different plants. A pond system consisting of three ponds with retention times longer than 24 hours was evaluated by Qiang et al. (2013) and these findings are presented in Table 1.7.

These results indicate variable removal efficiencies and it seems that pond systems with longer hydraulic retention times performed better. In this regard, the pond systems investigated by Qiang et al. (2013) were operated at longer retention times compared to the three evaluated by Pessoa et al. (2014). As sludge from the pond systems was not analysed, the fate of the compounds was not established. Depending on the design and operating conditions, sludge could stay for very long periods in the pond system.

Table 1.7: Removal efficiencies of stabilisation ponds for selected EDCs, as reported by different workers

Compound	% Removal	Reference
E1	65 / 41.4 / 31	(Pessoa et al., 2014)
E2	95 / 100 / 81	
E2-17A	100	
EE2	29.6 /100 / 58.4	
Estrone (E1)	97	(Qiang et al., 2013)
17b-estradiol (E2)	> 91	
estriol (E3)	> 95	
17a-ethinyl estradiol (EE2)	89	
bisphenol A (BPA)	98	
4-nonylphenol (NP)	98	

The bottom of a stabilisation pond is usually anaerobic, which can result in anaerobic biodegradation of the biosolids in the pond systems and, by implication, pond systems could display higher oestrogenic activity, according to research findings regarding anaerobic digestion.

1.5.3.3 Removal of EDCs during sludge stabilisation

The purpose of sludge stabilisation is to reduce the active biomass in sludge to a level where it can be disposed of without producing offensive odours or attracting nuisance insects (Metcalf and Eddy Inc., 2004). The two most commonly used stabilisation processes are anaerobic and aerobic digestion.

Anaerobic digestion is a multistage process. Biosolids are degraded in four steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) to carbon dioxide, methane and water (Metcalf and Eddy Inc., 2004). The process is commonly applied in the mesophilic temperature range of 32–38°C.

Studies have evaluated the removal of oestrogen hormones during anaerobic digestion. Muller et al. (2010) evaluated the removal of E1, E2 and E3 in an anaerobic digestion which was fed with a mixture of primary and waste activated sludge, and they found that about 30–40% of these compounds were removed during the process. Furthermore, Ifelebuegu (2011) has reported removal of E1, E2 and EE2 to be within the range 21–24%, 18–32% and 44–48%, respectively. Furlong et al. (2010) compared different sludge stabilisation processes and found that natural and synthetic oestrogen hormones were completely removed during aerobic digestion while anaerobic digestion was less successful. However, their bioassay analysis (YES test) established poor

reduction of oestrogenicity during aerobic digestion and an increase of oestrogenicity in anaerobically digested sludge. The increase in oestrogenicity during anaerobic digestion processes was attributed to an increase in alkylphenols compounds which display higher oestrogenicity than the ethoxylated precursors. The biotransformation of longer nonylphenol ethoxylates to di-ethoxylated nonylphenol (NP2EO), mono- ethoxylated nonylphenol (NP1EO) and NP during anaerobic digestion was demonstrated by Samaras et al. (2013). These findings imply that anaerobically digested sludge could pose serious health and environmental risks if considered for re-use.

Samaras et al. (2013) also analysed several other EDC compounds to evaluate how efficiently they are removed during anaerobic digestion. They found that mono-ethoxylated nonylphenol (NP1EO) and di-ethoxylated nonylphenol (NP2EO) were poorly biodegraded under anaerobic conditions, but there was more than an 80% reduction in ibuprofen and naproxen concentrations.

1.5.4 A mass balance approach to determine the fate of EDCs during wastewater treatment

Initially, most of the studies to determine whether EDCs are efficiently removed in WWTPs based their calculations on the concentrations of the compounds in the influent and effluent. Recently, more and more researchers have tried to establish the fate of these compounds by also evaluating the sludge streams in the treatment plants. However, when a mass balance is performed, where both the concentration and the flow rate of the different streams is considered, interpretation of data significantly improves, thus providing a more accurate picture.

Lee et al. (2004) have proposed a mass balance procedure where all streams are evaluated including the influents and effluents of each unit process in the treatment plant (Figure 1.3) as well as the flow rates in the different streams. However, in order to perform accurate mass balances, the following challenges have to be addressed (Lee et al., 2004), namely:

- The sludge streams contain both liquid and solid phases and each phase could have considerable amounts of EDCs, which should be taken into consideration when mass balance calculations are made.
- The formation of intermediate compounds. Some EDCs are removed but are replaced by other oestrogenic intermediates which are equally oestrogenic.
- The level of conjugation of EDCs. Oestrogens such as E1, E2 and EE2 enter WWTPs in conjugated form but are deconjugated during treatment, which will increase the E2 and EE2 concentrations.
- Conjugated forms of EDCs are biologically inactive and are not measured in bioassay methods (such as the e-screen method).

Lee et al. (2004) stressed the importance of taking composite samples over a 24-hour period. The substance of this point can be illustrated with the results obtained by Heidler and Halden (2007). They monitored triclosan hourly over a 24-hour period and two distinctive peak concentrations of the compound were observed around mid-day (Figure 1.4).

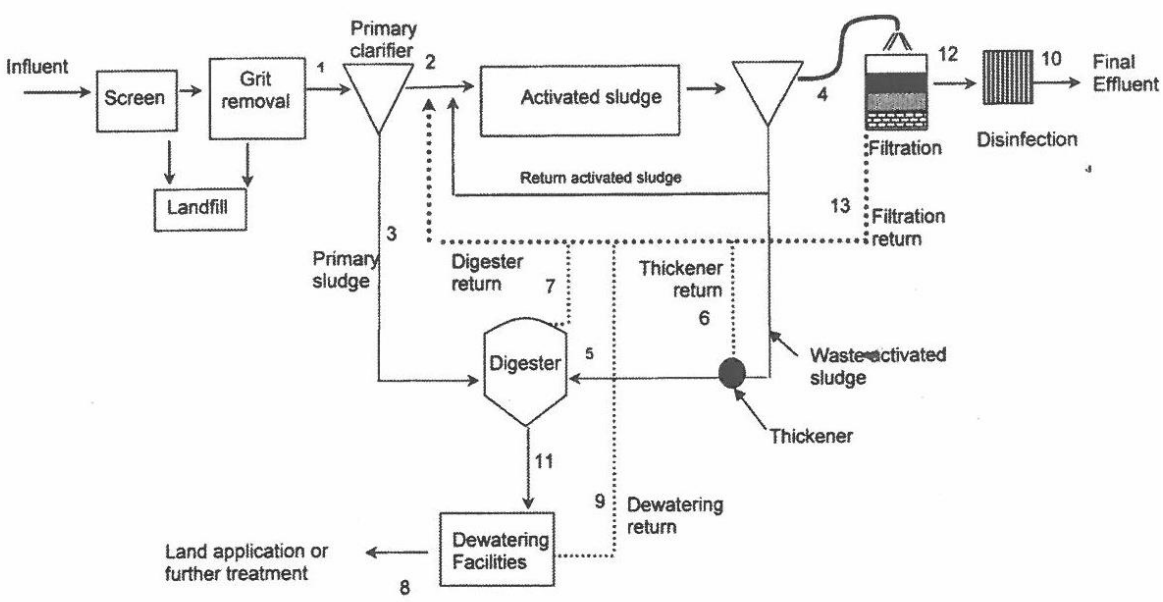


Figure 1.3: Location of sampling points in a wastewater treatment plant (Lee et al., 2004)

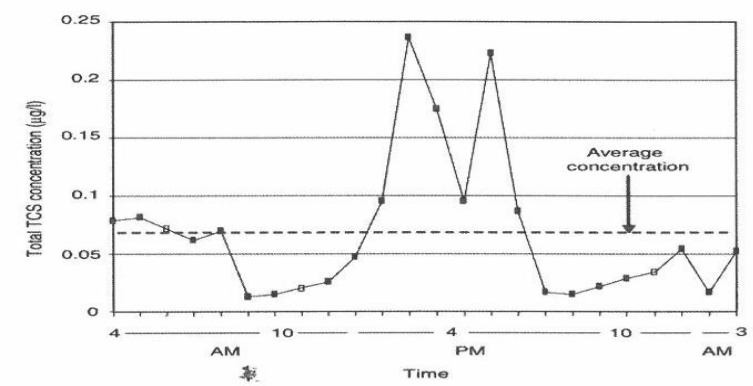


Figure 1.4: Variation in the concentration of triclosan in the effluent from a wastewater treatment plant over a 24-hour period (Heidler & Halden, 2007)

Heidler and Halden (2007) used a mass balance approach to assess triclosan removal in an activated sludge process. They collected composite samples before and after each unit process, noted the flow rates and performed mass balance calculations for triclosan. Their results indicated that 50% of the triclosan mass was in the sludge, 48% was transformed or lost and 2% was still present in the effluent.

Bertanza et al. (2011) determined the fate of nonylphenol and bisphenol A, together with the parent compounds mono- and di-ethoxylated nonylphenol, in a conventional activated sludge plant by means of a mass balance approach (Table 1.8). These compounds were primarily degraded in the activated sludge process with significant amounts still present in the effluent.

Table 1.8: The fate of selected EDCs in an activated sludge process using a mass balance approach (Bertanza et al., 2010)

Compound	Primary sludge %	Biodegraded in activated sludge process %	Waste activated sludge %	Effluent %
Nonylphenol	5.5	73.6	0.5	20.4
Bisphenol A	6.4	79.3	0.4	13.9
NP1EO	2.4	84.1	0.3	13.2
NP2EO	5.4	62.0	0.5	32.1

1.6 CONCLUSION

Research indicates variable removal efficiencies (from 0 to 100%) for different EDCs. These differences in removal efficiencies can be attributed to:

- Differences in the chemical structure of EDCs with different physicochemical properties. Chemical structures in the same application class may also differ. For example, compounds used as antibiotics displayed variable removal efficiencies.
- The type of treatment technology used and the operating conditions applied in the processes. Activated sludge processes were found to be more efficient than biological filtration systems. In activated sludge systems, it was found that sludge age is an important control parameter which has an effect on EDC removal efficiency. At longer sludge ages, EDC removal is more efficient. There is also an indication that nitrification promotes the removal of EDCs.

The main EDC removal mechanisms in WWTPs are adsorption and biodegradation. Thus, a large fraction of the EDCs are adsorbed onto the sludge surface. Aerobic digestion seems to reduce the concentrations better than anaerobic digestion. Findings also suggest that during anaerobic digestion some compounds are transformed to intermediates which either exhibit similar oestrogenic characteristics or are even more oestrogenic than the original compound.

Investigations into treatment technologies used in rural and remote areas are very limited. With regards to waste stabilisation pond systems, only evaluations for the removal of oestrogens, bisphenol A and 4-nonylphenol were found.

Studies on the removal of EDCs from WWTPs have mostly evaluated the influents and effluents from the treatment plants, and a few studies compare different technologies such as activated sludge and biological filters. Some findings make reference to the effect of sludge age on the removal of EDCs. However, there is a need to relate removal efficiency more closely to the operating conditions in the various unit processes in a plant by using a mass balance approach. These findings can assist treatment plant managers to optimise the removal of EDCs.

1.7 PROJECT AIMS AND OBJECTIVES

The project aims and objectives were:

- Screen the influent EDCs and monitor various stages of three selected types of WWTPs for target EDCs
- Determine and compare EDC removal efficiencies by using a mass balance approach
- Set up laboratory-scale activated sludge and anaerobic digestion reactors and determine their removal efficiency for each of the selected EDCs, during steady state operation.

Chapter 2

SCREENING AND MONITORING OF WASTEWATER TREATMENT PLANTS FOR ENDOCRINE DISRUPTING COMPOUNDS

2.1 INTRODUCTION

Effluent discharged from municipal WWTPs is the main source of EDCs in water resources (Caliman & Gavrilescu, 2009). WWTPs were originally designed to remove organic matter from wastewater, and, later, nitrogen and phosphorous were also included. Thus, WWTPs are not designed for the removal of EDCs and global findings indicate that trace quantities are being discharged into the aquatic environment. There is a need to institute screening and monitoring programmes at WWTPs to accurately assess the magnitude of the risks associated with these compounds. Moreover, EDCs have to be eliminated to prevent further contamination of water resources.

There is a vast array of trace organic compounds that are endocrine disrupting and which could be present in domestic wastewater (Schug et al., 2016). To establish the magnitude of the risks posed by EDCs, it is, firstly, essential to identify as many EDCs as possible which could be present in wastewater and, secondly, to quantify the compounds in terms of mass loads present in the influents and effluents from WWTPs. The mass load of the EDCs and amount of water flowing in the receiving river will determine the actual concentrations of the EDCs in the river. Findings have indicated oestrogenic effects in rainbow trout at E2 and EE2 concentrations of 10 and 0.5 ng/L, respectively (Purdom et al., 1994). Estimation of the mass loads entering receiving water resources will assist in establishing the magnitude of the risk.

The removal of EDCs during wastewater treatment occurs mainly by adsorption and biodegradation (Racz & Goel, 2010). EDCs may adsorb onto solid particles entering a wastewater treatment plant and be removed during the primary treatment stage, or they could adsorb onto the biomass generated during secondary treatment. The octanol water partition coefficient (K_{OW}) and the solid–water distribution coefficient (K_d) are frequently used to predict the degree to which an organic compound will be adsorbed onto solid particles in a WWTP (Carballa et al., 2004; Arvaniti & Stasinakis, 2015). The biodegradability of EDCs is influenced by the complexity of their organic molecules, as more complex molecules are often difficult to biodegrade. However, operating conditions in the plant also play a role in removal efficiency. Factors identified to date are: the diversity of the microbial community, sludge age, hydraulic retention time and the availability of oxygen (Luo et al., 2014). It becomes increasingly important to evaluate the elimination of different EDCs in relation to operating conditions in a plant, to establish a knowledge base for optimising removal efficiency.

The complete removal of EDCs during wastewater treatment will probability not be possible. However, if removal efficiency could be optimised without compromising the quality of the effluent with regard to the main objectives of the treatment, namely, removal of suspended solids, nutrients and pathogens, application of advanced treatment technologies could become economically viable.

The objectives of this section of the project report are to describe screening and monitoring of WWTPs for selected EDCs and to relate the removal efficiencies of different treatment technologies to the operating conditions in the WWTP by using a mass balance approach.

2.2 SAMPLING SITES

Three WWTPs were selected, based on the need to assess removal of EDCs by different technologies commonly used in the South African context. Table 2.1 lists the selected WWTPs. Two of the plants are in the City of Tshwane, which is a metropolitan municipality, while the third is in Phola, which is a rural community in the eMalahleni Local Municipality. Both the Daspoort and Zeekoegat treatment works utilise activated sludge processes, but the process design and operating conditions are different. At Daspoort and Zeekoegat, 24-hour composite samples were taken, while at Phola only grab samples were taken owing to lack of security and traveling distance.

Table 2.1: Selected WWTPs monitored for EDC removal

Municipality	Location	Major influent type	Type of treatment process	
City of Tshwane	Zeekoegat	Domestic/Industrial	**Activated sludge**	
			Process configuration	**Aeration type**
			Johannesburg	Diffused air (centrifugal blowers)
City of Tshwane	Daspoort	Domestic/Industrial	**Activated sludge**	
			Process configuration	**Aeration type**
			Three-stage Phoredox™	Surface aerators
eMalahleni Local Municipality	Phola	Domestic	Anaerobic ponds	Biological filters

2.2.1 The wastewater treatment plants

Daspoort Wastewater Treatment Plant

The Daspoort activated sludge plant uses the 3-stage Phoredox™ configuration, which has anaerobic, anoxic and aerobic zones. The process is operated at a sludge age of 26 days. The sludge in the primary settling tank (PST) is internally recycled to enhance volatile fatty acid (VFA) production for improved biological phosphate removal in the subsequent activated sludge process.

The plant draws influent wastewater at a near constant flow rate from a main collector sewer, which runs to Rooiwal WWTP. This sewer collects wastewater from the western part of Pretoria. The wastewater passes through the inlet works, comprising screens and grit chambers, to two PSTs. After the PSTs, the flow splits into three parallel, 3-stage Phoredox™ processes (Figure 2.1).

The effluents from six secondary settling tanks (SSTs) come together in one channel for a short distance, after which the flow splits into two streams. The one stream is disinfected with ultraviolet light, while the other one is treated with chlorine gas. After disinfection, the two effluent streams join and are discharged into the Apies River. The primary sludge from the PSTs is settled by gravity and then pumped to anaerobic digesters. The WAS is thickened in a dissolved air flotation thickener, after which it enters the anaerobic digester along with the primary sludge. The stabilised sludge is discharged to drying beds. Samples were taken from the water and the sludge streams at various points. The sampling points for the Daspoort activated sludge process are indicated in Figure 2.1. Samples from the wastewater stream were taken as follows: upstream of the PSTs (Point No. 1), downstream of the PSTs (Point No. 2), and downstream of the SSTs (Point no. 3), as indicated in Figure 2.1.

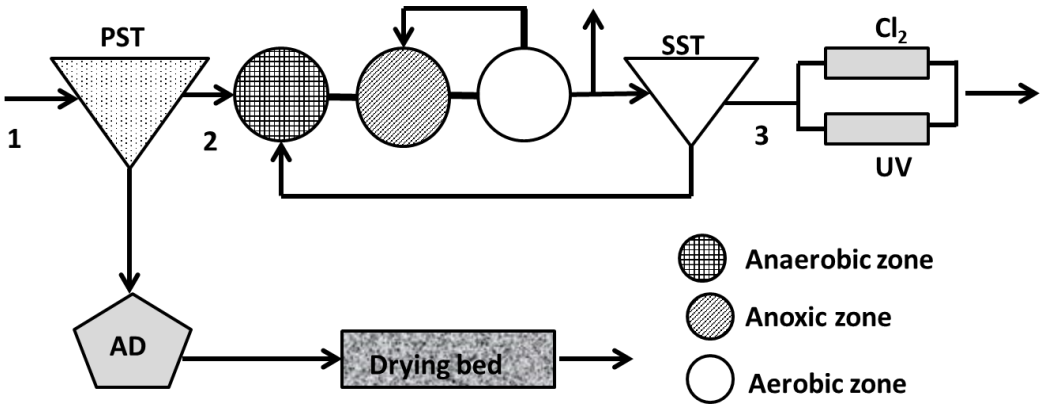


Figure 2.1: A schematic layout of Daspoort activated sludge plant. The numbers (1–3) indicate the sampling points

Zeekoegat wastewater treatment plant

At Zeekoegat WWTP, a number of configurations are used to achieve nutrient removal. At the time of sampling, the Johannesburg Process was being used. The Johannesburg configuration consists of pre-anoxic, anaerobic, anoxic and aerobic zones. The underflow of the SST flows to the pre-anoxic zone while the settled sewage from the balancing tank flows into the anaerobic zone. The plant was being operated at a sludge age of 35–45 days. The four PSTs are used on a batch basis to generate VFAs to enhance biological phosphate removal. The contents of a PST (sludge plus wastewater) is discharged every fourth day into the balancing tank, the contents of which flows into the activated sludge reactors. Table 2.1 compares the operating conditions of the Daspoort and Zeekoegat WWTPs.

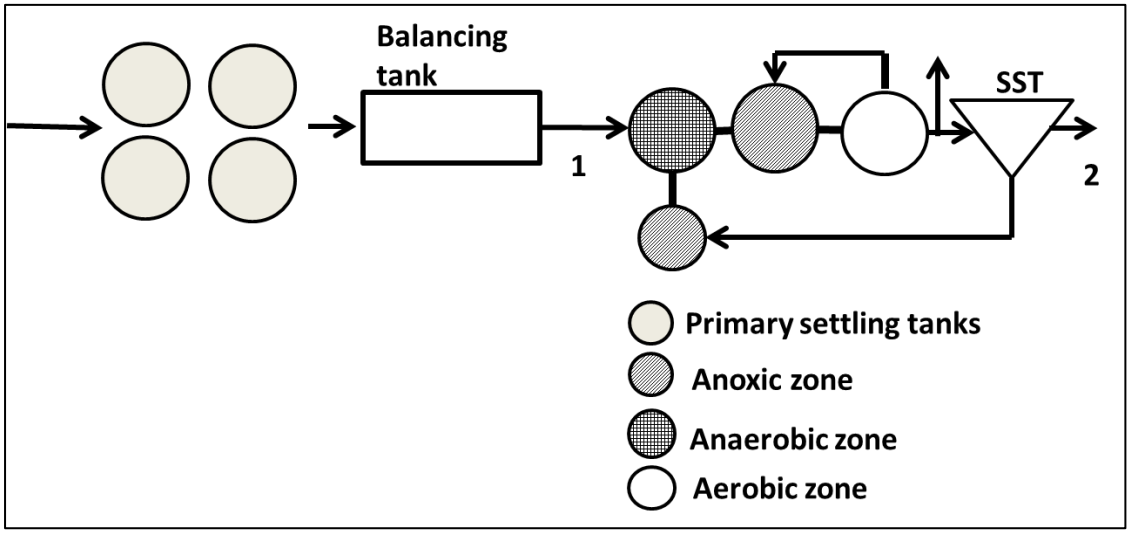


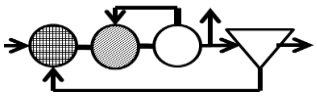
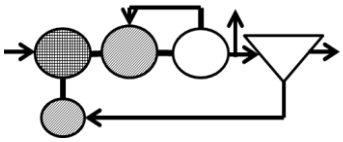
Figure 2.2: A schematic layout of Zeekoegat activated sludge plant. The numbers (1 and 2) indicate the sampling points

The sampling points for the Zeekoegat activated sludge process are indicated in Figure 2.2. Samples were taken as follows: downstream of the balancing tank (Point No. 1) and downstream of the SSTs (Point No. 2). At Zeekoegat, monitoring was mainly done to compare the performance of this activated sludge process with that employed at Daspoort as there is no desludging of the PSTs, and thus it was decided to sample only after the balancing tank and the SST.

Phola wastewater treatment plant

The Phola WWTP is located close to the town of Ogies which falls under the eMalahleni Local Municipality, Mpumalanga Province, and is about 140 km from Pretoria. The 2.8 ML/d plant serves the Phola Township near Ogies. The wastewater treatment process is an example of the pond enhanced treatment and operation (PETRO) system. This consists of a series of anaerobic ponds followed by a trickling filter. The raw wastewater flows through a set of screens (see Figure 2.3) and two vortex degritters, after which the flow splits into two parallel treatment units. Half of the flow is treated in two parallel series of anaerobic ponds. At the end of the pond system the effluents are combined and are treated in a biological trickling filter. Sludge from the anaerobic ponds is discharged onto drying beds every three weeks. The effluents from the humus tanks are combined and flow through the disinfection unit and into a reed bed before the water is discharged into the nearby river.

Table 2.2: Comparison of the operating conditions of the Daspoort and Zeekoegat activated sludge processes

Operating variables	Daspoort activated sludge process	Zeekoegat activated sludge process
Operating capacity, ML/d	38	45
BNR configuration		
Sludge age, days	30	35–45
Hydraulic retention time, hours	12	12
Average mixed liquid suspended solid concentration, mg/L	3 000	5 000
Waste activated sludge withdrawal	Direct from aeration tank	Direct from aeration tank
Method of VFA generation	Activated primary tank: sludge is internally recycled to maintain a sludge age of 4 days in the tank. The settled wastewater flows to the anaerobic zone.	Four PSTs are operated on a batch basis. Every fourth day the contents of the tank are discharged into the balancing tank.
Balancing tank	NO	YES

The sampling points at Phola WWTP are indicated in Figure 2.3. Samples from the wastewater stream were taken as follows: downstream of the degritters (Point No.1); downstream of the anaerobic ponds (Point no. 2) and at the discharge point of the trickling filter effluent (Point No. 3).

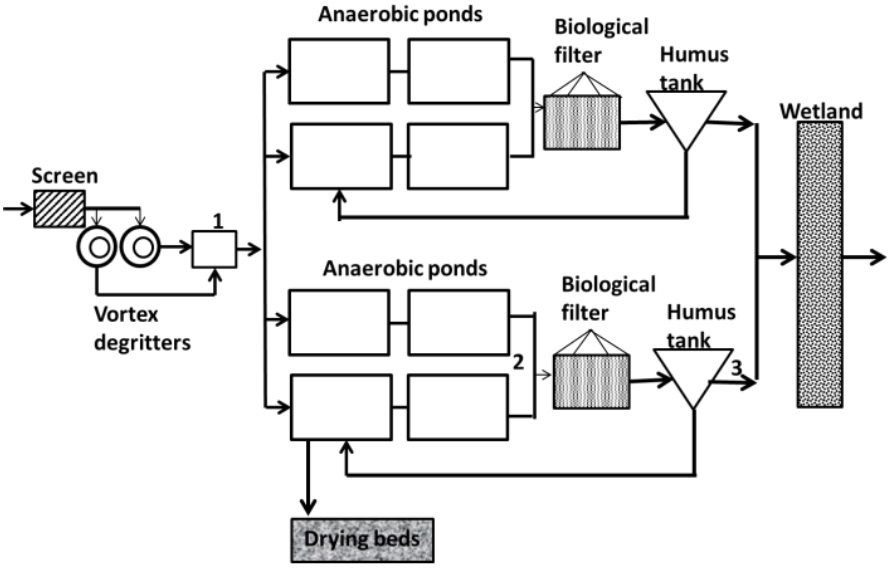


Figure 2.3: A schematic layout of the Phola Wastewater Treatment Plant. The numbers (1–3) indicate the sampling points

The operating conditions at Phola WWTP are indicated in Table 2.3.

Table 2.3: Operating conditions at Phola Wastewater Treatment Plant

PARAMETERS	**Treatment technologies**	
Flow rate, ML/d	**2.8**	
	Anaerobic ponds	**Biological filters**
Retention time, days	1.6	
Organic loading rate	0.23 kg BOD/m^3/d	3.7 g BOD/m^2/d
Nitrogen loading rate		0.9 g TKN/m^2/d

BOD – Biological oxygen demand

2.3 SAMPLING PROCEDURES

The raw wastewaters from Daspoort, Zeekoegat and Phola WWTPs were selected to perform the initial screening for the presence of EDCs. Composite sampling was performed and 24-hour composite samples were taken from the wastewater treatment processes at Daspoort and Zeekoegat WWTPs, while grab sampling was performed at Phola WWTP. The samplers were fitted with Teflon-lined tubing and amber glass bottles to protect the samples from sunlight and to prevent contamination by plastic material.

All glassware and funnels were triple rinsed with methanol and allowed to dry prior to sample collection. During transit to the laboratory, samples were kept on ice and stored at 4°C and analysed within 14 days of collection. Liquid samples were collected in glass-stoppered amber bottles (2.5 L) and acidified to a pH of < 3 to prevent the loss of the target EDCs via biological degradation and hydrolysis (Petrović and Barceló, 2000), while 1% (v/v) formaldehyde was also added for sample preservation.

Similar procedures were followed for the monitoring of the three WWTPs at the different sampling points, as indicated in Figures 2.1 to 2.3.

2.4 ANALYTICAL METHODS

2.4.1 Screening for the presence of EDCs in the wastewater

GC×GC-TOFMS analysis

The initial screening for the presence of EDCs in the influents to the three WWTPs was done with two-dimensional gas chromatography-time-of-flight mass spectrometry **(**GC×GC-TOFMS**),** which can be used to detect unknown pollutants in water. The drawback of this method is that only volatile organic compounds can be detected and non-target compounds should be present in the reference database. Raw wastewater samples were prepared for analysis according to the method described by Patrick et al. (2012) and analysed using GC×GC-TOFMS. The operating conditions for GC×GC-TOFMS analysis are given in Table 2.4, below.

Table 2.4: The operating conditions for GC×GC-TOFMS analysis

GC:	Agilent 7890A
Detector:	LECO Pegasus 4D Time-of-Flight Mass Spectrometer
Acquisition rate:	100 spectra/s
Mass range:	40 to 550 Da
Source temperature:	230°C, ionisation mode EI+
Detector voltage:	1 815 Volts
GC inlet temperature:	250°C
Inlet mode:	Split 50:1
Carrier gas:	Helium 1.4 ml/min, constant flow mode
Column 1:	Rxi-5Sil MS, 30 m x 0.25 mm ID x 0.25 µm film thickness
Column 2:	Rxi-17Sil MS, 0.97 m x 0.25 mm ID x 0.25 µm film thickness
Column 1 oven:	50 °C for 3 min to 300 °C at 10 °C/min, hold 5 min
Run time:	33 min
Column 2 oven offset:	10°C (relative to primary oven)
Modulator offset:	15°C (relative to secondary oven)
Modulation period:	3 s (hot pulse time 0.75 s)
Transfer line temperature:	300°C

LC-MS/MS analysis

Liquid chromatography-tandem mass spectrometry (LC/MS/MS**)** was used to screen for specific EDCs in the following classes: oestrogens, PFCs and pharmaceuticals. Table 2.5 shows the compounds which were included in the initial screening.

Table 2.5: EDCs screened for with LC/MS/MS

Class of EDC	Compound	Abbreviations
Oestrogens	Oestrone	E1
	17β-oestradiol	E2
	Estriol	E3
	17α-ethinyloestradiol	EE2
Perfluorinated hydrocarbons	Perfluorobutanoic acid	PFBA
	Perfluorodecanoic acid	PFDA
	Perfluorooctanoic acid	PFOA
	Perfluorohexanoic acid	PFHxA
	Perfluoro-1-butanesulfonate	PFBS
	Perfluoro-1-octanesulfonate	PFOS
	Perfluoro-1-hexanesulfonate	PFHxS
	Perfluorononanoic acid	PFNA
	Perfluoro-1-butanesulfonate	PFBS
	Perfluoro-1-Heptanesulfonate	PFHpS
	Perfluorononanesulfonate	PFNS
	Perfluorotetradecanoic Acid	PFTeDA
	Perfluorotridecanoic Acid	PFTrDA
	Perfluoroundecanoic Acid	PFUdA
	Perflyohexadecanoic Acid	PFHxDA
	Perfluoro-1-Dodecanesulfonate	PFDoS
	Perfluoro-1-Decanesulfonate	PFNS
	Perfluorononanesulfonate	PFTeDA
Pharmaceuticals	Nalidixic acid	
	Acetaminophen	
	Bezafribate	
	Carbamazepine	
	Lamivudine	
	Stavudine	

Sample preparation, extraction and clean-up

After collection, aqueous samples were filtered to remove particles that could interfere with the extraction procedure. Extraction of EDCs from liquid samples was based on solid phase extraction (SPE) with disposable cartridges. The SPE cartridges (Oasis HLB, 60 mg) were preconditioned with 6 mL of methanol and 6 mL of water. Thereafter, the samples (250 mL) were loaded onto the cartridges. After sample loading, the cartridges were washed with 6 mL of water and dried for 45 min under vacuum. The dried cartridges were eluted with 6 mL of methanol, and the extracts were evaporated to dryness under a gentle nitrogen stream. The dried samples were reconstituted with 1 mL of methanol (Vanderford et al., 2003). The samples were analysed using LC-MS/MS.

Liquid chromatography-tandem mass spectrometry

A binary pump (Agilent G1312A, Palo Alto, CA) and an autosampler (HTC-PAL, CTC Analytics, Zwingen, Switzerland) were used for all analyses. All analytes were separated using a 250 x 4.6 mm C_{12} column (Synergi Max-RP) with a 4 µm particle size. A binary gradient consisting of 0.1% aqueous formic acid (v/v) (A) and 100% methanol (B) at a flow rate of 700 µL/min was used. The gradient was as follows: 5% B held for 3.5 min, increased linearly to 80% by 10 min and held for 3 min, and finally stepped to 100% and held for 8 min. A 9-min equilibration step at 5% B was used at the beginning of each run to bring the total run time per sample to 30 min. An injection volume of 10 µL was used for all analyses. Mass spectrometry was performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). Optimisation of the mass spectrometer was done in three separate steps:

1. Determination of the best ionisation source and polarity;
2. Optimisation of compound-dependent parameters;
3. Optimisation of source-dependent parameters.

To determine the best ionisation source and mode for each analyte, all compounds were analysed using ESI positive/negative and atmospheric pressure chemical ionisation (APCI) positive/negative. For the electrospray ionisation (ESI) source, each analyte was infused directly into the mass spectrometer at a concentration of 100 ng/mL and a flow- rate of 10 µL/min. For the APCI source, each analyte was prepared at a concentration of 1 µg/mL in methanol and infused at 10 µL/min into a faster flow- rate of 700 µL/min methanol via a mixing tee, due to the higher flow rate required by the APCI source. During the infusions, the first quadrupole of the mass spectrometer (Q1) was scanned while the declustering potential was raised and lowered. Typically, Q1 was scanned from m/z 50 to [M + 100]. This allowed the most intense precursor ion to be selected for each source/mode. From these, the optimal source and polarity were selected. Once the best ionisation source/polarity had been established, the optimal compound-dependent parameters for each analyte were determined using that source/polarity (Vanderford et al., 2003).

2.4.2 Monitoring of EDC removal by different wastewater treatment plants

The three WWTPs, Daspoort, Zeekoegat and Phola, were monitored every three months over a one-year period. Samples were taken and prepared as described in Section 2.4.1 and the different sampling points were as indicated in Figures 2.1, 2.2 and 2.3. EDCs identified during the initial screening were mainly oestrogens, PFCs and pharmaceutical compounds. Compounds from the three classes were selected to be monitored as indicated in Table 2.6. Analyses were done with LC/MS/MS, as described above.

Table 2.6: EDCs monitored during this investigation

Class of EDC	Sub-class	Compound
Oestrogens		Estrone
		17β-estradiol
		17α-ethinylestradiol
Perfluorinated hydrocarbons		Perfluorobutanoic acid
		Perfluorodecanoic acid
		Perfluorooctanoic acid
		Perfluorohexanoic acid
		Perfluoro-1-butanesulfonate
		Perfluoro-1-octanesulfonate
		Perfluoro-1-hexanesulfonate
Pharmaceuticals	Antibiotic	Nalidixic acid
	Analgesic drug	Acetaminophen
	Lipid regulator	Bezafribate
	Epileptic drug	Carbamazepine
	Antiviral drug	Lamivudine
		Stavudine

2.5 MASS LOAD CALCULATIONS

The daily mass loads discharged from Zeekoegat, Daspoort and Phola WWTPs were calculated and were used to determine the percentage EDC removal. The mass load for Daspoort WWTP was calculated according to the following formulae:

Mass load (mg/d) = C_{eff} x Q_{eff}……(1)

and

$Q_{eff} = Q_{in} – Q_{Psludge} - Q_{WAS}$……..(2)

where:

C_{eff} = concentration of the compound in the effluent (mg/ML)

Q_{eff} = flow rate of the effluent (ML/d)

$Q_{PSludge}$ = flow rate of the primary sludge (ML/d)

Q_{WAS} = waste activated sludge flow rate (ML/d)

Q_{in} = Influent flow rate, (ML/d)

Q_{eff} was calculated to be 37.618 ML/d, based on an average influent flow rate of 38 ML/d, a PST sludge wasting rate of 0.08 ML/d and a wasting rate of activated sludge at a rate of 0.302 ML/d.

At Zeekoegat WWTP, the PST sludge is discharged together with the liquid portion into the balancing tank, thus, only WAS is removed during the activated sludge process. The mass balance calculation for Zeekoegat was done by using Equation (3).

$Q_{eff} = Q_{in} – Q_{WAS}$……(3)

where:

Q_{in} = influent flow rate, 55.5 ML/d

Q_{WAS} = waste activated sludge flow rate, 0.35 ML/d

2.6 THE PERFORMANCE EFFICACY AND EFFLUENT DISCHARGE STANDARDS

Both Daspoort and Zeekoegat are BNR plants. The plants are required to comply with effluent discharge standards for COD, ammonium, nitrate and phosphate. Both plants comply, 99% of the time, with the limits set by the Department of Water and Sanitation for nutrients in the effluent (DWA, 2013) as indicated in Table 2.7. The average nutrient concentrations of the two plants were as follows: COD *ca* 30 mg/L, ammonium < 1.0 mg N/L, and phosphate < 0.5 mg P/L. Nitrate concentration was between 5 and 7 mg N/L at Daspoort WWTP and between 3 and 5 mg N/L at Zeekoegat WWTP.

The main operational difference between the two plants is in the sludge age. Daspoort WWTP is operated at a sludge age of *ca* 26 days while Zeekoegat WWTP is operated at a sludge age of 35–45 days.

Table 2.7: Performance efficacy of the three WWTPs with regard to the effluent standards for nutrient removal

Parameter	Zeekoegat	Daspoort	Phola	General Standards*
COD, mg/L	30	30	< 75	75
Ammonia, mg N/L	< 1.0	< 1.0	< 6	6
Nitrate, mg N/L	3–5	5–7	10–15	15
Phosphate, mg P/L	< 0.5	< 0.5	11–14	1.0**

* DWA (2013)

** Special phosphate standard

Phola WWTP is small and consists of an integrated pond system primarily designed to remove COD and ammonium. Data about the performance of the plant was not readily available. Furthermore, the plant was frequently not operational as the operators were experiencing problems with the pump at the

pumping station; consequently, the plant received no inflow during down-times. Notwithstanding the operating problems experienced at the plant, the performance was found to be fairly good, based on analyses done by the team. The COD concentration was < 75 mg/L, ammonium concentration < 6 mg/L, and the nitrate concentration was between 10 and 15 mg/L. The phosphate concentration was in the range 11–14 mg/L.

2.6.1 Screening of the wastewater for EDCs

The raw wastewater entering Daspoort and Zeekoegat WWTPs contained more than 300 organic compounds while the raw wastewater entering Phola WWTP contained about 200 organic compounds. The Endocrine Disruption Exchange (TEDX) (2016) list was used to identify some of the common EDCs, as indicated in Table 2.8. A total of 44 compounds were identified. Common EDCs detected in all three plants included: triclosan, acetaminophen, caffeine, efavirenz, stigmastanol, etilefrine, levomenthol, aspirin, prednisolone, ibuprofen, norephedrine, bezafribate, carbamazepine, lamivudine, nalidixic acid, stavudine, coumarin, boldenone, androsterone, oestrone (E1), 17β-oestradiol (E2), oestriol (E3), 17α-ethinyloestradiol (EE2), octylmethoxycinnamate (OMC), oxybenzone, n-propyl - p- hydroxybenzoate (propylparaben), o,p-DDT, pyrimethanil, bisphenol A, butylbenzylphthalate(BBP), di-(2-ethylhexyl) phthalate (DEHP), di-n-butylphthalate (DBP), diethyl phthalate (DEP), perfluorobutanoic acid (PFBA), perfluorodecanoic acid (PFDA), perfluorohexanoic acid (PFHxA), perfluoro -1-butanesulfonate (PFBS), perfluoro-1-heptanesulfonate (PFHpS), perfluoro-1-octanesulfonate (PFOS), perfluoro-1-hexanesulfonate (PFHxS), perfluoro-n-pentanoic acid (PFPeA), perfluorooctanoic acid (PFOA), perfluorohexadecanoic acid (PFHxDA) and perfluorononanoic acid (PFNA).

The composition of the wastewaters entering the two plants in the City of Tshwane was very similar (Table 2.4), with only a few differences. In the wastewater received by Daspoort WWTP, the pharmaceutical compound, prednisolone (used to treat a variety of inflammatory and autoimmune conditions), was also detected while ibuprofen (analgesic) was only detected in the raw wastewater entering Zeekoegat WWTP. Pyrimethanil (a wide-spectrum fungicide), was only detected in the wastewater entering Zeekoegat WWTP. PFOA was only present in the wastewater entering the Daspoort WWTP.

Phola township is situated in a rural district of Mpumalanga, close to the town of Ogies. The number of organic compounds identified in the raw wastewater entering Phola WWTP was significantly less than the number identified in the wastewater generated in the City of Tshwane. No pesticides and fewer plasticisers were detected in the wastewater entering Phola WWTP. Boldenone (an anabolic-androgenic steroid), and androsterone (a steroid hormone) were not present in the wastewater entering Phola. Norephedrine, a decongestant and an ingredient in cough and cold medicines, which can be bought without a prescription, was present only in the wastewater entering Phola WWTP. PFHxDA and PFNA were only present in the wastewater entering Phola WWTP.

Following screening of influents for the three WWTPs, the identified EDCs were classified into five groups, namely oestrogens, perfluorinated chemicals (PFCs) and pharmaceuticals, pesticides, personal care products and plasticisers. However, the majority of the EDCs present were oestrogens, PFCs and pharmaceuticals.

Table 2.8: The most common EDCs detected in the raw wastewaters entering Daspoort, Zeekoegat and Phola Wastewater Treatment Plants

Compounds	Zeekoegat	Daspoort	Phola
Disinfectant*:*			
Triclosan	x	x	x
Pharmaceuticals:			
Levomenthol	x	x	x
Etilefrine	x	x	x
Stigmastanol	x	x	x
Acetaminophen	x	x	x
Aspirin	x	x	x
Efavirenz	x	x	x
Caffeine	x	x	x
Prednisolone		x	
Ibuprofen	x		
Norephedrine			x
Nalidixic acid	x	x	x
Bezafribate	x	x	x
Carbamazepine	x	x	x
Lamivudine	x	x	x
Stavudine	x	x	x
Fragment and fabric conditioner:			
Coumarin	x	x	x
Personal care products:			
Octyl-methoxycinnamate (OMC)		x	
Oxybenzone	x		x
n-Propyl p-hydroxybenzoate (Propylparaben)	x		
Pesticides:			
o,p-DDT	x	x	
Pyrimethanil	x		
Steroids:			
Boldenone	x	x	
Androsterone	x	x	
Oestrone	x	x	x
17β-oestradiol	x	x	x
Estriol	x	x	x
17α-ethinyloestradiol	x	x	x
Plasticisers:			
Bisphenol A	x	x	
Butyl benzyl phthalate (BBP)	x	x	
Di-(2-ethylhexyl) phthalate (DEHP)	x	x	x
Di-n-butyl phthalate (DBP)	x	x	
Diethyl phthalate (DEP)			x
Perfluorocompounds (PFCs)			
Perfluorobutanoic acid (PFBA)	x	x	
Perfluorodecanoic acid (PFDA)	x	x	
Perfluorooctanoic acid (PFOA)		x	
Perfluorohexanoic acid PFHxA	x	x	
Perfluoro-1-butanesulfonate (PFBS)	x	x	
Perfluoro-1-heptanesulfonate (PFHpS)	x	x	
Perfluoro-1-octanesulfonate (PFOS)	x	x	
Perfluoro-n-pentanoic acid (PFPeA)	x	x	
Perfluoro-1-octanesulfonate (PFHxS)	x	x	
Perfluorohexadecanoic acid (PFHxDA)			x
Perfluorononanoic acid (PFNA)			x

2.6.2 Evaluation of EDC removal by different wastewater treatment plants

The mass loads were calculated for the influents and effluents from the plants as indicated in Section 2.4. The results are shown in Table 2.9.

2.6.2.1Perfluorinated chemicals

The mass load of PFOS was found to be present in the highest concentration in all three plants. The influent mass load for PFOS into Daspoort was 19±10 g/d, which was significantly higher than the mass loads observed at Zeekoegat and Phola, namely 8±11 g/d and 0.2±0.3 g/d, respectively. The degradation of a number of PFCs into PFOS as end-product (Sinclair & Kannan, 2006) explains the high concentration of PFOS in the wastewater.
For Daspoort WWTP, the mass load for the different PFCs was in the range 0.03±0.02 to 19±10 g/d and they were found to be present in the following order, arranged from the lowest mass load to the highest:

PFDA< PFBA< PFPeA<PFOA< PFHxA< PFHxS<PFOS

At Zeekoegat WWTP, the PFC mass load ranged from 0.05±0.05 g/d to 8.±11 g/d and they were found to be present in the following order:

PFDA< PFOA<PFBA< PFHxA< PFPeA< PFHxS<PFOS

At Phola WWTP, the PFC mass load ranged from 0.002±0.001 g/d to 0.2±0.3 g/d, and they were found to be present in the following order:

PFDA< PFOA<PFBA<PFPeA<PFHxA<PFHxS<PFOS

The total PFC mass load into Daspoort WWTP was the highest, namely 24±7 g/d, compared to 11±3 g/d into Zeekoegat WWTP and 0.4±0.1 g/d into Phola WWTP. Daspoort received a higher PFC mass load than Zeekoegat although the inflow into Daspoort WWTP, which is 38 ML/d, is less than than the inflow into Zeekoegat WWTP, which is 55 ML/d.

PFC removal was inadequate in all three plants and PFCs were detected in the effluents of all three plants (shown in Table 2.9). The highest PFC removal efficiencies were observed at Zeekoegat WWTP, where the percentage PFC removal was as follows: PFDA (27%), PFOA (63%), PFBA (38%), PFHxA (43%), PFPeA (23%), PFHxS (75%), and PFOS (94%).

The removal efficiencies at Daspoort WWTP were as follows: PFHxS (17%), PFDA (44%), PFOA (54%), PFHxA (45%), PFPeA (23%), and PFOS (85%). The PFBA mass load was found to be 13% higher in the effluent than in the influent at Daspoort WWTP.

The removal efficiencies at Phola WWTP were lower compared to those of the other two plants with a maximum removal of 76% for PFOS, followed by 74% for PFHxS, 68% for PFHxA, 63% for PFOA, 55% for PFPeA, 35% for PFDA, and 17% for PFBA.

Biodegradation of precursor compounds can contribute to the increased concentrations observed for PFBA at Daspoort WWTP (Schultz et al., 2006).

Table 2.9: The mass loads entering and leaving Zeekoegat, Daspoort and Phola WWTPs for the EDCs monitored

EDC group	WWTP	Influent daily mass loads (g/d±SD)	PS + AS (%)	Effluent daily mass loads (g/d±SD)	WWTP	Influent daily mass loads (g/d±SD)	PS + AS (%)	Effluent daily mass loads (g/d±SD)	WWTP	Influent daily mass loads (g/d±SD)	AP + BF (%)	Effluent daily mass loads (g/d±SD)
PFCs:	**Zeekoegat**				**Daspoort**				**Phola**			
PFBA		0.4±0.4	38	0.2±0.2		0.1±0.1	-13	0.1±0.1		0.01±0.01	17	0.01±0.002
PFDA		0.05±0.05	27	0.04±0.04		0.03±0.02	44	0.02±0.01		0.002±0.001	35	0.001±0.001
PFOA		0.4±0.1	63	0.1±0.1		0.4±0.3	54	0.2±0.2		0.02±0.01	63	0.006±0.003
PFHxA		0.4±0.1	43	0.3±0.3		0.7±0.3	45	0.4±0.5		0.03±0.03	68	0.008±0.002
PFOS		8.1±10.6	94	0.5±0.2		19±10	85	3±2		0.2±0.3	76	0.06±0.05
PFPeA		0.5±0.4	23	0.4±0.2		0.6±0.4	23	0.4±0.2		0.02±0.02	55	0.008±0.003
PFHxS		1.2±0.8	75	0.3±0.2		3.0±1	17	2±2		0.05±0.05	74	0.012±0.008
Total PFCs		**11±3**	**84**	**1.8±0.1**		**24±7**	**75**	**6±1**		**0.4±0.1**	**75**	**0.1±0.02**
Oestrogens:												
E1		14±14	44	8±6		5±3	28	4±3		1.2±1.0	61	0.5±0.5
E2		0.1±0.1	12	0.05±0.04		0.06±0.01	34	0.04±0.02		0.01±0.01	76	0.003±0.002
EE2		0.5±0.3	61	0.2±0.2		0.4±0.4	55	0.2±0.1		0.05±0.03	65	0.02±0.01
Total Oestrogens		**15±8**	**47**	**8±5**		**6±3**	**33**	**4±2**		**1.2±0.7**	**70**	**0.5±0.3**
Pharmaceuticals:												
Nalidixic acid		8±1	-1.2	8±2		5.0±0.5	13	4±0.7		0.6±0.2	35	0.4±0.3
Bezafibrate		127±144	94	8±4		58±36	90	6±5		5±7	88	0.5±0.6
Acetaminophen		97±77	87	13±11		22±19	76	5±5		60±24	94	4±4
Carbamazepine		68±35	58	29±17		36±28	56	16±25		2±2	41	1±1
Stavudine		291±227	72	82±55		169±170	41	100±136		9±5	74	2±1
Lamivudine		36±18	100	nd		11±15	100	nd		4±2	52	2±1
Total Pharmaceuticals		**628±101**	**78**	**140±33**		**300±61**	**56**	**131±38.4**		**81±23**	**87**	**10±1**

PS – Primary Settling; **AS** – Activated Sludge; **AP** – Anaerobic Pond; **BF** – Biofilter; **SD** – Standard Deviation

The percentage removal of total PFCs varied between 23 and 94% for Zeekoegat, 0 and 85% for Daspoort and between 17 and 76% for Phola, which implied that PFCs were discharged from the three plants. The daily effluent mass loads were: Zeekoegat: 1.8±0.1 g/d; Daspoort: 6±1 g/d;. Phola: 0.10±0.02 g/d.

Removal efficiency for the different PFCs monitored varied significantly, as indicated above. In PFCs, hydrogen atoms are replaced with fluorine atoms in an alkyl carbon chain (Du et al., 2014) which creates chemically very stable compounds, generally considered to be non-biodegradable (Arvaniti & Stasinakis, 2015). Therefore, adsorption is regarded as the main removal mechanism for these compounds. PFCs have a strong hydrophobic chain which promotes adsorption onto solid particles. The adsorption capacity of individual PFCs is influenced by functional groups and the length of the carbon-fluorine chain. The adsorption capacities of long-chain perfluoroalkyl carboxy acids are better than short chain PFCs (Du et al., 2014). Researchers also found that PFOSs have a higher adsorption capacity than do PFOAs due to the longer carbon chain and the presence of sulfonic acid which has a higher acidity than PFOA (Arvaniti & Stasinakis, 2015). The results of this study also showed that PFOS was removed more efficiently than PFOA.

As PFC removal occurs mainly through adsorption, optimisation of solid separation processes will enhance PFC removal. Total removal was 75% at Phola and Daspoort WWTPs and 84% at Zeekoegat WWTP. Comparing the different treatment technologies employed in the three WWTPs revealed that the anaerobic pond system at Phola is designed to remove 80% of the BOD, where the secondary ponds enhance settlement of solids to provide a relatively solids-free effluent for the trickling filter system (Shilton, 2006). The hydraulic retention time in the pond system is 1.6 days (Table 2.3). The solids from the humus tanks are also recycled back to the pond system and wasting only occurs bimonthly. Essentially, the bulk of the sludge is effectively trapped in the anaerobic pond system, which enhanced PFC removal.

Comparisons between the two activated sludge systems revealed that PFC removal was better at Zeekoegat WWTP. Operating conditions at Zeekoegat, which could have contributed to the improved PFC removal efficiencies, were the longer sludge age and the higher MLSS concentration in the reactors. Zeekoegat WWTP is operated at a sludge age of 45 days, with an average MLSS content of 5 000 mg/L while the average sludge age at Daspoort is 26 days, with an average MLSS content of 3 000 mg/L. The higher MLSS increases the available surface area for adsorption of PFCs (Arvaniti et al., 2014).

Adsorption of PFCs onto solid particles is also influenced by the characteristics of the wastewater. Two parameters identified in the literature that enhance adsorption capacity (Du et al., 2014) are lower pH values and the presence of divalent cations (e.g. calcium ions). The pH and the concentration of cations in the wastewater were not considered during this investigation but need to be evaluated in future studies.

2.6.2.2 Oestrogen removal

Oestrone (E1)

The oestrogen that was present in the highest concentration in all three plants was oestrone (E1). The concentrations varied between 92 and 913 ng/L. The mass of E1 entering the three WWTPs was as follows: Zeekoegat: 14±14 g/d; Daspoort: 5±3 g/d; and Phola: 1.2±1.0 g/d. The removal efficiencies were 44%, 28% and 61%, respectively, with 8±6 g/d, 4±3 g/d and 0.5±0.5 g/d discharged in the effluents, respectively, for Zeekoegat, Daspoort and Phola.

17β-oestradiol (E2)

The mass loads of 17β-oestradiol (E2) entering the plants were as follows: 0.1±0.1 g/d for Zeekoegat, 0.06±0.01 g/d for Daspoort and 0.01±0.01 g/d for Phola, while 0.05±0.04 g/d, 0.04±0.02 g/d and 0.003±0.002 g/d, respectively, were discharged from the three plants. The best E2 removal, 76%, was

observed from Phola WWTP, compared to the 12% and 34% obtained from Zeekoegat and Dasport WWTPs, respectively.

17α-ethinyloestradiol (EE2)

The mass loads of 17α-ethinyloestradiol (EE2) entering and leaving Zeekoegat were 0.5±0.3 g/d and 0.2±0.2 g/d, respectively; for Daspoort, they were 0.4±0.1 g/d and 0.2±0.1 g/d, respectively; and for Phola they were 0.05±0.03 g/d and 0.02±0.01 g/d, respectively.

The removal efficiencies for EE2 were 61%, 55% and 65% for Zeekoegat, Daspoort and Phola, respectively.

The total mass of oestrogens was 15±8 g/d into Zeekoegat WWTP, 6±3 g/d into Daspoort WWTP and 1.3±0.7 g/d into Phola WWTP. The mass of total oestrogens discharged from the three plants was 8±5 g/d at Zeekoegat, 4±2 g/d at Daspoort, and 0.5±0.3 g/d at Phola WWTPs.

The removal of all oestrogens in the activated sludge process at Zeekoegat WWTP was 47%, which was better than the 33% removal achieved at Daspoort WWTP. At Daspoort WWTP, the removals were in the range of 28–55%, while at Zeekoegat WWTP the removal percentages varied between 12 and 61%. The integrated pond and biological filtration process as Phola performed better, where the removal efficiencies were in the range 61-76%, and 61% removal of all oestrogens.

The results obtained for oestrogens during this study confirmed the variable nature of the concentrations present in the influent and the degree of removal that can be achieved, as reported by other researchers. Hamid and Eskicioglu (2012) conducted a literature review on the concentrations of oestrogens found in the influents of WWTPs and also compared removal efficiencies achieved by different plants. In the influents, the E1 concentrations were found to vary from as little as 2.4 up to 670 ng/L, E2 concentrations varied from 4 up to 150 ng/L, and EE2 concentrations from 0.4 up to 14.4 ng/L. The removal percentages in the activated sludge plants were found to vary, for E1 from 22 to 95%, for E2 from 59 to 98%, and for EE2 from 52 to 100%. Chimchirian et al. (2007) reported E1 removal using a biological filter to be 41–89% (based on grab sample analysis).

Oestrogens are removed by a combination of adsorption and biodegradation (Racz & Goel, 2010). However, from the literature, it is evident that only a small percentage (10%) of the oestrogens are removed by adsorption which implies that biodegradation is the main removal mechanism.

Estimation of the efficiency of wastewater treatment at removing oestrogens is complicated as several biological transformations occur during treatment (Racz & Goel, 2010), namely:

- Deconjugation of oestrogens to the active unconjugated form;
- Biological conversion of E2 to E1;
- Two metabolic pathways exist whereby E2 is biodegraded, i.e. E2 can be co-metabolised and, under those conditions, the removal of E1 is unlikely. E2 can also be actively metabolised as a substrate under conditions where concentration of total organic carbon in the plant is low.

According to the literature, a longer sludge age and the presence of a nitrifying biomass enhances oestrogen removal, and removal efficiencies between 80 and 100% have been reported (Hashimoto et al., 2007). Both Daspoort and Zeekoegat WWTPs are operated under similar conditions but despite this fact, their performance was not as good as those reported in the literature. This implies that other factors also play a role in oestrogen removal. Longer hydraulic retention times are reported to enhance oestrogen removal (Belhaj et al., 2014). Both the activated sludge plants have HRTs of 12 hours. Belhaj et al. (2014) examined activated sludge flocs and suggests that an abundance of filamentous bacteria could play a role in poor oestrogen removal. Thus, the importance of sludge morphology needs to be investigated further. The marginally better removal at Zeekoegat WWTP could be ascribed to the use of diffused air aeration that provides better oxygen transfer rates than the surface aerators used at

Daspoort. Stadler (2016) reported that EE2 removal improves as dissolved oxygen concentration increases.

2.6.2.3 Pharmaceuticals

Of the three different classes of EDCs monitored, pharmaceutical mass loads were the highest entering all three plants. The mass load entering Zeekoegat was 628±101 g/d, into Daspoort, 300±61 g/d and flowing into Phola, 81±23 g/d.

An antibacterial drug - Nalidixic acid

Nalidixic acid is an antibacterial drug used for treating urinary tract infections (Drugs.com, 2017). The drug was detected in the influents and effluents of all three plants. The average mass loads in the influents were 8±1 g/d for Zeekoegat WWTP and 5.0±0.45 g/d for Daspoort WWTP. Mass loads in the effluents were 8±2 g/d and 4±0.7 g/d for Zeekoegat and Daspoort WWTPs, respectively (Table 2.9). At Phola WWTP the average influent mass load was 0.6±0.2 g/d and the average mass load in the effluent was 0.4±0.3 g/d.

The removal efficiencies for nalidixic acid were very low, i.e. no removal took place at Zeekoegat with 13% and 35% at Daspoort and Phola WWTPs, respectively. Ghosh et al. (2009) investigated the removal efficiencies for antibiotics in WWTPs in Japan and reported nalidixic acid removal of between 53 and 100% which is significantly higher than the removals noted during this study. The Japanese authors also found enhanced removals in BNR plants operated at HRTs between 11 and 13 hours and STRs between 7 and 18 days. These findings were in strong contrast to the poorer removal efficiencies found during this study. Daspoort and Zeekoegat WWTPs have similar HRTs, namely, 12 days. However, both were operated at longer sludge ages. The reasons for the poor removal of nalidixic acid in these two plants needs to be further investigated.

A lipid regulator - Bezafibrate

The mass of bezafibrate entering Zeekoegat WWTP was 127±144 g/d and the mass in the effluent was 8±4 g/d. The removal efficiency was 94%.

The influent to Daspoort WWTP contained 58±36 g/d with an average of 6±5 g/d in the effluent; the removal was 90%. Phola WWTP was found to have an average bezafibrate mass load of 5±7 g/d in the influent and 0.5±0.6 g/d in the effluent, with 88% removal efficiency. Kasprzyk-Hordern et al. (2009) reported 71% removal of bezafibrate in activated sludge plants and 45% in trickling filters, while Sipma et al. (2010) reported 21–99% removal in conventional activated sludge plants.

Bezafibrate is essentially biodegradable and has a log K_{OW} value of 4.25 (Rojas et al., 2012). Verlicchi et al. (2012) reported that bezafibrate removal increases when exposed to older sludge ages. Thus the older sludge age at Zeekoegat plant could have contributed to the improved removal of bezafibrate. Sui et al. (2016) conducted laboratory-scale experiments and found that the removal of bezafibrate increased with a decreased initial concentration of the drug, as the reactor removal rate followed first order kinetics (Sui et al., 2016). The average concentration flowing into the Zeekoegat plant (2 284±2 586 ng/L) was higher than the concentration in the inflow to Daspoort (1 520±938 ng/L)

An analgesic drug – Acetaminophen

Acetaminophen, a commonly used analgesic drug, also known as paracetamol, was detected in relatively high concentrations in the influent entering the Phola WWTP. The concentration was on average 21.7±5.0 µg/L, compared to the concentrations entering the Zeekoegat and Daspoort WWTPs,

that averaged 1.7±0.03 µg/L and 0.6±0.04 µg/L, respectively. This resulted in average mass loads of 97±77 g/d for Zeekoegat, 22±19 g/d for Daspoort, and 60±24 g/d for Phola.

The removal efficiencies for the three plants were 87%, 76% and 94%, respectively, for Zeekoegat, Daspoort and Phola WWTPs. Kasprzyk-Hordern et al. (2009) reported 99% removal of acetaminophen in activated sludge plants and 94% in trickling filters. The effluents contained 13±11 g/d, 5±5 g/d and 4±4 g/d for Zeekoegat, Daspoort and Phola, respectively.

Acetaminophen has a log K_{OW} value of 0.46, thus will not readily adsorb onto sludge and is regarded as highly biodegradable (Rojas et al., 2012). The authors also found that both HTR and STR do not have an effect on acetaminophen removal. However, Stadler (2016) found that the biodegradation rate of acetaminophen is higher as dissolved oxygen concentration increases. Zeekoegat uses diffused air aeration, which results in higher oxygen transfer rates compared to the surface aeration systems used at Daspoort WWTP (MetCalf and Eddy Inc, 2004), which could explain the improved removal observed at Zeekoegat.

An anti-epileptic drug – Carbamazepine

The mass loads of carbamazepine entering the three plants were 68±35 g/d for Zeekoegat WWTP, 36±28 g/d for Daspoort and 2±2 g/d for Phola WWTP. The removal percentages were 58%, 56% and 41%, respectively, for Zeekoegat, Daspoort and Phola WWTPs, with 29±17 g/d, 16±25 g/d and 1.2±1.1 g/d discharged in the effluent.

The percentage removals achieved by the three WWTPs were higher than what is reported in the literature. In general, very low carbamazepine removal in WWTPs has been reported in the literature. A literature review by Onesios et al. (2009) indicated that most studies reported removal percentages of between 0 and 20% and only two instances where the removal percentages were between 20 and 40% and 10 and 53%. In contrast to the general findings, Komesli et al. (2015) reported more than 90% removal in a BNR plant operated for 25 days.

A literature review by Verlicchi et al. (2012) indicated that carbamazepine removal is not affected by STR, HRT or even pH. Carbamazepine has a low K_{OW} value (Rojas et al., 2013), i.e. a low adsorption capacity and is difficult to biodegrade. The removals achieved at Daspoort and Zeekoegat were in the same range, thus the differences in operating conditions did not affect removal efficiency.

Antiviral drugs – Stavudine and Lamivudine

The average concentrations for stavudine entering the three plants varied between 966 and10 911 ng/L and for lamivudine, between 40 and 2 265 ng/L. The mass loads of stavudine entering Zeekoegat and Daspoort presented the highest loads of all the compounds monitored during this study, namely loads of 291±227 g/d and 169±170 g/d, respectively. At Phola WWTP, an average load of 9±5 g/d was present in the influent. The removal percentages were 72%, 41% and 74%, respectively, for Zeekoegat, Daspoort and Phola WWTPs.

Lamivudine mass loads into the three plants were 36±18 g/d, 11±15 g/d and 4±2 g/d for Zeekoegat, Daspoort and Phola WWTPs respectively. Lamivudine was not detected in the effluents of Zeekoegat and Daspoort WWTPs; however, an average mass load of 2±1 g/d was observed in the effluent of Phola WWTP. The removal for lamivudine in Phola WWTP was 52%, while 100% removal was attained in the two activated sludge processes.

Prasse et al. (2010) monitored activated sludge treatment plants in Germany and found removals for both stavudine and lamivudine to be from 87 to > 99%. However, the compounds were present in lower concentration ranges, from 220–720 ng/L for lamivudine and from 12–23 ng/L for stavudine, compared to the concentration ranges detected during this study. No data could be found in the literature relating the removal efficiencies for the two antiviral drugs to operational conditions in the plants concerned.

The percentage removal of all the pharmaceuticals monitored varied from 0–100% for Zeekoegat, 13–100% for Daspoort and 35–94% for Phola. Apart from nalidixic acid and lamivudine, the removal efficiencies for the pharmaceuticals were higher in the Zeekoegat plant.

On average, 134±33 g/d, 131±38 g/d and 10±1 g/d of total pharmaceuticals were discharged from Zeekoegat, Daspoort and Phola WWTPs, with removal efficiencies of 78%, 56% and 88% respectively. Again, the activated sludge plant at Zeekoegat performed better than the plant at Daspoort.

EDC removal efficiencies were found to be variable. Although other researchers related operating factors such as SRT, HRT and pH to removal efficiency there are other factors that should also be considered. The characteristics of the biomass are important factors that contribute to EDC removal efficiencies at different WWTPs (Cirja et al., 2007). The structure of the biomass flocs is directly related to the specific surface area of the MLSS which was found to have an effect on enzyme activities of the biomass (Cirja et al., 2007).

2.7 CONCLUSIONS

During the initial screening of the influent wastewaters entering Daspoort, Zeekoegat and Phola WWTPs, a total of 44 EDCs were identified. These could be classified as oestrogens, PFCs, pharmaceuticals, pesticides, personal care products and plasticisers. The main groups detected were oestrogens, PFCs and pharmaceuticals.

The removal efficiencies for the different classes of EDCs monitored in this study varied significantly, even for compounds belonging to the same class. The degree of removal is influenced by the chemical structure of the compound. The removal of EDCs by different treatment processes are related to the removal mechanisms involved. Two main mechanisms are involved, namely, adsorption and biodegradation/biotransformation. From the literature it is evident that PFCs are removed mainly by adsorption where the other EDCs monitored are removed mainly by biodegradation.

The chemical structures of the individual EDCs predict to what extent a compound will be adsorbed onto sludge particles and biomass. PFCs are mostly removed by adsorption and therefore optimisation of the water/sludge separation systems will enhance PFC removal. Thus, for PFCs:

- The integrated pond system at Phola performed similarly to the activated sludge process at Daspoort, probably due to the long HTR in the pond system.
- The higher MLSS concentration and longer sludge age at Zeekoegat enhanced removal.

The removal of oestrogens was found to be lower than generally reported in the literature. The integrated pond system at Phola WWTP performed better than the two activated sludge processes; the Zeekoegat plant performed marginally better that the Daspoort plant.

The operating conditions at Zeekoegat WWTP provided enhanced removal for most of the pharmaceuticals, the only exception being nalidixic acid which was not removed, and lamivudine removal which was similar to that found at Daspoort WWTP where it was not detected in the effluent. Important operational conditions at Zeekoegat which could have contributed to the enhanced removal of most of the EDCs at Zeekoegat were:

- Longer sludge age;

- Higher MLSS concentration;
- The diffused air aeration system providing better oxygen transfer efficiencies.

The performance of the integrated pond system at Phola WWTP was similar to the removal efficiencies detected at the Daspoort activated sludge plant.

The large variation in removal efficiencies for the same compounds, in the current study and as reported in the literature, suggests that there are other factors which contribute to removal efficiency that need to be addressed in future investigations. One such factor is biomass morphology involving the influence of an abundance of filamentous bacteria.

In general, the EDC groups studied were found to be discharged in the order: pharmaceuticals > oestrogens > PFCs. Significant amounts of pharmaceuticals and oestrogens enter the environment daily. The main contributors to the high pharmaceutical loads were stavudine in the effluents from Zeekoegat (82 g/d) and Daspoort (100 g/d) and acetaminophen, with a mass load of 4 g/d, contributing the highest portion of the daily mass loads discharged from the Phola WWTP.

Chapter 3
EVALUATION OF LABORATORY-SCALE REACTORS FOR THE REMOVAL OF ENDOCRINE DISRUPTING COMPOUNDS

3.1 INTRODUCTION

The monitoring of three WWTPs in this study revealed that significant amounts of EDCs enter the environment daily; this trend is also observed globally. Another observation, from monitoring the three WWTPs, was that the removal efficiency of activated sludge processes differed from the removal efficiency of an integrated pond and biological filter system. Furthermore, there were differences between the removals achieved in the activated sludge plants (Zeekoegat and Daspoort) for the same compound. Literature studies have shown similar trends. For example, for carbamazepine, most publications reported between 20% and 40% removal (Onesios et al., 2009), while Komesli et al. (2015) found more than 90% removal. During the present investigation, the average removal was found to be 55%. These types of results indicate that several factors play a role in the elimination of EDCs in WWTPs. To optimise the removal of EDCs it is necessary to understand the extent to which different operational parameters contribute to removal efficiency. This is where laboratory-scale experiments can make a significant contribution.

Laboratory-scale reactors are run under controlled conditions and can be used to determine the removal efficiency for a specific chemical and also to predict the concentration that will be discharged into receiving waters. Furthermore, numerous tests under different conditions can be conducted to establish relationships between each and the effect on the removal efficiency for a specific EDC. This in turn can be used to predict how the operation of full-scale plants should be changed to enhance removal. Investigators around the globe perform laboratory studies which simulate different water treatment technologies. Therefore, to ensure comparable results, the Organization for Economic Cooperation and Development (OECD) has developed guidelines for the setup and simulation of laboratory-scale reactors in order to standardise the laboratory procedures to be used.

It is evident from the literature that EDCs are mostly removed by a combination of adsorption and biodegradation. Simulation of activated sludge processes and anaerobic digestion, as the two most widely used processes for wastewater and sludge treatment in South Africa, can contribute knowledge for the optimisation of full-scale plants to eliminate EDCs. The OECD Guideline, 303A, (OECD, 2007) makes provision for determining the biodegradability of a compound in the laboratory, using standardised procedures.

The aim of this part of the investigation was to evaluate the EDC removal efficiency of a laboratory-scale activated sludge reactor and an anaerobic digestion process, using selected EDCs.

3.2 REMOVAL OF ENDOCRINE DISRUPTING COMPOUNDS IN A LABORATORY-SCALE ACTIVATED SLUDGE PROCESS

3.2.1 Materials and methods

3.2.1.1 The reactor setup and operation

A behrotest® laboratory-scale wastewater plant, which complies with the international standard set by the OECD (2007) for biodegradability tests, was used. The system consists of three different glass containers, connected in series, each with a working volume of four litres (Figures 3.1 and 3.2). The three glass containers were filled with activated sludge and operated with settled wastewater from Daspoort WWTP. The operating conditions are described in Table 3.1.

The laboratory-scale plant was operated according to the procedural framework set by OECD Guideline 303A (OECD, 2007). Owing to the variable concentrations of EDCs in the influent, the wastewater was spiked with 5.0 µg/L each, of oestrogens, pharmaceuticals and PFCs, as indicated in Table 3.2. The reactor was run until steady state conditions had been reached. Influent and effluent samples were collected and analysed for the selected EDCs.

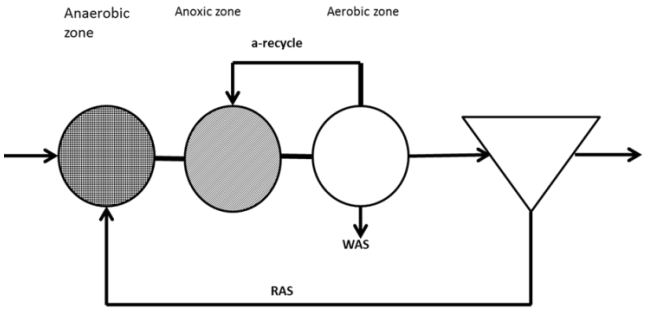


Figure 3.1: Schematic layout of the laboratory-scale activated sludge reactor

Table 3.1: Operating conditions in the laboratory-scale activated sludge reactor

PARAMETER	VALUE
Flow rate (mL/h)	320
Recycle ratio: RAS	1:1
a-recycle (for mixed liquor)	1:4
Dissolved oxygen in the aeration tank (mg/L)	1–2
Sludge age (days)	30

Figure 3.2: The laboratory-scale activated sludge reactor used during this investigation

Table 3.2: EDCs spiked during the laboratory-scale activated sludge experiment

Class of EDC		Compound
Oestrogen		17α-ethinyloestradiol (EE2)
Pharmaceuticals	Analgesic drug	Acetaminophen
	Lipid regulator	Bezafibrate
	Anti-epileptic	Carbamazepine
	Antiviral	Lamivudine
Perfluorinated chemicals		Perfluorodecanoate(PFDA) Perfluoro-1-octanesulfonate (PFOS)

3.2.1.2 Determination of chemical and physical properties

The dissolved oxygen concentration was determined with a Hach dissolved oxygen meter, and the pH with a Hach pH meter. The COD concentration was determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). The ammonia, nitrate and phosphate concentrations were determined with a Hach DR/890 Colorimeter according to the methods described by the manufacturer (HACH, 2009).

3.2.1.3 Analysis of the EDC concentrations

The wastewaters were analysed for EDCs as described in Chapter 2.

3.2.2 Results and discussion

The chemical and physical parameters for the raw and treated wastewaters are indicated in Table 3.3, below. Typically, a 3-stage Phoredox™ process can produce an effluent low in nutrients, similar to the average concentrations achieved at Daspoort WWTP, which are also indicated in Table 3.3.

Complete phosphate removal was not possible during the laboratory-scale operation and the process stabilised at an average phosphate concentration of 5±1mg P/L. A low concentration of readily biodegradable COD in the influent and difficulty in maintaining complete anaerobic conditions in the anaerobic zone were probably the reasons for the failure to achieve an improved phosphate removal. However, COD, ammonium and nitrate concentrations were within the wastewater discharge limits as set by the Department of Water and Sanitation (DWA, 2013), namely, < 75 mg/L, < 6 mg/L and < 15 mg/L, respectively, and compared favourably with results obtained at Daspoort activated sludge plant (see Table 3.3).

Table 3.3: Chemical characteristics of the influent and treated wastewaters in the laboratory-scale reactor

Parameter	Influent	Effluent	Daspoort effluent
COD (mg/L)	328±124	28±7	33±19
Phosphate (mg P/L)	14±2	5±1	<1
Ammonium (mg N/L)	37±3	0.5±0.5	<1
Nitrate (mg N/L)		8±2	7±2
pH	7.6±0.2	7.4±0.2	7.9±0.2

The mass loads for the test compounds were calculated and are indicated in Table 3.4.

Table 3.4: Mass loads of the target EDCs in the effluent from the laboratory-scale reactor

EDC group	EDC	Mass load Influent, µg/d	Mass load Effluent, µg/d	Removal %
PFCs	PFDA	38	0.040±0.001	100
	PFOS	38	12±4	70
	Total PFCs	**77**	**12**	**84**
Oestrogens	EE2	38	0.05±0.01	100
	Total oestrogens	**38**	**0.05**	**100**
Pharmaceuticals	Bezafibrate	38	nd	100
	Acetaminophen	38	nd	100
	Carbamazepine	38	17±2	57
	Lamivudine	38	nd	100
	Total pharmaceuticals	**154**	**17**	**89**

nd - not detected

The mass load of the total pharmaceutical compounds entering the laboratory-scale reactor was 154 µg/d followed by 77 µg/d PFCs and 38 µg/d oestrogens. The effluent contained 17 µg/d pharmaceuticals, 12 µg/d PFCs and 0.05 µg/d oestrogens. PFC removal varied between 70 and 100%,while between 57 and 100% of the pharmaceuticals were removed and nearly 100% of the oestrogens. The operating conditions in the laboratory-scale reactor were similar to those of Daspoort WWTP. The SRT in the laboratory-scale reactor was slightly longer than the SRT in the Daspoort plant (30 days compared to 26 days in Daspoort plant). The MLSS concentrations ($2800_{\text{Lab scale}}$, $3\,000_{\text{Daspoort}}$) were comparable, while the dissolved oxygen concentrations for both were between 1 and 2 mg/L. However, Daspoort uses mechanical surface aerators while aeration through a ceramic disc was used in the laboratory reactor, which implies that oxygen transfer was better in the laboratory reactor.

Comparison of the removal efficiencies of the two reactors revealed that the following EDCs were removed better in the laboratory-scale reactor, namely PFDA ($100\%_{\text{Lab scale}}$,$14\%_{\text{Daspoort}}$), EE2 ($100\%_{\text{Lab scale}}$, $42\%_{\text{Daspoort}}$), bezafibrate ($100\%_{\text{Lab scale}}$, $89\%_{\text{Daspoort}}$) and acetaminophen ($100\%_{\text{Lab scale}}$, $75\%_{\text{Daspoort}}$). Similar removal rates for carbamazepine were observed in both laboratory-scale and Daspoort WWTP reactors, namely 57% and 55%, respectively. Lamivudine was completely removed in both laboratory and full-scale plants. Only for PFOS was the percentage removal in the laboratory reactor less (70%) than the removal achieved at the Daspoort WWTP (80%). Thus, for most of the EDCs in the three groups, the results were comparable to the results obtained in the full-scale plant. The enhanced removals could be ascribed to better control of operational variables.

Onesios et al. (2009) conducted a literature review where they collected data from both laboratory studies and full-scale plant studies. They also found that results obtained from laboratory-scale reactors were not always the same as those found in full-scale plants because it is seldom possible to precisely simulate operating conditions. This notwithstanding, laboratory experiments still contribute to gaining understanding of the removal of EDCs using activated sludge processes.

3.2.3 Conclusion

For most of the compounds evaluated, the removal efficiencies obtained using the laboratory-scale reactor were found to be in the same range as those achieved in the Daspoort WWTP. The only exceptions were PFDA and EE2 where removal in the laboratory-scale reactors was significantly better than in the Daspoort WWTP.

3.3 REMOVAL OF ENDOCRINE DISRUPTING COMPOUNDS BY ANAEROBIC DIGESTION

3.3.1 Materials and methods

3.3.1.1Sources of sludge
Batch anaerobic reactors were operated with:

- Waste activated sludge (WAS);
- Primary settling tank (PS) sludge, and
- Mixture of PS and WAS (MS).

Sludges were obtained from the Daspoort WWTP.

3.3.1.2 Operation of the batch reactors
The experimental procedures with the laboratory-scale anaerobic digesters were carried out as described by Mathew et al. (2014). The reactors were seeded with 100 mL of anaerobic digester sludge. The sludge in each flask was adjusted to pH 7.2 and purged with nitrogen gas. The reactors were covered with aluminium foil to exclude light and were kept at 36°C in a shaking water bath. After a retention time of 20 days, the batch experiments were terminated and biogas production was determined from the volumes of acid liquid displaced during the experiment (Samaras et al*.,* 2014). The initial and final EDC concentrations of the target compounds were determined and the percentage EDC removals calculated. The EDCs evaluated are indicated in Table 3.5.

Table 3.5: The EDCs evaluated during the anaerobic digestion experiments

Class of EDC	Compound	Abbreviations
Oestrogens	17α-ethinyloestradiol	EE2
Perfluorinated chemicals	Perfluorodecanoic acid	PFDA
	Perfluoro-1-octanesulfonate	PFOS
Pharmaceuticals	Analgesic drug - acetaminophen Lipid regulator - bezafibrate Anti-epileptic - carbamazepine Antiviral drugs - lamivudine	

3.3.1.3 Analysis of the EDCs in the sludge

The sludge samples were dried and prepared as described by Samaras et al. (2014) followed by SPE and analysed using LC/MS/MS as described in Chapter 2.

3.3.2 Results and discussion

The efficiency of sludge digestion in the batch experiments was measured by calculating the reduction of volatile solids (VS) content. Typically, between 40 and 60% reduction in VS is expected during anaerobic digestion of sludge for 20 days and at 36ºC (Ross et al., 1992). The average VS reduction for the WAS was 55%, and for the PS, 57%. However, the MS displayed inadequate VS reduction, at

20%, which was probably due to the very high total solids (TS) concentration in the sludge sample obtained from Daspoort (37 g/L) that resulted in an overload of VFAs in the first stage of anaerobic digestion and a decrease in the pH and activity of methane-producing bacteria (Ross et al., 1992).

The average biogas volumes produced over 20 days for the WAS, PS and MS digestions were 700±357 mL (WAS), 1 747±920 mL (PS) and 1 452±266 mL (MS). Typically, 1 000 L biogas is produced for every 1 000 g VS destroyed (Ross et al*.,* 1992). The concentrations of the selected EDCs are expressed as g/ton, as indicated in Table 3.6.

Table 3.6: EDC removal results for anaerobic digestion of WAS, PS and MS sludges

Sludge Type	EDC group	EDC	Before digestion (g/ton±SD)	After digestion (g/ton±SD)	Removed (%)
***WAS**	**PFCs**	PFDA	5.1±0.04	0.5±0.1	91
		PFOS	322±86	141±8	56
		Total PFCs	**327**	**142**	**57**
	Oestrogen	Oestrogen, EE2	2.2±0.02	2.3±0.3	**0**
		Total Oestrogens	**2.2**	**2.3**	**0**
	Pharmaceuticals	Bezafibrate	345±17	231±29	33
		Acetaminophen	608±31	346±37	43
		Carbamazepine	136±3	91±3	33
		Total Pharmaceuticals	**1089**	**668**	**39**
***PS**	**PFCs**	PFDA	3.2±0.01	2±0.1	55
		PFOS	603±13	217±3	64
		Total PFCs	**606**	**219**	**64**
	Oestrogen	Oestrogen, EE2	1.8±0.50	0.1±0.1	93
		Total Oestrogens	**1.8**	**0.1**	**93**
	Pharmaceuticals	Bezafibrate	593±51	231±32	61
		Acetaminophen	354±77	259±24	27
		Carbamazepine	163±16	137±41	16
		Total Pharmaceuticals	**1110**	**627**	**44**
***MS**	**PFCs**	PFDA	4±0.3	0.5±0.1	89
		PFOS	398±16	223±10	44
		Total PFCs	**402**	**224**	**44**
	Oestrogen	Oestrogen, EE2	2.3±0.6	0.5±0.2	78
		Total Oestrogens	**2.3**	**0.5**	**78**
	Pharmaceuticals	Bezafibrate	424±59	142±2	66
		Acetaminophen	309±24	204±64	34
		Carbamazepine	149±7	119±1	20
		Total Pharmaceuticals	**882**	**465**	**47**

*WAS – waste activated sludge, PS – primary sludge, MS – mixed WAS/PS sludge, SD – standard deviation

The three groups of EDCs evaluated were present in the following order, from highest to lowest concentrations: Pharmaceuticals > PFCs > oestrogens.

The total concentrations of pharmaceuticals in the raw sludges were as follows: 1 089 g/ton, 1 110 g/ton and 882 g/ton while the anaerobically digested sludges contained 668 g/ton, 627 g/ton and 465 g/ton, respectively, for WAS, PS and MS. There were 327 g/ton, 606 g/ton and 402 g/ton of PFCs in the raw WAS, PS and MS, respectively. The digested sludges, WAS, PS and MS, contained 142 g/ton, 219 g/ton and 224 g/ton PFCs, respectively. The oestrogen concentration was 2.2 g/ton in the raw WAS and 2.3 g/ton in the digested WAS, 1.8 g/ton in the raw PS and 0.2 g/ton in the digested PS, 2.3 g/ton in the raw MS and 0.46 g/ton in the anaerobically digested MS.

Oestrogens

The concentration for the synthetic hormone, 17α-ethinyloestradiol (EE2) was 2.2±0.02 g/ton and 2.3±0.3 g/ton before and after digestion of WAS; 1.8±0.5 g/ton and 0.1±0.1 g/ton before and after digestion of PS, and 2±0.6 g/ton and 0.5±0.2 g/ton before and after digestion of MS. The percentage removals were 0%, 93% and 78% respectively by WAS, PS and MS.
Removal efficiencies reported in the literature indicate variable and sometimes contradictory results. Carballa et al. (2007) reported 85% removal of E1, E2 and EE2 in mixed sludge (20 d, 4–400 µg/L), while Paterakis et al. (2012) found 79% and 70% removal of E1 and 34% and 4% removal in PS and mixed sludge, respectively. No results were found for work done on E1, E2 and EE2 removal in WAS alone.

Perfluorinated chemicals

Among the PFCs, perfluoro-1-octanesulfonate (PFOS) was found to be present in the highest concentrations in the wastewater (see Chapter 2), and was also found to be present in the sludge in much higher concentrations, and notably higher than perfluorodecanoic acid (PFDA). The PFOS concentration in the PS was 603±13 g/ton, 398±17 g/ton in the MS and 322±86 g/ton in the WAS. The digested sludges contained 217±3 g/ton, 223±13 and 141±8 g/ton, respectively, for PS, MS and WAS. The best anaerobic removal efficiency (64%) was achieved with PS, followed by WAS with 56% removal. Only 44% removal was obtained from digestion of the MS.

PFDA concentrations for raw and digested WAS were, respectively, 5.0±0.1 g/ton and 0.5±0.1 g/ton, for raw and digested MS, respectively; 4±0.3 g/ton and 0.5±0.1 g/ton, for raw and digested PS, respectively, and for PFDA, 3±0.1 g/ton and 1.5±0.1 g/ton. The removals were 91%, 89% and 55%, respectively, for WAS, MS and PS sludge.

Only a few articles related to PFC removal during anaerobic digestion have been published. Schultz et al. (2006) evaluated the removal of PFOS and PFDA during anaerobic digestion. The authors detected PFCs in both raw and digested sludge. PFDA was detected but no removal was noted after anaerobic digestion. Schultz et al. (2006) observed an increase in PFOS after anaerobic digestion, which they attributed either to differences in HRTs between the unit processes or to the degradation of precursors to PFOS. An increase in PFOS concentration after anaerobic digestion was also reported byGómez-Canela et al. (2012).

Pharmaceuticals

No lamivudine was detected in the raw WAS, PS and MS sludges. As reported in Chapter 2, there was also no lamivudine detected in the effluents from the two activated sludge plants, Daspoort and Zeekoegat. Thus, it seems that this compound is readily biodegradable and does not adsorb onto sludge particles.

The bezafibrate (lipid regulator) concentrations were 345±17 g/ton and 231±29 g/ton, respectively, for raw and digested WAS; for raw and digested MS, the bezafibrate concentrations were 424±59 g/ton and 142±2 g/ton, respectively; and for raw and digested PS, the bezafibrate concentrations were 593±51 g/ton and 231±51 g/ton, respectively. The removals obtained for bezafibrate were 33%, 66% and 61%, respectively, for anaerobic digestion of WAS, MS and PS.

The concentrations of the analgesic drug, acetaminophen (paracetamol), before and after digestion, i.e. for raw and digested WAS were, respectively, 608±31 g/ton and 346±37 g/ton; for raw and digested MS the acetaminophen concentrations were 309±24 g/ton and 204±64 g/ton, respectively; and for raw and digested PS, the acetaminophen concentrations were 354±77 g/ton and 259±24 g/ton, respectively. The removals obtained for acetaminophen were 43%, 34% and 27%, respectively, for digestion of WAS, MS and PS.

The concentrations of carbamazepine (an anti-epileptic and anti-depression drug) in raw and digested WAS were, respectively, 136±3 g/ton and 91±3 g/ton; for raw and digested MS the carbamazepine concentrations were 149±7 g/ton and 119±1 g/ton, respectively; and for raw and digested PS the concentrations were 163±16 g/ton and 137±41 g/ton, respectively. The anaerobic removals obtained for carbamazepine were 33%, 20% and 16%, respectively, for WAS, MS and PS.

Scant reference was found in the literature to anaerobic digestion of the pharmaceutical compounds evaluated during this study. Onesios et al. (2009) reported no removal of carbamazepine carried out at laboratory scale. A study done in Turkey (Komesli et al., 2015) on the removal of acetaminophen and carbamazepine by anaerobic digestion of WAS indicated incomplete removal of these compounds, although a significant reduction in the carbamazepine concentration in the digested sludge was observed. Martin et al. (2015) investigated the removal of acetaminophen, carbamazepine and bezafibrate during anaerobic digestion of mixed sludge. They found that acetaminophen was present, but below the detection limit, in WAS and the digested sludge, and that both carbamazepine and bezafibrate were removed to a significant extent. However, removal of these compounds was considered inadequate. Unfortunately, there was no indication of the operating conditions in the digesters.

Elimination of the different EDC groups found in the present study was inadequate, with 57%, 64% and 44% removal of total PFCs; 0%, 93% and 78% removal of oestrogens; and 39%, 44% and 47% removal of pharmaceuticals from WAS, PS and MS, respectively. As a result, substantial quantities of EDCs were still present in the anaerobically digested sludge which could pose environmental risks following subsequent disposal and/or usage of the sludge.

According to baseline studies on waste generation in South Africa (DEA, 2012), a total of 673 360 tons of dry sludge are generated per annum. Fifty-seven percent of the generated sludges are anaerobically digested (Snyman, 2004). Dried sludge is commonly used in agriculture as a soil conditioner and source of nutrients.

Guidelines set by the Department of Water and Sanitation governing the application of sludge for agricultural purposes state that the application rate should not exceed the plant nutrient requirements, with a maximum application rate of 10 ton dry sludge/hectare/year (WRC, 2006). Using the maximum application rate and the mass of EDCs still present in the anaerobically digested mixed sludges (PS and WAS mixture commonly co-digested in anaerobic digesters) it is estimated, according to this DWS guideline, that 7.0 kg/hectare/year pharmaceuticals, 2.3 kg/hectare/year PFCs and 1.3 kg/hectare/year oestrogens, would possibly be applied to agricultural land. This could pose an environmental risk and a risk to food security. Two different investigations have been reported in the literature which confirm that there are reasons for concern. A study done by Dodgen et al. (2013) established that EDCs

accumulate in leafy vegetables, and Vogel et al.(2003) demonstrated leaching of EDCs by run-off water after application of sewage sludge to agricultural land.

3.3.3 Conclusion

The EDCs present in sludges that were targeted in the present study were inadequately removed by anaerobic digestion, which implies that these compounds would be present in the sludges discharged from plants and may, therefore, potentially pose environmental risks. Pharmaceuticals were present in the highest concentrations in all three sludge types studied, followed by PFCs and oestrogens. The best removal was achieved for EE2 in the anaerobic digestion of primary sludge, while for the PFCs the highest reduction was noted for PFDA in WAS. Carbamazepine was poorly removed from all three sludge types.

Chapter 4
CONCLUSIONS AND RECOMMENDATIONS

From the initial screening of raw influent wastewaters entering Daspoort, Zeekoegat and Phola WWTPs, a total of 42 EDCs were identified. The identified EDCs belonged to the following five groups: oestrogens, PFCs, pharmaceuticals, pesticides, personal care products and plasticisers, with most of the EDCs falling into the oestrogen, PFC and pharmaceutical groups.

Pharmaceuticals were identified as the main contributors to the daily mass loads of EDCs entering the three WWTPs. The mass loads of oestrogens were the second highest in the influents to Zeekoegat and Phola WWTPs, while the daily influent mass load of PFCs at Daspoort was higher than the daily influent mass load of oestrogens. Most of the EDCs were not completely eliminated during the treatment processes and the untreated EDCs were thus being discharged into the receiving water bodies. The daily mass loads were calculated and it was found that significant amounts of pharmaceuticals and oestrogens entered the environment on a daily basis.

The removal efficiencies in each EDC group varied from 'not removed' to > 99% removal. Removal efficiencies also varied with the different treatment technologies. The integrated ponds system at Phola achieved better removal of oestrogens and PFCs, with PFOS as the exception. Comparison of the two activated sludge plants indicated that for most of the EDCs removal was more effective in the Zeekoegat plant than at Daspoort. Factors that could have contributed to the higher removals at Zeekoegat were the longer SRT, higher MLSS content and the improved oxygen transfer.

Laboratory-scale activated sludge and anaerobic digestion reactors were run to evaluate the removal of selected EDCs under controlled conditions using representative EDCs from the three groups monitored at the full-scale WWTPs. The elimination of EDC groups in laboratory-scale studies was found be comparable, but were mostly marginally better, than the removals achieved in the full-scale plants. It was also found that EDC elimination was incomplete following anaerobic digestion. The most poorly removed were from the pharmaceuticals, followed by the PFCs and oestrogens.

EDCs include a multitude of organic compounds with widely ranging functional groups, which complicates optimisation of the removal of these compounds by wastewater treatment processes. It is apparent that other factors, apart from those already identified, e.g. SRT and HRT in activated sludge processes, also play a role in the removal of these compounds from wastewater. These unknown factors need to be identified and investigated in future studies. For activated sludge processes, important factors to consider are biomass morphology and sludge bacterial species diversity.

Laboratory-scale experiments can make a significant contribution towards understanding the role that different variables play in the removal of EDCs. Some of the compounds were fairly well removed in the integrated pond system and the role of anaerobic ponds needs to be evaluated. The sludge from the integrated pond system should also be assessed to compare with removals achieved by anaerobic digestion.

Another factor which affects accurate estimation of EDC removal is the degradation of certain compounds, e.g. oestrogen E2 is converted to E1 during treatment. Furthermore, parent compounds can break down to metabolites, which could also be endocrine disrupting. This also needs to be considered in future studies.

This study partially quantified the risks resulting from discharging EDCs into receiving water bodies. As only a few EDCs were evaluated, there is a need to study additional groups of these compounds. Thus, more in-depth studies are needed to gain better insight into the magnitude of the eco-toxicological effects on the environment and the potential risks to users of the discharged water and the disposed sludge from wastewater treatment plants.

REFERENCES

ANECK-HAHN, N.H., BORNMAN & DE JAGER, C. 2008. Preliminary assessment of oestrogenic activity in water sources in Rietvlei Nature Reserve, Gauteng, South Africa. *African Journal of Aquatic Science,* 33**,** 249–254.

APHA (ed.) 2005. *Standard Methods for the Examination of Water and Waste Water,* Washington, D.C: American Public Health Association.

AURIOL, M., FILALI-MEKNASSI, Y., TYAGI, R.D., ADAMS, C.D. & SURAMPALLI, R.Y. 2006. Endocrine disrupting compounds removal from wastewater, a new challenge. *Process Biochemistry,* 41**,** 525–539.

BARNHOORN, I.E.J., VAN DYK, J.C., PIETERSE, G.M. & BORNMAN, M.S. 2010. Intersex in feral indigenous freshwater Oreochromis mossabicus, from various parts in the Luvuhu River, Limpopo Province, South Africa. *Ecotoxicology and Environmental Safety,* 74**,** 1537–1542.

BENOTTI, M.J., TRENHOLM, R.A., VANDERFORD, B.J., HOLADY, J.C., STANFORD, B.D. & SNYDER, S.A. 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environ Sci Technol,* 43**,** 597–603.

BERGMAN, A., HEINDAL, J.J., JOBLING, S., KIDD, K.A. & ZOELLER, R.T. 2012. *State of the Science of Endocrine Disrupting Chemicals*, UNEP and WHO.

BERTANZA, G., PEDRAZZANI, R., DAL GRANDE, M., PAPA, M., ZAMBARDA, V., MONTANI, C., STEIMBERG, N., MAZZOLENI, G. & LORENZO, D. 2011. Effect of biological and chemical oxidation on the removal of estrogenic compounds (NP and BPA) from wastewater: An integrated assessment procedure. *Water Res,* 45**,** 2473-2484.

BERTANZA, G., PEDRAZZANI, R., ZAMBARDA, V., GRANDE, M. D., ICARELLI, F. & BALDASSARRE, L. 2010. Removal of endocrine disrupting compounds from wastewater treatment plant effluents by means of advanced oxidation. *Water Sci Technol,* 61**,** 1663-71.

BIRKETT, J.W. & LESTER, J.N. 2003. *Endocrine disrupters in wastewater and sludge treatment processes.*, Lewis Publishers.

CALIMAN, F.A. & GAVRILESCU, M. 2009. Pharmaceuticals, personal care products and endocrine disrupting agents in the environment – a review. *Clean,* 37**,** 277–303.

CARBALLA, M., OMIL, F., TERNES, T. & LEMA, J.M. 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Res,* 41**,** 2139-50.

CASTIGLIONI , S., BAGNATI, R., OFANELLI, R., COPOMATI, F., ECALAMARI, D. & ZUCCATO, E. 2006. Removal of Pharmaceuticals in Sewage Treatment Plants in Italy. *Environmental Science and Technology,* 40**,** 357-363.

CHANG, H., CHOO, K., LEE, B. & CHOI, S. 2009. The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water. *Journal of Hazardous Materials,* 172**,** 1-12.

CHEMBASE.EN. 2017. *Chemical structure of PFOS* [Online]. Available: http://en.chembase.cn/molecule-106194.html [Accessed 30/05/2017 2017].

CHIMCHIRIAN, R.F., SURI, R.P.S. & FU, H. 2007. Free Synthetic and Natural Estrogen Hormones in Influent and Effluent of Three Municipal Wastewater Treatment Plants. *Water Environment Research,* 79**,** 969-974.

CIRJA, M., IVASHECHKIN, P., SCHÄFFER, A. & CORVINI, P.F.X. 2007. Factors affecting the removal of organic micropollutants from wastewater in conventional treatment plants (CTP) and membrane bioreactors (MBR). *Reviews in Environmental Science and Bio/Technology,* 7**,** 61-78.

DEA 2012. *National Waste Information Baseline Report.,* Pretoria, South Africa, Department of Environmental Affairs.

DODGEN, L.K., LI, J., PARKER, D. & GAN, J.J. 2013. Uptake and Accumulation of Four PPCP/EDCs in Two Leafy Vegetables. *Environmental pollution (Barking, Essex : 1987),* 182**,** 150-156.

DRUGS.COM. 2017. *Nalidixic acid* [Online]. [Accessed 15 May 2017].

DWA. 2013. *Government Gazette: Revision of General Authorisations in terms of Section 39 of the National Water Act , 1998 (Act no. 36 of 1998).* [Online]. Pretoria, RSA. Available: www.gpwonline.co.za/Gazettes/Gazettes/36820_6-9_WaterAffairs [Accessed 20 June 2017].

ECKENFELDER, W.W., FORD, D.L. & ENGLANDE, A.J. 2009. *Industrial Water Quality,* New York, McGraw Hill.

FURLONG, E.T., GRAY, J.L., QUANRUD, D.M., TESKE, S.S., ESPOSITO, K., MARINE, J., ELA, W.P., STINSON, B., KOLPIN, D.W. & PHILLIPS, P.J. 2010. *Fate of estrogenic compounds during municipal sludge stabilization and dewatering.* London: WERF.

GHOSH, G.C., OKUDA, T., YAMASHITA, N. & TANAKA, H. 2009. Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. *Water Science and Technology,* 59**,** 779-786.

GÓMEZ-CANELA, C., BARTH, J.A.C. & LACORTE, S. 2012. Occurrence and fate of perfluorinated compounds in sewage sludge from Spain and Germany. *Environmental Science and Pollution Research,* 19**,** 4109-4119.

GRADY, C.P.L., DAIGGER, G.T. & LIM, H.C. 1999. *Biological Wastewater Treatment,* New York, Marcel Dekker, Inc.

HACH 2009. *Hach DR/890 Procedures manual*, Hach Company.

HAMID, H. & ESKICIOGLU, C. 2012. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. *Water Research,* 46**,** 5813-5833.

HEBERER, T. 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters,* 131**,** 5-17.

HEIDLER, J. & HALDEN, R.U. 2007. Mass balance assessment of triclosan removal during conventional sewage treatment. *Chemosphere,* 66**,** 362-369.

HENZE, M. & VAN LOOSDRECHT, M.C.M. 2008. *Biological wastewater treatment: principles, modelling and design,* London, IWA Publishing.

IFELEBUEGU, A.O. 2011. The fate and behavior of selected endocrine disrupting chemicals in full scale wastewater and sludge treatment unit processes. *Int. J. Environ. Sci. Tech,* 8**,** 245-254.

KABIR, E.R., RAHMAN, M.S. & RAHMAN, I. 2015. A Review on Endocrine Disruptors and their Possible Impacts on Human Health. *Environmental Toxicology and Pharmacology*.

KASPRZYK-HORDERN, B., DINSDALEB, R.M. & GUWY, A.J. 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Researh,* 43**,** 363-380.

KIKANDI, S.N. 2008. *Understanding the Mode of Action of Estrogenic Compounds & Development of Novel Electroless Plating Methods*, State University of New York at Binghamton.

KJELDSEN, L.S. & BONEFELD-JORGENSEN, E.C. 2013. Perfluorinated compounds affect the function of sex hormone receptors. *Environ Sci Pollut Res Int,* 20**,** 8031-44.

KO, E.J., KIM, K.W., KANG, S.Y., KIM, S.D., BANG, S.B., HAMM, S.Y. & KIM, D.W. 2007. Monitoring of environmental phenolic endocrine disrupting compounds in treatment effluents and river waters, Korea. *Talanta,* 73**,** 674-83.

KOMESLI, O.T., MUZ, M., AK, M.S., BAKıRDERE, S. & GOKCAY, C.F. 2015. Occurrence, fate and removal of endocrine disrupting compounds (EDCs) in Turkish wastewater treatment plants. *Chemical Engineering Journal,* 277**,** 202-208.

KRUGER, T., BARNHOORN, I., JANSEN VAN VUREN, J. & BORNMAN, R. 2013. The use of the urogenital papillae of male feral African sharptooth catfish (Clarias gariepinus) as indicator of exposure to estrogenic chemicals in two polluted dams in an urban nature reserve, Gauteng, South Africa. *Ecotoxicol Environ Saf,* 87**,** 98-107.

LEE , Y.M., OLESZKIEWICZ , J.A., CICEK, N. & LONDRY, K. 2004. Endocrine Disrupting Compounds (EDC) in Municipal Wastewater Treatment: A Need for Mass Balance. *Environmental Technology,* 25**,** 635-645.

LIU, Z. H., KANJO, Y. & MIZUTANI, S. 2009. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment - physical means, biodegradation, and chemical advanced oxidation: a review. *Sci Total Environ,* 407**,** 731-48.

LOFRANO 2012. Removal of Emerging Contaminants from Water and Wastewater by Adsorption Process. *In:* LOFRANO (ed.) *Emerging Compounds Removal from Wastewater.* Springer Briefs in Green Chemistry for Sustainability.

LUO, Y., GUO, W., NGO, H.H., NGHIEM, L.D., HAI, F.I., ZHANG, J., LIANG, S. & WANG, X.C. 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci Total Environ,* 473-474**,** 619-41.

MANICKUM, T. & JOHN, W. 2014. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). *Sci Total Environ,* 468-469**,** 584-97.

MARTIN, J., SANTOS, J.L., APARICIO, I. & ALONSO, E. 2015. Pharmaceutically active compounds in sludge stabilization treatments: anaerobic and aerobic digestion, wastewater stabilization ponds and composting. *Sci Total Environ,* 503-504**,** 97-104.
METCALF AND EDDY INC. 2004. *Wastewater Engineering, Treatment, Disposal and Reuse,* New York., McGraw-Hill.
MNIF, W., DAGNINO, S., ESCANDE, A., PILLON, A., FENET, H., GOMEZ, E., CASELLAS, C., DUCHESNE, M.J., HERNANDEZ-RAQUET, G., CAVAILLES, V., BALAGUER, P. & BARTEGI, A. 2010. Biological analysis of endocrine-disrupting compounds in Tunisian sewage treatment plants. *Arch Environ Contam Toxicol,* 59**,** 1-12.
MULLER, M., COMBALBERT, S., DELGENÈS, N., BERGHEAUD, V., ROCHER, V., BENOÎT, P., DELGENÈS, J.-P., PATUREAU, D. & HERNANDEZ-RAQUET, G. 2010. Occurrence of estrogens in sewage sludge and their fate during plant-scale anaerobic digestion. *Chemosphere,* 81**,** 65-71.
NIE, Y., QIANG, Z., ZHANG, H. & BEN, W. 2012. Fate and seasonal variation of endocrine-disrupting chemicals in a sewage treatment plant with A/A/O process. *Separation and Purification Technology,* 84**,** 9-15.
OECD. 2007. *OECD GUIDELINE FOR THE TESTING OF CHEMICALS* [Online]. Available: http://www.oecd.org/chemicalsafety/testing/39574397.pdf [Accessed 11 November 2017].
ONESIOS, K. M., YU, J. T. & BOUWER, E. J. 2009. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. *Biodegradation,* 20**,** 441-466.
PATRICK, J., BINKLEY, J. & HEIM, J. 2012. A Broad Spectrum GC x GC–TOFMS Analysis for Endocrine Disruptor Compounds in a Midwestern U.S. Watershed. *chromatographyonline* [Online]. Available: http://www.chromatographyonline.com/broad-spectrum-gcgc%E2%80%93tofms-analysis-endocrine-disruptor-compounds-midwestern-us-watershed [Accessed 24 April 2016].
PESSOA, G.P., DE SOUZA, N.C., VIDAL, C.B., ALVES, J.A.C., FIRMINO, P.I.M., NASCIMENTO, R. F. & DOS SANTOS, A.B. 2014. Occurrence and removal of estrogens in Brazilian wastewater treatment plants. *Science of The Total Environment,* 490**,** 288-295.
PETROVIĆ, M. & BARCELÓ, D. 2000. The stability of non-ionic surfactants and linear alkylbenzene sulfonates in a water matrix and on solid-phase extraction cartridges. *Fresenius' Journal of Analytical Chemistry,* 368**,** 676-683.
POOL, E.J. 2008. The estrogenicity of sewage effluent entering the Eerste-Kuils river catchment system. WRC Report No. 1590. Water Research Commission, Pretoria, South Africa.
PRASSE, C., SCHLÜSENER, M.P., SCHULZ, R. & TERNES, T.A. 2010. Antiviral Drugs in Wastewater and Surface Waters: A New Pharmaceutical Class of Environmental Relevance? *Environmental Science & Technology,* 44**,** 1728-1735.
PURDOM, C.E., HARDIMAN, P.A., BYE, V.V.J., ENO, N.C., TYLER, C.R. & SUMPTER, J.P. 1994. Estrogenic Effects of Effluents from Sewage Treatment Works. *Chemistry and Ecology,* 8**,** 275-285.
PURI, D. 2014. *Textbook of Medical Biochemistry*, Elsevier Health Sciences APAC.
QIANG, Z., DONG, H., ZHU, B., QU, J. & NIE, Y. 2013. A comparison of various rural wastewater treatment processes for the removal of endocrine-disrupting chemicals (EDCs). *Chemosphere,* 92**,** 986-992.
RACZ, L. & GOEL, R.K. 2010. Fate and removal of estrogens in municipal wastewater. *Journal of Environmental Monitoring,* 12**,** 58–70.
REN, Y., NAKANO, K., NOMURA, M., CHIBA, N. & NISHIMURA, O. 2007. Effects of bacterial activity on estrogen removal in nitrifying activated sludge. *Water Research,* 41**,** 3089-3096.
ROH, H., SUBRAMANYA, N., ZHAO, F., YU, Y., SANDT, J. & CHU, K.-H. 2009. Biodegradation potential of wastewater micropollutants by ammonia-oxidizing bacteria. *Chemosphere,* 77**,** 1084–1089.
ROJAS, M.R., LEUNG, C., BONK, F., ZHU, Y., EDWARDS, L., ARNOLD, R.G., SÁEZ, A.E. & KLEČKA, G. 2013. Assessment of the Effectiveness of Secondary Wastewater Treatment Technologies to Remove Trace Chemicals of Emerging Concern. *Critical Reviews in Environmental Science and Technology,* 43**,** 1281-1314.
ROSS, W.R., NOVELLA, P.H., PITT, A.J., LUND, P., THOMSON, B.A., KING, P.B. & FAWCETT, K.S. 1992. Anaerobic digestion of waste-water sludge: Operating guide. Pretoria, Sout Africa: WRC.
RUSHTON, L. 2004. *The endocrine system,* New York, Infobase Publishing.
SAMARAS, V.G., STASINAKIS, A.S., MAMAIS, D., THOMAIDIS, N.S. & LEKKAS, T.D. 2013. Fate of selected pharmaceuticals and synthetic endocrine disrupting compounds during wastewater

treatment and sludge anaerobic digestion. *Journal of Hazardous Materials,* 244–245**,** 259-267.
SAMARAS, V.G., STASINAKIS, A.S., THOMAIDIS, N.S., MAMAIS, D. & LEKKAS, T.D. 2014. Fate of selected emerging micropollutants during mesophilic, thermophilic and temperature co-phased anaerobic digestion of sewage sludge. *Bioresource Technology,* 162**,** 365-372.
SAWYER, C.N., MCCARTY, P.L. & PARKIN, G.F. 1994. *Chemistry for environmental engineering,* New York, McGraw-Hill Inc.
SCHUG, T.T., JOHNSON, A.F., BIRNBAUM, L.S., COLBORN, T., GUILLETTE, L.J., JR., CREWS, D. P., COLLINS, T., SOTO, A.M., VOM SAAL, F.S., MCLACHLAN, J.A., SONNENSCHEIN, C. & HEINDEL, J.J. 2016. Minireview: Endocrine Disruptors: Past Lessons and Future Directions. *Mol Endocrinol,* 30**,** 833-47.
SCHULTZ, M.M., HIGGINS, C.P., HUSET, C.A., LUTHY, R.G., BAROFSKY, D.F. & FIELD, J.A. 2006. Fluorochemical mass flows in a municipal wastewater treatment facility. *Environ Sci Technol,* 40**,** 7350-7.
SCIENCES, N.I.O.E.H. 2016. *<Perfluorinated Chemicals (PFCs)* [Online]. Available: https://www.niehs.nih.gov/health/materials/perflourinated_chemicals [Accessed 30/05/2017 2017].
SHILTON, A. 2006. *Pond Treatment Technology*, IWA Publishing.
SIPMA, J., OSUNA, B., COLLADO, N., MONCLÚS, H., FERRERO, G., COMAS, J. & RODRIGUEZ-RODA, I. 2010. Comparison of removal of pharmaceuticals in MBR and activated sludge systems. *Desalination,* 250**,** 653–659.
SNYMAN, H.G., HERSELMAN, J.E. AND KASSELMAN, G. 2004. *A metal content survey of South African sewage sludge and an evaluation of analytical methods for their determination in sludge.,* Pretoria, South Africa, WRC.
STASINAKIS, A.S., THOMAIDIS, N.S., ARVANITI, O.S., ASIMAKOPOULOS, A.G., SAMARAS, V.G., AJIBOLA, A., MAMAIS, D. & LEKKAS, T. D. 2013. Contribution of primary and secondary treatment on the removal of benzothiazoles, benzotriazoles, endocrine disruptors, pharmaceuticals and perfluorinated compounds in a sewage treatment plant. *Science of The Total Environment,* 463–464**,** 1067-1075.
TEDX. 2016. *The TEDX list of potential endocrine disruptors.* [Online]. Available: http://endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/search-the-tedx-list [Accessed 9 August 2016].
TIJANI, J.O., FATOBA, O.O. & PETRIK, L.F. 2013. A Review of Pharmaceuticals and Endocrine-Disrupting Compounds: Sources, Effects, Removal, and Detections. *Water, Air, and Soil Pollution,* 224**,** 29.
VANDERFORD, B.J., PEARSON, R.A., REXING, D.J. & SNYDER, S.A. 2003. Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in Water Using Liquid Chromatography/Tandem Mass Spectrometry. *Analytical Chemistry,* 75**,** 6265-6274.
VERLICCHI, P., AL AUKIDY, M. & ZAMBELLO, E. 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Science of the Total Environment,* 429**,** 123-155.
VOGEL, D., GEHRING, M., TENNHARDT, L., WELTIN, D., BILITEWSKI, B. 2003. Mobility and Fate of Endocrine Disrupting Compounds (EDCs) in Soil after Application of Sewage Sludge to Agricultural Land. *In:* PULLAMMANAPPALLIL, P., MCCOMB, A., DIAZ, L. F., BIDLINGMAIER, W., ed. Proc. 4 th Int. Conference ORBIT Ass. on Biol. Process. Organics: Adv. Sustain. Soc.,April 30 - May 02 2003 Perth, Australia. 241-250.
WANG, X., ZHANG, R., ZHANG, H., HE, L., SHEN, J., CHAI, Z., YANG, B. & WANG, Y. 2016. Impact of Biological Treatment Techniques on Perfluoroalkyl Acids Emissions in Municipal Sewage. *Water, Air, & Soil Pollution,* 227**,** 149.
WRC 2006. Guidelines for the utilisation and disposal of wastewater sludge. Requirements for the agricultural use of wastewater sludge. Pretoria, South Africa: Water Research Commission.
ZHANG, Z., FENG, Y., GAO, P., WANG, C. & REN, N. 2011. Occurrence and removal efficiencies of eight EDCs and estrogenicity in a STP. *Journal of Environmental Monitoring,* 13**,** 1366-1373.