University of Fort Hare
Together in Excellence

REMOVAL RATE OF ENDOCRINE DISRUPTORS (PHTHALATES AND PHENOLIC COMPOUNDS) IN EFFLUENTS OF SELECTED WASTEWATER TREATMENT PLANTS OPERATED UNDER DIFFERENT TREATMENT TECHNOLOGIES IN THE EASTERN CAPE, SOUTH AFRICA.

TAOFEEK GBENGA, SALAUDEEN

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE AND AGRICULTURE

UNIVERSITY OF FORT HARE

ALICE 5700, SOUTH AFRICA

MARCH, 2017

REMOVAL RATE OF ENDOCRINE DISRUPTORS (PHTHALATES AND PHENOLIC COMPOUNDS) IN EFFLUENTS OF SELECTED WASTEWATER TREATMENT PLANTS OPERATED UNDER DIFFERENT TREATMENT TECHNOLOGIES IN THE EASTERN CAPE, SOUTH AFRICA.

BY

TAOFEEK GBENGA SALAUDEEN

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(CHEMISTRY)

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE AND AGRICULTURE

UNIVERSITY OF FORT HARE

ALICE 5700

SOUTH AFRICA

Supervisor:	**Professor Omobola O. Okoh**
Co-supervisor:	**Professor Anthony I. Okoh**

MARCH, 2017

DECLARATION

I, the undersigned, do declare that this thesis entitled “Removal rate of endocrine disruptors (phthalates and phenolic compounds) in effluents of selected wastewater treatment plants operated under different treatment technologies in the Eastern Cape, South Africa” submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Chemistry in the Faculty of Science and Agriculture, and the work contained herein is a record of my own work with the exception of the citations, and that this work has not been submitted to any other University partially or entirely for the award of any degree.

Name: **Taofeek Gbenga Salaudeen**

Signature: …………………………………….

Date: ……………………………………..

DECLARATION ON PLAGIARISM

I, **Taofeek Gbenga Salaudeen**, student number 201415520 hereby declare that I am fully aware of the University of Fort Hare's policy on plagiarism and I have taken every precaution to comply with the regulations.

Signature……………………………….. Date…………………………………….

CERTIFICATION

This thesis entitled “Removal rate of endocrine disruptors (phthalates and phenolic compounds) in effluents of selected wastewater treatment plants operated under different treatment technologies in the Eastern Cape, South Africa” meets the regulation governing the award of degree of Doctor of Philosophy of the University of Fort Hare and is approved for its contribution to scientific knowledge and literary presentation.

……………………………………………….. ……………………………..

Prof O. O. Okoh Date

Major Supervisor

DEDICATION

This work is dedicated to the Glory of Almighty Allah (SWT) the Lord of the universe for His grace, guidance, infinite mercies and protection throughout the turbulent periods of this work.

ACKNOWLEDGMENTS

I am indeed grateful to my supervisors Prof Omobola O. Okoh and Prof Anthony I. Okoh for their inspiration, guidance, advice, support, and understanding. Their wealth of experience and valuable contributions to this work cannot be appreciated enough.

Worthy of recognition are the members of the two vibrant research groups to which I belong: Synthetic, Environmental and Applied Chemistry Research Group (SEACREG) and Applied and Environmental Microbiology Research Group (AEMREG). Their constructive criticisms, valuable advice and contributions gave this work the best of outcomes. My profound appreciation also goes to the lecturers in the Department of Chemistry, Biochemistry and Microbiology: Prof. L.V. Mabinya, Prof. U.U. Nwodo, Prof. Ezekiel Green, Dr. Ben Iweriebor (AEMREG), Prof. O.O. Oyedeji, Dr. Blessing Aderibigbe, Dr. David Katwire, Prof. Alexander Sadimenko and Prof. P. Ajibade (HOD) in the Department of Chemistry. Their words of encouragement and motivation were indeed a strong motive for the challenging time.

This research study would have been more difficult or not reached a logical conclusion but for the financial supports of South African Medical Research Council (SAMRC) and the Water Research Commission (WRC). Their financial supports in terms of a bursary and the purchase of the major equipment for this work (gas chromatography (GCs) with an array of detectors) are highly appreciated.

My special thanks and appreciation also go to my colleagues, Dr. Olagoke Adeniji, Dr. Abdulrazaq Yahaya, Mr. Olufemi Ademoyegun, Mr. Mike Ojemaye, Mr. Rotimi Larayetan, Mr. Ezekiel Agoro, Miss Dorcas Mutukwa and Mrs. Remi Gbede. Their words of encouragement motivated me the more.

I would not have considered this part complete without acknowledging and appreciating the valuable input and support of Dr. Okaiyeito, Kunle, Mr Olaniyan, Lamidi, Dr Adewuyi,

Sheriff, Dr Adeniji, Olagoke, Mr Adeboye, Azeez and Dr Babatope Akinyemi (Department of Agric. Economics). Likewise, Dr Mishi (GMRDC) for his supports in statistical analysis. May the God Almighty contribute the best of values to your affairs as well.

I also sincerely acknowledge and express my profound gratitude to the Executive Director of Forestry Research Institute of Nigeria, Ibadan (both the present and the immediate past) for giving me the opportunity to embark on the program. In the same vein, I equally appreciate the Head of Administration of FRIN, Mr. Ganiyu Akinade, Dr Olajide Adejoba, my HOD and chairman of the Training Committee and Barrister Tosin.

Thanks go also to my special friends; Dr Lawal Ibrahim, Dr Azeez Abdulfatai, Mrs Olasupo, Olusola, Mr Oyolola Idris for their special support and motivation. Worthy of recognition are Mrs Wahab Monsurah, Imam Rofiat, Amoo Mutiu, Ajibade Soliu, Dr Azeez Jamiu, Lukman Olawoyin, Soboyede Adeola, Kilaso Lukman, Idris Olatunbosun, Adekanmi, Kamar, Ruqayya Tijani, Fatimah Adebayo-Aare and a host of others. Members of Forestry Muslim Community, Ibadan, members of Muslim Students Association (Alice, branch, South Africa), Ajadi Muslim Community and others, May Allah reward your moral support and prayers. My special thanks also go to Prof Sulaiman Yusuf and Alhaji Kazeem Alonge for their special financial interventions.

I am also very grateful to my lovely and precious parents Alhaji Basheer and Alhaja Fausat Salaudeen for being there for me always with prayers, words of encouragements and support. May Almighty Allah bless you both more than you have blessed me from childhood and may you reap the fruit of your labour in shaa Allah. To my siblings and their families; Ummu Sofiya and Udhaifa, Taoheed, Tajudeen and Ibrahim Salaudeen may Allah reward your immeasurable support and reward you all abundantly.

Finally, to you my adorable and lovely wife Mrs Khadijah Salaudeen (Nee Ahmed) for your patience, love and understanding that gave me the right state of mind to be away for so long

since we got married. Your sincerity of purpose and support for my success in life shall never go unnoticed. I equally acknowledge the understanding of my children Abdulrahman, Hassan and Muhammad Taofeek. I hope and promise to make it up with you all in shaa Allah. Most importantly, I’m grateful to Almighty Allah for taking me this far and for His wisdom. All glory belongs to Almighty Allah.

LIST OF ACRONYMS

AEMREG	Applied and Environmental Microbiology Research Group
ANOVA	Analysis of Variance
AS	Activated Sludge
BBP	Benzyl butyl phthalate
CPSIA	Consumer Product Safety Improvement Act
DBP	Di-n-butyl phthalate
DEHP	Di(2-ethyl hexyl) phthalate
DEP	Diethyl phthalate
DMP	Dimethyl phthalate
DOP	Di-n-octyl phthalate
EC	European Community
EQS	Environmental Quality Standard
EU	European Union
FD	Frequency of detection
HPLC	High Performance Liquid Chromatography
LFB	Laboratory fortified blank
LFM	Laboratory fortified matrix sample
OP	Oxidation Pond
PAE	Phthalate Esters
PCP	Pentachlorophenol
SEACREG	Synthetic, Environmental and Applied Chemistry Research Group
SPE	Solid Phase Extraction
TF	Trickling Filter

USEPA	United State Environmental Protection Agency
WWTPs	Wastewater Treatment Plants
2,4,6-TCP	2,4,6-trichlorophenol
2,4-DCP	2,4-dichlorophenol
2,4-DMP	2,4-dimethylphenol
2,4-DNP	2,4-dinitrophenol
2-CP	2-chlorophenol
2-M-4,6-DNP	2-methyl-4,6-dinitrophenol
2-NP	2-nitrophenol
4C-3MP	4-chloro-3-methylphenol
4-NP	4-nitrophenol

TABLE OF CONTENTS

LIST OF TABLES

Tables Page

LIST OF FIGURES

GENERAL ABSTRACT

The presence of phthalate esters (PAEs) and certain phenolic compounds widely known as endocrine disruptors in environmental waters such as treated wastewaters constitutes health hazard to human and aquatic lives. Unfortunately, wastewater treatment plants (WWTPs) only partially remove these synthetic chemical compounds from wastewater. In order to forestall the health challenge faced by rural dwellers, which rely on surface water for their daily needs, the present study embarked on investigating these endocrine disruptors in Municipal wastewater in the Amathole and Buffalo Districts in the Eastern Cape, South Africa and their removal rate by different WWTP technologies. One WWTP each from Adelaide, Alice, Bedford, Berlin and Seymour, using activated sludge (AS), trickling filter (TF), and oxidation pond (OP) technology were randomly selected. Some physicochemical parameters of these wastewaters were determined *on-site* using standard methods and the extraction method for endocrine disrupting chemicals (EDCs) in water was validated using solid phase extraction (SPE). Extracts were analysed using gas chromatography-mass spectrometer (GC-MS). Nine phenolic compounds; phenol (PH), 2-chlorophenol (2-CP), 2,4-dimethylphenol (2,4-DMP), 2,4-dichlorophenol (2,4-DCP), 4-chloro-3-methylphenol (4-C-3MP), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), pentachlorophenol (PCP), 2,4,6-trichlorophenol (2,4,6-TCP) and six priority PAEs namely; dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP), di(2-ethyl hexyl) phthalate (DEHP), and di-n-octyl phthalate (DOP) were the investigated EDCs. PAEs were extracted from dried sludge samples in an ultrasonic bath using dichloromethane. Some physicochemical parameters of the wastewater assessed revealed that treatment processes of AS, TF, and OP reduced turbidity, total suspended solids (TSS), total dissolved solids (TDS), and electrical conductivity (EC) while dissolved oxygen (DO) was increased. There was no significant influence on temperature and pH across the sampling points. Except for turbidity, the quality of effluent released mostly falls within South Africa standard limits for domestic and recreational water. The nine phenolic compounds were detected across the sampling points for all the WWTPs at different frequencies. The prominent phenolic compounds were 2-NP, 4-C-3MP, PCP, and 2,4-DMP with concentrations ranging from 3.3 (2,4-DMP) – 83.0 µgL^{-1} (4-C-3MP) in the influents. However, their concentrations in the effluents and receiving water bodies were below tolerable limits of 5 µgL^{-1} set by the US Environmental Protection Agency (USEPA) and the European Union (EU) for domestic use. The removal capacities of the WWTPs varied between 33 and 96%. The six PAEs were detected in all water samples from all the WWTPs. DBP was the most abundant compound in all the sampling points and sometimes DEHP in some receiving waters. The maximum detection for DBP in influent, effluent, downstream and sludge were 2,488 µgL^{-1} (Alice), 26.47 µgL^{-1} (Adelaide), 115.3 µgL^{-1} (Seymour) and 1,249 µg/g dw (Alice), respectively. DEHP was the highest detected PAE in the upstream 17.53 µgL^{-1} (Seymour). There was a notable reduction of all PAEs in the final effluent with a removal efficiency which varied as much as 61.9 – 99.5% except for AS in Seymour which operated a single tank system (27.3 - 93.7%). Removal mechanisms continued more on adsorption on settling particles and sludge than biodegradation as this study found a high positive correlation between TSS, turbidity and PAEs removal. The concentrations of PAEs detected in the receiving waters were above 1.3 and 3µgL^{-1} limit standard set by the EU and USEPA, respectively for DEHP in surface water. Similarly, the average concentrations of DBP, BBP, DEHP and DOP which vary as much as 25.97 (BBP) – 1249 µg/g d.w (DBP) in sludge samples were above EU legislation of 100 µg/g d.w. for agricultural use. AS technology, showed a better performance in the removal of PAEs (77 - 99%), followed by TF (76 - 98%) and OP (61 - 98%). In conclusion, the PAE concentration in the WWTP effluents impacted negatively on the receiving water bodies and sewage sludge unlike the phenolic compounds that were notably reduced below the

acceptable limits. Perhaps, due to the meagre amounts of the phenolic compounds that was detected entering the WWTPs. In order to avert the potential health risk to aquatic organisms' and rural dwellers, it is exigent that constituted authorities gather more information on micro-pollutants in the environment as a basis for regulations on the use of these dangerous chemicals in industries.

CHAPTER ONE

GENERAL INTRODUCTION

1.0. Introduction

In the last century, the world has benefited immensely both socially and economically from the application of a number of synthetic chemicals developed in the fields of science, agriculture (in terms of increased crop yield), medicines and industries. However, many of these chemicals persist in the environment as a result of their widespread uses (Danzo, 1998; Kookana *et al.*, 2007).

Some exogenous synthetic or natural chemicals that alter endocrine functions and consequently cause adverse health effects in organisms or their progeny are referred to as endocrine disrupting chemicals (EDCs) (Damstra *et al.*, 2002; Kookana *et al.*, 2007; Schiliro *et al.*, 2009; WHO-UNEP, 2013).

Previous studies have shown that these chemicals have multiple uses in residential, industrial and agricultural applications also make them ubiquitous in the environment, especially in surface water, rain or storm-water, sediments, soils and sewage sludge (Peijnenburg and Struijs, 2006; Roslev *et al.*, 2007; Adeniyi *et al.*, 2008; Gasperi *et al.*, 2008; Schiliro *et al.*, 2009; Fatoki *et al.*, 2010; Gao *et al.*, 2014). In addition, their refractory tendency to microbial degradation and their potential bioaccumulation in aquatic organisms increase their widespread presence in the environment (Abdel daiem *et al.*, 2012).

Human and aquatic exposure to some of these chemicals is also known to produce carcinogenic, mutagenic and teratogenic effects and other types of diseases in humans (Park *et al.*, 2012; Gao and Wen, 2016). Thus, the potential environmental hazards that these compounds pose have necessitated an increasing global attention to their fate in the environment (Becker *et al.*, 2004; Horn *et al.*, 2004; Park *et al.*, 2012; Gao and Wen, 2016). This has also made some European Union member countries and the USA, under the Consumer Product Safety Improvement Act (CPSIA), restrict or ban their uses (Hileman, 2007; Gao *et al.*, 2014). Different classes of endocrine disrupting chemicals (EDCs) include:

alkylphenols, alkylphenolethoxylates, phthalates, pesticides, bisphenol A, pharmaceutical products, polybrominated compounds and steroid sex hormones, most of which are widely reported to be detected in wastewater facilities (Roslev *et al.*, 2007; Dargnat *et al.*, 2009; Olujimi *et al.*, 2012). Phthalates esters (PAEs) and phenolic compounds have also been included in the list of priority pollutants by the US Environmental Protection agency (EPA) and the European Union due to their activities on aquatic and terrestrial animals (Llompart *et al.*, 2002; Olujimi *et al.*, 2010).

Chemically, PAEs are esters of phthalic acid that are among the most important industrial chemicals because of their increasing production rate and eco-toxicological potential (ESIS, 2009; Clara *et al.*, 2010). They are synthetic compounds widely known in the industry for wide applicability as plasticizers in polymers and additives in a number of various industrial products such as insecticides, paints, coverings, insulators in electric disposals, personal care products, and cosmetics, amongst others (Abdel daiem *et al.*, 2012). The global production of PAEs is approximately 6 million tons per year in spite of their application restrictions in some areas (Mackintosh *et al.*, 2006; Peijnenburg and Struijs, 2006; Abdel daiem *et al.*, 2012; Guo *et al.*, 2012). However, PAEs are not chemically bonded with their matrices when applied in products and may therefore be dispersed or leached easily into the environment either during their production, use or after disposal (Kotowska *et al.*, 2006; Yang *et al.*, 2006). Bis(2-ethylhexyl) phthalate (DEHP), is considered the most widely used PAE, being the dominant plasticizer used in PVC production (ATSDR, 2000; ATSDR, 2006; EU-RAR., 2008; Clara *et al.*, 2010). Human and aquatic animals are unavoidably exposed to these organic pollutants through drinking contaminated water. With the rural dwellers depending more on stream and river water for their daily domestic and agricultural use, they are more prone to the resultant health problems (Fatoki *et al.*, 2010). Thus, health risk assessment as a result of PAE uses has become a global concern.

Phenolic compounds at very low concentrations have serious effects on the taste and odour of water. They have also been found to be toxic to fish and other aquatic lives (Suliman *et al.*, 2006). Water pollution and the destruction of ecosystems have continued to increase because of contaminants produced and discarded into the water system as a result of the rapid increase in population, urbanization, industrialization, globalization and warfare, combined with increased wealth and more luxurious lifestyles (UN-Water, 2006).

The presence of EDCs (among which are a number of natural chemicals and an array of anthropogenic such as personal care products, pharmaceuticals, steroid hormones, pesticides) in the aquatic environment, such as sewage, groundwater, surface water and drinking water is emerging as a new challenge to environmental scientist and the scientific community (Fent *et al.*, 2006; Pruden *et al.*, 2006; Luo *et al.*, 2014). The release of treated effluent and the variation in removal efficiencies of wastewater treatment plants (WWTPs) that serve as primary barriers against the spread of these micro-pollutants remained the primary source of these non-conventional pollutants in the aquatic environment (Kolpin *et al.*, 2002; Loraine and Pettigrove, 2006; Gao *et al.*, 2014). A better understanding and modelling of the fate of these micro-pollutants after being released into surface water is essential for the effective prediction of their impact on receiving water bodies (Kümmerer, 2009; Luo *et al.*, 2014; Cesaro and Belgiorno, 2015). Complications are also associated with the detection and analysis of these diverse compounds in low concentrations (ng/L - µg/L) and create a challenge for water and wastewater treatment facilities. These organic contaminants, (phthalate and phenolic compounds) often escape from the conventional WWTPs, which are not designed specifically to degrade them and thus make the removal performance of WWTPs unsatisfactory (Nakada *et al.*, 2006; Xue *et al.*, 2010). Aquatic organisms are often exposed to the phthalates and phenolic compounds that may have escaped from WWTPs and this consequently introduce a hazard to the food chain (Kraigher *et al.*, 2008; Balabanici *et* *al.*, 2012). Sources of these EDCs in municipal wastewater among other routes include domestic and industrial effluent water, atmospheric deposit, and urban or agricultural run-offs. In some developed countries, additional processes such as optimization of the existing operations, or incorporation of new specific treatment operations specific to micro-pollutants removals are being adopted to improve the performance of WWTPs against removal of these EDCs (Batt *et al.*, 2007; Cai *et al.*, 2012).

The need to recycle and reuse the scarce water resources has necessitated an increase in the number of WWTPs in many countries (including South Africa) with a view to removing the conventional pollutants (such as ammonia and phosphate) and forestall outbreaks of environmental pollution and the spread of diseases (Wang *et al.*, 2005; Sun *et al.*, 2008; Olujimi *et al.*, 2012). This water scarcity, as in many other countries in the world, is becoming a major problem in South Africa, as dams in recent years have been less than 30% full (Marcucci and Tognotti, 2002; Malley *et al.*, 2009; Qiao *et al.*, 2009). As an alternative, river water, ground water, effluents from wastewater treatment plants are considered as suitable water sources for communities' daily household use and portable drinking water, especially in the rural areas (Blignaut and Van Heerden, 2009). Water quality monitoring and enforcement in these areas has been a major challenge as there are no interim guidelines for permissible levels of certain chemicals such as phthalate esters (PAEs) in fresh water systems. This has given rise to indiscriminate pollution of fresh water systems through industrial discharge, which in turn, has challenged water consumption trends in South Africa (Fatoki *et al.*, 2012). The presence of PAEs in treated and untreated wastewater, and sewage sludge of full scale WWTPs has been widely reported in many countries such as South Africa (Fatoki and Noma, 2001; Olujimi *et al.*, 2012), France in Europe (Gasperi *et al.*, 2008; Dargnat *et al.*, 2009) and China (Gao *et al.*, 2014).

This is attributable to the continued escape of PAEs and phenolic compounds from conventional WWTPs which has increased their presence in aquatic environments through discharged effluents (Xue *et al.*, 2010; Gao *et al.*, 2014). These compounds tend to be transformed or removed through physical processes (such as coagulation, filtration, and sedimentation), chemical or biological means in the WWTPs and as a result, they tend to be concentrated in wastewater sludge thereby creating disposal problems or constraining the beneficial uses of these solids (Barnabé *et al.*, 2008). Precautions and monitoring actions such as physicochemical parameters in water resources are therefore inevitable tools that have to be well established in most of the WWTPs to assess the quality and any potential impediment to the protection of the environment and public health (Okoh *et al.*, 2007; Leech *et al.*, 2009; Luo *et al.*, 2014). Especially now, there is a better understanding that exposure to even very low concentrations of EDCs can increase the risk of effects (WHO-UNEP, 2013). The growing realization that wastewater effluents may play a role in causing increase in exposure to endocrine disruptors, and at a time when water reclamation is coming under more scrutiny as a way to conserve water resources, make it imperative to assess the efficacy of water reclamation systems in removing potential EDCs (WERF, 2006). Interestingly, previous study has shown that the removal of PAEs and phenolic compounds from WWTPs can vary from 60% to 100% (Oliver *et al.*, 2005).

In South Africa, there has been paucity of information on the level of EDCs in the final effluents of municipal wastewater that is being released to the environment, especially, for aquatic life and to land-fill sites. Likewise, there is little information on the efficacy of the exiting wastewater treatment plants for the removal of the EDCs (Fatoki *et al.*, 2010; Olujimi *et al.*, 2010; Olujimi *et al.*, 2012). This calls for more monitoring and control as several research outcomes have revealed a high level of contamination of such treated water with EDCs.

1.2. Statement of research problem

There is growing demand for safe water in South Africa as the economy expands and the population increases. However, vast communities in the Eastern Cape Province of South Africa are predominantly rural and still rely heavily on surface water for their domestic, irrigation and recreational needs. In order to protect these communities from the risk of water borne diseases and several health risk, emphasis should be placed on the quality of effluents from WWTPs being released into the water bodies (Momba *et al.*, 2006; Luo *et al.*, 2014). Moreover, PAEs have been reported in water, and sediment, as well as in fish tissue samples at a level that could trigger endocrine disruption in humans and wildlife (Adeniyi *et al.*, 2008; Fatoki *et al.*, 2010). The impact of exposure to chemicals causing endocrine disruption has been on the increase especially in aquatic life, wildlife and humans. South Africa is one of the countries reported to have used and abused most chemicals listed by developed and developing countries as EDCs (Olujimi *et al.*, 2010; Olujimi *et al.*, 2012).

The increase in industrialization, urbanization, and the malfunctioning of wastewater treatment plants in some cities in South Africa have also steadily increased contamination in rivers at a level that is toxic even to aquatic organisms (Fatoki and Ogunfowokan, 1993; Fatoki *et al.*, 2010). Poor management and inadequate maintenance of wastewater and municipal sewage treatment infrastructure in Dimbaza, East London, Alice and Fort Beaufort all in the Eastern Cape, South Africa have also been identified as part of reasons for the pollution of their respective receiving watersheds upon which most rural communities depend (Momba *et al.*, 2006). Little or no data are available concerning the efficiency of the WWTPs in the removal of the phthalates and selected phenolic endocrine disrupting compounds.

1.3. Hypothesis

This study was based on the hypothesis that EDCs (phthalate and phenolic compounds) are not effectively removed from municipal wastewater by WWTPs (in the Eastern Cape Province, South Africa) using activated sludge, oxidation pond, and trickling filters technologies and as such, these micro-pollutants are discharged into the water systems.

1.4. Research aim and objectives

Aim

This research is aimed at determining the removal rate of endocrine disruptors (phthalate esters and phenolic compounds) in municipal wastewaters from WWTPs operated under three different treatment technologies (activated sludge, oxidation pond, and trickling filter) in the Eastern Cape Province, South Africa.

Specific Objectives:

- To validate analytical methods suitable for the identification and quantification of the selected phthalates and phenolic compounds from water:
- To collect wastewater samples and assess compliance in the physicochemical qualities of the treatment plant’s influent, effluent and receiving water bodies to national standards
- To determine the occurrence and concentrations of the selected six phthalate esters and USEPA priority phenolic compounds in the influents, and final effluents of the municipal WWTPs in Adelaide, Alice, Bedford, Berlin and Seymour all in the Eastern Cape, South Africa as well as in their receiving water bodies (up-stream and down-stream):

- To determine the effectiveness of the removal of the targeted micro-pollutants by the different wastewater treatment technologies and the impact of the final effluents on the organic load of the receiving water sheds:
- To assess the occurrence and concentration of phthalate esters in WWTPs sludge samples.

References

Abdel daiem, M. M., Rivera-Utrilla, J., Ocampo-Pérez, R., Méndez-Díaz, J. D. and Sánchez-Polo, M. (2012) ‘Environmental impact of phthalic acid esters and their removal from water and sediments by different technologies - A review’, *Journal of Environmental Management*, 109, 164–178. doi: 10.1016/j.jenvman.2012.05.014.

Adeniyi, A., Dayomi, M., Siebe, P. and Okedeyi, O. (2008) ‘An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo Province, South Africa.’, *Chemistry Central Journal*, 2, 9. doi: 10.1186/1752-153X-2-9.

ATSDR. (Agency forToxic Substances and diseases Registry). (2000) *Toxicological profile for di(2-ethylhexyl)phthalate (DEHP). Atlanta, GA: ATSDR,.*

ATSDR, (Agency for Toxic Substances and Disease Registry). (2006) *U.S. Department of Health and Human Services: Atlanta, Georgia*.

Balabanici, D., Hermosilla, D., Merayo, N., Klemencic, A. K. and Blanco, Á. (2012) ‘Comparison of different wastewater treatments for removal of selected endocrine disruptors from paper mill wastewaters’, *Journal of environmental science and health. Part A, Toxic/hazardous substance & environmental engineering*, 47(10), 1350–63. doi: 10.1111/cobi.12645.This.

Barnabé, S., Beauchesne, I., Cooper, D. G. and Nicell, J. A. (2008) ‘Plasticizers and their degradation products in the process streams of a large urban physicochemical sewage treatment plant’, *Water Research*, 42(1–2), 153–162. doi: 10.1016/j.watres.2007.07.043.

Batt, A. L., Kim, S., and Aga, D. S. (2007) ‘Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations.’, *Chemosphere*, 68(3), 428–435.

Becker, K., Seiwert, M., Angerer, J., Heger, W., Koch, H. M., Nagorka, R., Roßkamp, E., Schlüter, C., Seifert, B. and Ullrich, D. (2004) ‘DEHP metabolites in urine of children and

DEHP in house dust.’, *International Journal of Hygiene and Environmental Health*, 20, 409–417.

Blignaut, J. and Van Heerden, J. (2009) ‘The impact of water scarcity on economic development initiatives’, *Water SA*, 35(4), 415–420.

Cai, K., Elliott, C. T., Phillips, D. H., Scippo, M. L., Muller, M. and Connolly, L. (2012) ‘Treatment of estrogens and androgens in dairy wastewater by a constructed wetland system.’, *Water Research.*, 46(7), 2333–2343.

Cesaro, A. and Belgiorno, V. (2015) ‘Removal of endocrine disruptors from urban wastewater by advanced oxidation processes (AOPs): a review’, *The Open Biotechnology Journal*, 9(1), 1–28. doi: 10.2174/1874070720151112E012.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C. and Chovanec, A. (2010) ‘Chemosphere Occurrence of phthalates in surface runoff , untreated and treated wastewater and fate during wastewater treatment’, *Chemosphere* 78(9), 1078–1084. doi: 10.1016/j.chemosphere.2009.12.052.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R. J. and and Van der Kraak, G. (2002) *Global assessment of the state-of-the-science of endocrine disruptors, WHO/PCS/EDC/02.2, International Programme on Chemical Safety, World Health Organisation.*

Danzo, B. J. (1998) ‘The effects of environmental hormones on reproduction.’, *Cellular and Molecular Life Sciences*, 54, 1249–1264.

Dargnat, C., Teil, M. J., Chevreuil, M. and Blanchard, M. (2009) ‘Phthalate removal throughout wastewater treatment plant. Case study of Marne Aval station (France)’, *Science of the Total Environment*. 407 1235–1244. doi: 10.1016/j.scitotenv.2008.10.027.

ESIS (European Chemical Substances Information System). (2009) *European Chemical Substances Information System. <http:// Ecb.jrc.ec.europa.eu/esis/> (22.01.17).*

EU-RAR. (2008) *European Union Risk Assessment Report on Bis(2-ethylhexyl) Phthalate*

(DEHP)., Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau, 2nd Priority List, vol. 80. <http://ecb.jrc. Ec.europa.eu/Documents/Existing-Chemicals/risk_assessment/report/ Dehpreport042.pdf>.accessed 11/01/2017.

Fatoki, O., Olujimi, O., Odendaal, J. and Genthe., B. (2012) *Health Risk Assessment of Plasticizer in Wastewater Effluents and Receiving Freshwater Systems*, *InTechOpen*.

Fatoki, O. S., Bornman, M., Ravandhalala, L., Chimuka, L., Genthe, B. and Adeniyi, A. (2010) 'Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects', *Water SA*, 36(1), 117–126. doi: 10.4314/wsa.v36i1.50916.

Fatoki, O. S. and Noma, A. (2001) 'Solid Phase Extraction Method for Selective Determination of Phthalate Esters in the Aquatic Environment', *South African journal of chemistry*, 54, 69–83.

Fatoki, O. . and Ogunfowokan, A. O. (1993) 'Determination of phthalate ester plasticizers in the aquatic environment of South Western Nigeria.', *Environment International*, 19, 619–623.

Fent, K., Weston, A., and Carminada, D. (2006) 'Ecotoxicology of human pharmaceuticals.', *Aquatic Toxicology*, 76, p. 122–59.

Gao, D., Li, Z., Wen, Z. and Ren, N. (2014) 'Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China', *Chemosphere*. 95, 24–32. doi: 10.1016/j.chemosphere.2013.08.009.

Gao, D. W. and Wen, Z. D. (2016) 'Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes', *Science of the Total Environment*. 541, 986–1001. doi: 10.1016/j.scitotenv.2015.09.148.

Gasperi, J., Garnaud, S., Rocher, V. and R., M. (2008) 'Priority pollutants in wastewater and combined sewer overflow', *Science of the Total Environment*, 407(1), 263–272.

Guo, Y., Zhang, Z., Liu, L., Li, Y., Ren, N. and Kannan, K. (2012) ‘Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure.’, *Journal of Agricultural and Food Chemistry*, 60, 6913–6919.

Hileman, B. (2007) *California bans phthalates in toys for children, Chem. Eng. News*.

Horn, O., Nalli, S., Cooper, D. and Nicell, J. (2004) ‘Plasticizer metabolites in the environment.’, *Water Research.*, 38, 3693–3698.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. T. (2002) ‘Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance.’, *Environmental Science & Technology*, 36, 1202–1211.

Kookana, R., Williams, M., and Waller, N. (2007) *Endocrine- disrupting chemicals in the Australian environment, Land & Water Australia*.

Kotowska, U., Garbowska, K., and Isidorov, V. (2006) ‘Distribution coefficients of phthalates between absorption fiber and water and its using in quantitative analysis.’, *Analytica Chimica Acta*, 560, 110–117.

Kraigher, B. ., Kosjek, T. ., Heath, E. ., Kompare, B. and Mandić-Mulec, I. (2008) ‘Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors’, *Water Research.*, 42 4578–4588.

Kümmerer, K. (2009) ‘Antibiotics in the aquatic environment - A review - Part I.’, *Chemosphere*, 75, 417–434.

Leech, D. M., Snyder, M. T., and Wetzel, R. G. (2009) ‘Natural organic matter and sunlight accelerate the degradation of 17ß-estradiol in water’, *Science of the Total Environment*, 407(6), 2087–2092.

Llompart, M., Lourido, M., Landin, P., Garcia-Jares, C., And and R. Cela, J. (2002) ‘Optimization of a derivatization-solid-phase microextraction method for the analysis of thirty phenolic pollutants in water samples.', *Journal of Chromatography A*, 963(1–2), 137–148.

Loraine, G. A. and Pettigrove, M. E. (2006) 'Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California.', *Environmental Science & Technology*, 40, 687–695.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S. and Wang, X. C. (2014) 'A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment', *Science of the Total Environment*. 473–474, 619–641. doi: 10.1016/j.scitotenv.2013.12.065.

Mackintosh, C. E., Maldonado, J. A., Ikonomou, M. G. and Gobas, F. A. P. C. (2006) 'Sorption of 40, phthalate esters and PCBs in a marine ecosystem.', *Environmental Science & Technology*, 40(11), p. 3481–3488.

Malley, Z. J. U., Taeb, M., Matsumoto, T., and Takeya, H. (2009) 'Environmental sustainability and water availability: Analyses of the scarcity and improvement opportunities in the Usangu plain, Tanzania.', *Physics and Chemistry of the Earth*, 34, 3–13.

Marcucci, M. and Tognotti, L. (2002) 'Reuse of wastewater for industrial needs: the Pontedera case.', *Resources, Conservation and Recycling*, 34, 249–259.

Momba, M. N. B., Osode, A. N., and Sibewu, M. (2006) 'The impact of inadequate wastewater treatment on the receiving water bodies - Case study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province', *Water SA*, 32(5 SPEC. ISS.), 687–692. doi: 10.4314/wsa.v32i5.47854.

Nakada, N., Tanishima, T., Shinohara, H. and Kiri, K. (2006) 'Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment', *Water Research*, 40(17), 3297–3303. doi: 10.1016/j.watres.2006.06.039.

Okoh, A. I., Odjadjare, E. E., Igbinosa, E. O., and Osode, A. N. (2007) 'Wastewater treatment plants as a source of microbial pathogens in the receiving watershed.', *Africa Journal of Biotechnology*, 6, 2932–2944.

Oliver, R., May, E., And and Williams, J. (2005) 'The occurrence and removal of phthalates in a trickle filter STW.', *Water Research*, 39, 4436–4444.

Olujimi, O., Fatoki, S., Odendaal, J., and Daso, A. P. (2012) 'Chemical monitoring and temporal variation in levels of endocrine disrupting chemicals (priority phenols and phthalate esters) from selected wastewater treatment plant and freshwater systems in Republic of South Africa', *Microchemical Journal*. 101, 11–23. doi: 10.1016/j.microc.2011.09.011.

Olujimi, O. O., Fatoki, O. S., Odendaal, J. P. and Okonkwo, J. O. (2010) 'Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: A need for more monitoring', *Water SA*, 36(5), 671–682. doi: 10.4314/wsa.v36i5.62001.

Park, M. A., Hwang, K. A., Lee, H. R., Yi, B. R., Jeung, E. B. and Choi, K. C. (2012) 'Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. http://dx.doi.org/10.3892/mmr.2011.712.', *Molecular Medicine Reports*, 5(3), 761–766.

Peijnenburg, W. and Struijs, J. (2006) 'Occurrence of phthalate esters in the environment of the Netherlands.', *Ecotoxicology and Environmental Safety*, 63(2), 204–215.

Pruden, A., Pei, R., Storteboom, H. and Carlson, K. (2006) 'Antibiotic resistance genes as emerging con- taminants: studies in Northern Colorado.', *Environmental Science & Technology*, 40, 445–50.

Qiao, G., Zhao, L., and Klein, K. K. (2009) 'Water user associations in Inner Mongolia: Factors that influence farmers to join.', *Agricultural Water Management*, 96, 822–830.

Roslev, P., Vorkamp, K., Aarup, J., Frederiksen, K. and Nielsen, P. H. (2007) 'Degradation of phthalate esters in an activated sludge wastewater treatment plant', *Water Research*, 41(5), 969–976. doi: 10.1016/j.watres.2006.11.049.

Schiliro, T., Pignata, C., Rovere, R., Fea, E. and Gilli, G. (2009) ‘The endocrine disrupting activity of surface waters and of wastewater treatment plant effluents in relation to chlorination’, *Chemosphere*. 75(3), 335–340. doi: 10.1016/j.chemosphere.2008.12.028.

Suliman, F. E. O., Al-kindi, S., Al-kindy, S. and Al-lawat, H. (2006) ‘Analysis of phenols in water by high-performance liquid chromatography using coumarin-6-sulfonyl chloride as a fluorogenic precolumn label.’, *Journal of Chromatography A*, 1101, p. 179–184.

Sun, Q., Deng, S., Huang, J., Shen, G. and Yu, G. (2008) ‘Contributors to estrogenic activity in wastewater from a large waterwater treatment plant in Beijing, China’, *Environmental Toxicology and Pharmacology*, 25, 20–26.

UN-Water- (UNESCO) (2006) ‘Water: A Shared Responsibility The United Nations World Water Development Report’, *Water Resources*, (3), 120–156. doi: 10.7748/nm.21.4.12.s12.

Wang, Y., Hu, W., Cao, Z., Fu, X. and Zhu, T. (2005) ‘Occurrence of endocrine-disrupting com- pounds in reclaimed water from Tianjin, China’, *Analytical and Bioanalytical Chemistry*, 383, 857–863.

WERF, (Water Environment Research Foundation) (2006) ‘Final report on Removal of Endocrine Disrupting Compounds in water reclamation processes, IWA publishing, IWAP ISBN; 184339-758-7.’, *Water SA*, 35(3), p. 289–299.

WHO-UNEP (2013) *State of the Science: Endocrine Disrupting Chemicals - 2012, Summary for Decision Makers*. Available at: http://apps.who.int/iris/bitstream/10665/78102/1/WHO_HSE_PHE_IHE_2013.1_eng.pdf?ua=1 (acessed 17/2/2017).

Xue, W. C., Wu, C. Y., Xiao, K., Huang, X., Zhou, H. D., Tsuno, H. and Tanaka, H. (2010) ‘Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation', *Water Research.*, 44, 5999–6010.

Yang, M., Park, M. S., and Lee, H. (2006) 'Endocrine Disrupting Chemicals: Human Exposure and Health Risks', *Journal of Environmental Science and Health, Part C*, 24(2), 183–224. doi: 10.1080/10590500600936474.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

In recent times, the world is beginning to realise the resultant adverse health effects of the use of synthetic and natural chemicals that have immensely benefitted human and the ecosystem. In order to ensure that the inauspicious effects of these chemicals do not outweigh the apparent benefits that transcend the field of science, agriculture, medicine and industries, researchers are now paying more attention to the menace of these chemicals as environmental pollutants (Gao and Wen, 2016). The environmental fate of the endocrine disrupting chemicals (EDCs) that are widely known for their potential alteration or interruption of the functions of the endocrine system and their consequent adverse health effects on organisms and their progeny is now drawing increasing global attention (Park *et al.*, 2012). Their multiple uses in residential, industrial and agricultural areas and their refractory disposition to microbial degradation coupled with their potential accumulation in aquatic organisms as suggested by many researchers have made them ubiquitous in the environment, especially in surface water, rain or storm-water, groundwater, drinking water, sediments, soils and sewage sludge as reported in the literature (Boyd *et al.*, 2003; Adeniyi *et al.*, 2008; Schiliro *et al.*, 2009; Gao *et al.*, 2014).

This chapter, therefore, reviews the properties, and environmental fate of phthalate esters (PAEs) and phenolic compounds as examples of EDCs in water.

2.2. Endocrine disrupting chemicals (EDCs)

EDCs are used to describe natural chemical compounds or man-made substances that are capable of interfering with or mimicking the function of hormones in the body, thereby affecting the normal functions of body tissues and organs (Matsui, 2008). This has been linked to developmental, reproductive, neural and immune related problems in wildlife and laboratory animals (Matsui, 2008). Other researchers have also considered them exogenous substances that can cause the transmission of adverse health effects in parent organisms to their progeny (as a result of alteration in the endocrine function) (Damstra *et al.*, 2002; Kookana *et al.*, 2007; Schiliro *et al.*, 2009; WHO-UNEP, 2013).

The physiological systems affected by this disruption may include all hormonal systems ranging from the development and function of reproductive organs to adult onset diabetes or cardiovascular disease (Park *et al.*, 2012; WHO-UNEP, 2013; Gao and Wen, 2016). Although, there has been sufficient evidence to conclude that wildlife species are adversely affected by endocrine-active chemicals, the available evidence for the same effects in humans is weak (WHO-UNEP, 2013). The most widely studied class of EDCs is the estrogen receptor agonist, and chemicals in this group include: plasticizers (bisphenol A and phthalates), natural estrogen (17β-estradiol and metabolites estrone and estriol), synthetic estrogens (17α-ethinylestradiol used as active ingredient of contraceptives), and surfactants (alkylphenol degradation products nonylphenol and octylphenol) and disruption in other hormone systems which are not commonly observed (Roslev *et al.*, 2007; Dargnat *et al.*, 2009; Olujimi *et al.*, 2010). Various sources of exposure to these disruptors on a daily basis include the diet, air, skin and water through the food and beverages that are consumed, pesticides and cosmetics that are applied as well as drugs that are taken. Research outcomes have speculated on their contributions to the incidence of some diseases which include obesity, diabetes, endometriosis, cancers and reduced fertility in humans (Matsui, 2008). Phthalates, phenolic and compounds that are considered EDCs have been included on the list of priority pollutants by the US Environmental Protection agency (EPA) and the European Union because of their activities on aquatic and terrestrial animals (Llompart *et al.*, 2002; Olujimi *et al.*, 2010).

2.2.1. Categories of EDCs

The broad compounds that have been identified as EDCs can be classified into three categories:

- Natural compounds which are required in the routine functioning of the endocrine system (estrogens, estrone and androgens).
- Synthetic steroidal hormones specifically designed to target the endocrine system (antiestrogens).
- Synthetic chemicals which represent the most diverse range of chemicals produced for variety of uses (pesticides, alkyphenol, phthalate esters, organohalogens, bisphenol A, heavy metals) (Safe and Gaido, 1998; Baker, 2001; Kookana *et al.*, 2007).

2.2.2. How do endocrine disruptors work?

Various research studies on animals have revealed the mechanism through which endocrine disruptors alter the normal functions of the hormone and, in turn, influence the endocrine system. EDCs could do the following:

- Make the body fail to respond properly when the normal signal carried by the hormones is intercepted by the binding of the EDC to their receptors and consequently preventing the binding of the endogenous hormone (Jiao and Cheng, 2008; Jung *et al.*, 2012).
- Partly or completely imitate naturally occurring hormone in the body such as estrogens (the female sex hormone), androgens and thyroid hormones, resulting in overstimulation (Matsui, 2008).
- Influence or control the natural ways of the hormones or their receptors, for example, by altering their metabolism in the liver (Sumpter, 2005; Matsui, 2008).

2.2.3. Routes of exposure of the aquatic environment to EDCs

Water still remains the major reservoir for all micro-pollutants as it receives effluent from WWTPs, landfill leachate, agricultural runoffs and atmospheric deposition (Chen *et al.*, 2012). There are diverse sources of micro-pollutants in the environment particularly in water bodies. They originate mainly from mass-produced materials and commodities (Luo *et al.*, 2014). In recent times, various research investigations on micro-pollutants in different types of water (wastewater, groundwater, drinking water) revealed significant spatial and temporal variations in their concentration, depending on different factors such as rate of production, water consumption per day, metabolism (excretion rate), specific sales and services and the size and removal efficiency of WWTPs (Petrovic *et al.*, 2009; Jelic *et al.*, 2012). Variation in the daily composition of domestic sewage that is discharged into municipal systems is usually influenced by what is being excreted with urine from the daily hormones and or ingested synthetic steroids (Cesaro and Belgiorno, 2015). The direct contamination of groundwater by EDCs as a result of percolation from agricultural areas is also worrying (Lapworth *et al.*, 2012).

Some environmental factors such as rainfall, temperature and level of sunlight can also influence the concentration of EDCs in discharged effluents (Kasprzyk-Hordern *et al.*, 2009). Major sources of EDC contaminants discharged into the aquatic environment include atmospheric deposition, domestic and industrial wastes and urban or agricultural runoffs (Boyd *et al.*, 2003; Schiliro *et al.*, 2009). Other sources of these contaminants in the aquatic milieu include: pharmaceuticals, veterinary and illicit drugs, synthetic chemicals and metals (Zheng *et al.*, 2007). All these compounds constitute organic contaminants in the WWTPs. Their low solubility in water and the operational treatment at WWTPs have brought about the concentration of some EDCs in sewage sludge, resulting in the exposure of soil microbes, plants and animals to the EDCs, through the food chain (Vikelsøe *et al.*, 2002; Dargnat *et al.*, 2009). Additional sources include accidental spills, storm-water runoff, flow of water through agricultural areas where agrochemicals are extensively used to improve crops, ingredients in cosmetics and, personal care products, food supplements and their metabolites (Suliman *et al.*, 2006; Falconer *et al.*, 2006; Olujimi *et al.*, 2010). Polyvinyl chloride (PVC) waste and other PAEs in municipal landfills also migrate through the soil to groundwater (Castillo and Barceló, 2001; Abdel daiem *et al.*, 2012).

The presence of these micro-pollutants in the aquatic environment have been associated with a number of negative effects which include, short-term and long-term toxicity, endocrine disrupting effects and antibiotic resistance of microorganisms (Fent *et al.*, 2006; Pruden *et al.*, 2006; Luo *et al.*, 2014).

2.2.4. Phthalate esters (PAEs) as an example of EDCs

PAEs are liquid organic chemicals that resemble vegetable oil in appearance but differ in chemical structure and molecular weights (Sibali *et al.*, 2013). They are by far the most massively produced organic chemicals in the world because of their uses (ATSDR, 2006; Sibali *et al.*, 2013). PAEs are non-halogenated esters of phthalic acid which are colourless or a yellowish oil-like liquid, with a melting point below 25 ^{o}C and boiling points ranging from 284 - 384 ^{o}C (Table 2.1). These properties contributed to their suitability as plasticizers (Abdel daiem *et al.*, 2012).

They are diakyl or alkyl esters of 1,2-benzene carboxylic acid e.g. dimethyl phthalate (DMP), dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP) formed as a product of a reaction between alcohol (methanol, ethanol) and the carboxylic groups on a benzene ring of phthalic acids. They vary in structure and length (Figure 2.1) and the length and the branching of the dialky or alkyl/aryl side chain determines their physical properties and industrial applications (Latini, 2005; Yang *et al.*, 2006; Adeniyi *et al.*, 2008). Many studies have reported their ubiquitous presence in the environment (Koch *et al.*, 2003; Yang *et al.*, 2006; Gasperi *et al.*, 2008; Gao *et al.*, 2014) and their metabolites in human body fluids and other matrices such as serum, urine, seminal fluid and breast milk (Hogberg *et al.*, 2008; Romero-Franco *et al.*, 2011; Luo *et al.*, 2014). The continuous release of these highly consumed organic compounds and their resistance to microbial degradation have also made them ubiquitously present in water and other environmental matrices (Fierens *et al.*, 2012; Kang *et al.*, 2012; Liu *et al.*, 2014). Their occurrence in various environmental matrices both in developed and developing countries have been documented (Dargnat *et al.*, 2009; Clara *et al.*, 2010; Gao *et al.*, 2014). In Africa as well, their occurrence have been reported in rivers, (Fatoki *et al.*, 2010; Olujimi *et al.*, 2012), effluents from WWTPs, (Ogunfowokan *et al.*, 2006; Olujimi *et al.*, 2012), sediments and sludge (Adeniyi *et al.*, 2008).

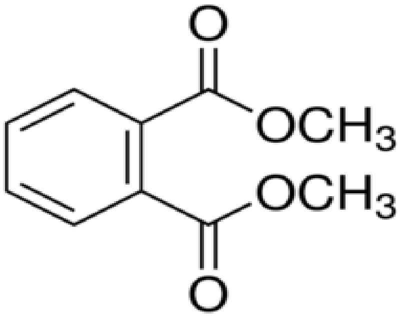
They have been widely used as plasticizers to improve the mechanical properties of plastic resins (flexibility and softness) in the production of polyvinyl chloride (PVC) base plastics; rubber, adhesives, styrene, coatings, film, pulp and paper (Sun *et al.*, 2008; Xue *et al.*, 2010). Other uses include, pesticide formulation, plumbing, non-ionic surfactants, construction materials, vinyl upholstery, table cloths and shower curtains (Yuan *et al.*, 2002; Cortazar *et al.*, 2005; Kayali *et al.*, 2006; Adeniyi *et al.*, 2008; Sun *et al.*, 2008; Xue *et al.*, 2010). In every finished product, the phthalate content usually ranges from 10 - 60 % (IARC, 2000; Abdel daiem *et al.*, 2012). However, PAEs are easily leached or released during the life cycle of plastic products because they are not chemically bound to the resin when they are used as plasticizers (Kotowska *et al.*, 2006; Yang *et al.*, 2006).

Table 2.1 Characteristics of the six investigated phthalate esters

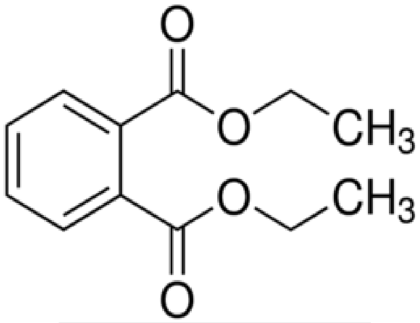
Compound	**Formula**	**Alkyl chain**	**MW**	**Sw(mgL^{-1})**	**logKow**	**Melting pt. (^{O}C)**	**Boiling pt. (^{o}C)**
DMP	$C_{10}H_{10}O_4$	1	194.19	400	1.61	2	284
DEP	$C_{12}H_{14}O_4$	2	222.24	1080	2.38	-3	299
DBP	$C_{16}H_{22}O_4$	4	278.34	10	4.45	-35	340
BBP	$C_{19}H_{20}O_4$	4	312.37	2.8	4.84	-35	370
DEHP	$C_{24}H_{38}O_4$	8	390.57	0.003	7.5	-50	385
DOP	$C_{24}H_{38}O_4$	8	390.57	0.022	8.1	-50	384

*MW=Molecular weight; Sw=Solubility in water; K_{ow}= Octanol-water partition coefficient. DMP = di-methyl phthalate; DEP = diethyl phthalate; DBP = dibutyl phthalate; BBP = benzyl butyl phthalate; DEHP = di(2-ethyl hexyl) phthalate; DOP = di-n-octyl phthalate

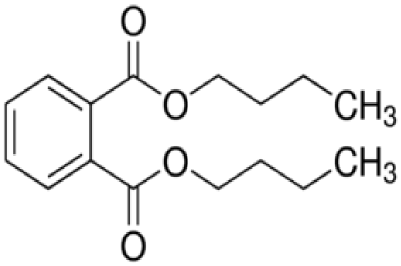
Data taken from (EU-RAR., 2007, 2008; Clara *et al.*, 2010).



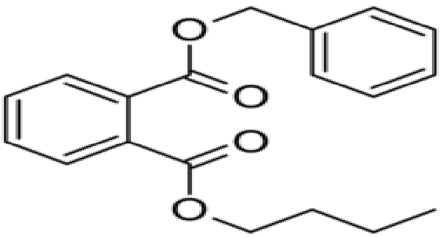
Dimethyl phthalate



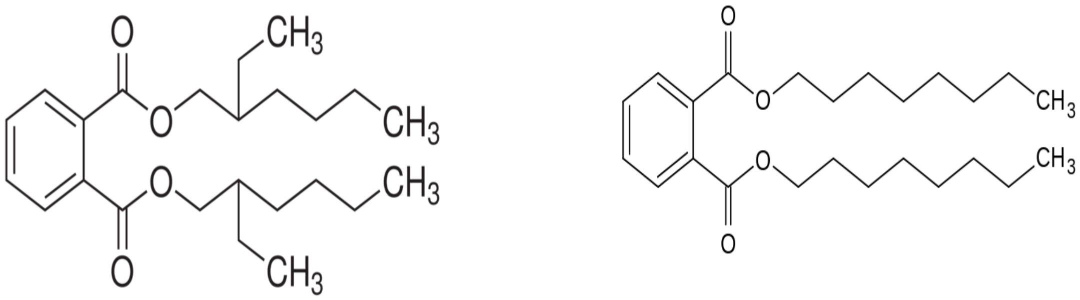
Diethyl phthalate



Di-n-butyl phthalate



Benzyl butyl phthalate



Di-(2-ethyl hexyl) phthalate phthalate

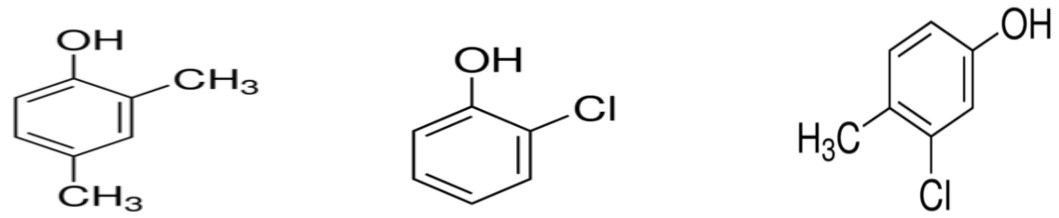
Di-n-octyl phthalate

Figure 2.1 Chemical structures of the selected phthalates (www.chemicalland.com).

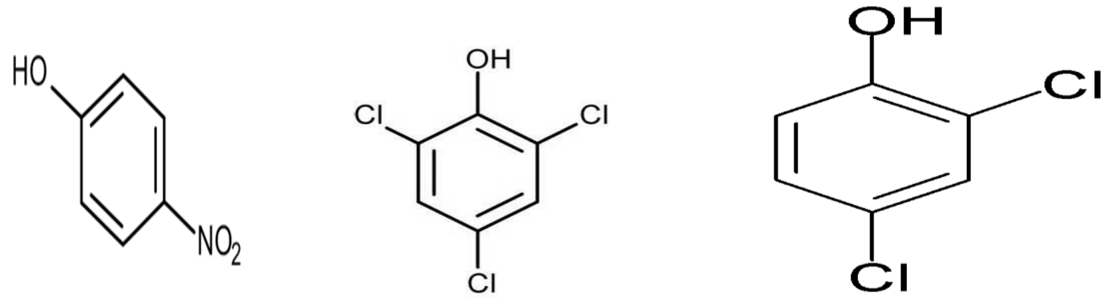
2.2.5. Phenolic compounds

Phenolic compounds are aromatic molecules containing hydroxyl (OH), methyl (CH_3) or sulphonic group attached directly to the benzene ring structure (Figure 2.2) (Xue *et al.*, 2010). Like PAEs, they are important pollutants widely present in the environment and are used in several industrial processes to manufacture chemicals such as pesticides, dye, insecticides, herbicides, explosives, for pulp bleaching with chlorine, in wood preservatives, antioxidants, plastics, adhesives and synthetic intermediate (Suliman *et al.*, 2006; Santana *et al.*, 2009; Olujimi *et al.*, 2012). The most widely studied groups such as nitrophenol, alkylphenols, bisphenols, chlorophenols and methylyphenols are classified according to their physical and chemical properties (Table 2.2) and include (Padilla-Sanchez *et al.*, 2010). Like other EDCs, some phenolic compounds have also been reported in various research findings as being highly toxic, carcinogenic, mutagenic, estrogenic and anti-androgenic (Michalowicz and Duda, 2007; Olujimi *et al.*, 2010). That is why some phenolic compounds particularly chlorophenols and nitrophenols have been classified as priority pollutants by the World Health Organisation (WHO), USEPA and European Community (USEPA, 1982; EC, 2001; WHO, 2009). These compounds are found in rivers, groundwater and soil directly through industrial effluent discharge or indirectly through natural or synthetic chemicals and find access to the environment through various sources of which industrial, domestic and vehicle emissions are the most important (Kumar *et al.*, 2014). They are likewise in the environment through the production and use of pesticides containing compounds such as 2,4-dichloro-phenoxyacetic acid, 2,4,5-trichloro-phenoxyacetic acid, dinitrophenol (dinoseb) and phenolic biocides (e.g. pentachlorophenol) (Michalowicz and Duda, 2007; Kumar *et al.*, 2014).

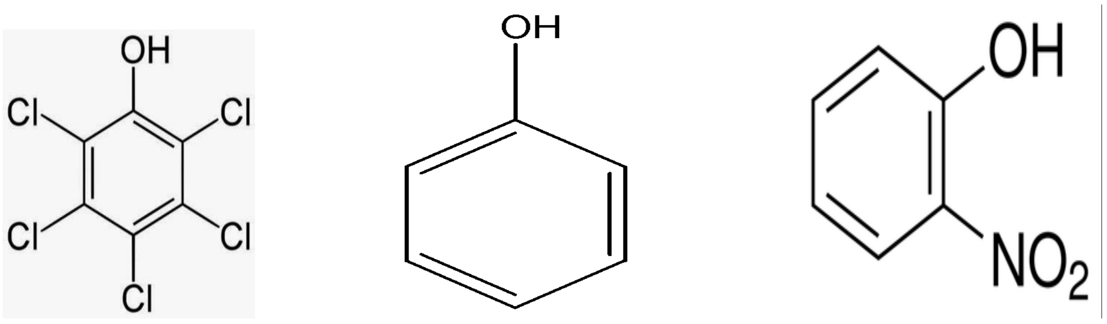
Eighty-five percent of phenolic compounds released from treatment plants are in the form of potentially estrogenic degradation products (Petrovic *et al.*, 2009; Olujimi *et al.*, 2010). Phenolic compounds have been found to be toxic to fish and other aquatic life and have adverse effects on the taste and odour of water even at a very low concentrations (ng/L - µg/L) (Suliman *et al.*, 2006). Guedes and Leitao (2012) reported the inhibitory potential of phenol and phenolic compounds in biological activities during wastewater treatments.



2,4-dimethylphenol 2-chlorophenol 4- chloro-3- methylphenol



4-nitrophenol 2, 4, 6-trichlorophenol 2,4-dichlorophenol



Pentachlorophenol Phenol 2-nitrophenol

Figure 2.2 Molecular structures of selected phenolic compounds (www.sigmaaldrich.com).

Table 2.2 Physical properties of selected phenolic compounds.

Compound	Formula	MW (g/mol)	$\log K_{ow}$	Melting pt. (ºC)	Boiling pt. (ºC)	Density (g/cm^3)
Phenol	C_6H_5OH	94.11	1.47	40.5	181.7	1.07
2-CP	C_6H_5ClO	128.56	2.15	9.4	174.9	1.26
2,4-DMP	$(CH_3)_2C_6H_3OH$	122.17	2.43	22-23	211	1.01
4-C-3-MP	C_7H_7ClO	142.58	3.1	63-65	235	1.37
2,4-DCP	$C_6H_4Cl_2$	163	3.17	42-43	209	1.38
2-NP	$C_6H_5NO_3$	139.11	1.77	43-45	214	1.5
2,4,6-TCP	$C_6H_2Cl_3OH$	197.45	6.15	69	246	1.68
4-NP	$C_6H_5NO_3$	139.11	2.04	113-114	279	1.5
PCP	C_6HCl_5O	266.34	5.34	190	309	1.98

*MW = Molecular weight, phenol =PH, 2-chlorophenol = 2-CP, 2,4-dimethylphenol = 2,4-DMP, 2,4-dichlorophenol = 2,4-DCP, 4-chloro-3- methylphenol = 4-C-3MP, 2-nitrophenol = 2-NP, 4-nitrophenol = 4-NP, pentachlorophenol = PCP, 2,4,6-trichlorophenol = 2,4,6-TCP

Data taken from (Dean *et al.*, 1995)

2.3. Various wastewater treatment plant technologies and their capacities for the removal of EDCs

The essence and design of WWTPs is to enable the disposal of wastewater (domestic or industrial) effluents without posing danger to human health or damage to the natural environment. In order to reduce contaminants to a level that nature can handle, different WWTP technologies are designed with processes for different levels of treatments (Ravi *et al.*, 2010). The three basic treatment technologies commonly used are; activated sludge (AS), which is the most widely used, trickling filter (TF) (Biofilter) and oxidation pond (OP). However, conventional wastewater treatment plants are not specifically designed to remove EDCs from wastewater and so the degree to which they are removed during primary and secondary treatment varies from nearly complete to very little. Although, it is not clear if variation in concentration and activities of EDCs are the results of single unit processes, operational parameters or sequences of processes employed during wastewater treatment (WERF, 2006).

At the primary treatment stage, where the wastewaters are retained in the grit chamber, the removal of EDCs is suggested to be through adsorption on the settling particles. The already sorbed EDCs and the newly sorbed fractions are eventually removed through the settling of particles in the primary clarifier (Marttinen *et al.*, 2003; Dargnat *et al.*, 2009).

In a similar vein, studies have shown that the removal mechanisms of EDCs at the secondary stage of treatment processes are the combined efforts of biodegradation and adsorption on particles (Gani and Kazmi, 2016). The low molecular weight compounds tend to be more easily degraded than the larger ones or sometimes they volatilize (Marttinen *et al.*, 2003; Gani and Kazmi, 2016). Berkett and Lester, (2003) listed four main removal pathways for EDCs in WWTPs as adsorption onto suspended solids; aerobic and anaerobic biodegradation; chemical (abiotic) degradation via processes such as hydrolysis; volatilization. In general, research has strongly supported the removal of EDCs through adsorption and biodegradation (Dargnat *et al.*, 2009; Clara *et al.*, 2010).

As a consequence of partial removal, the discharge of final effluent from WWTPs into surface water such as rivers, has been shown to adversely affect surrounding wildlife and aquatic organisms, as investigations suggest that these effluents are mainly responsible for increasing estrogenic activity in many aquatic bodies (Sprynskyy *et al.*, 2007).

2.3.1. Oxidation pond

The oxidation pond, also referred to as the stabilization pond, is one of the oldest methods of treating wastewater through a lagoon which uses bacteriological waste stabilization and a long retention time to decompose organic wastes (Figure 2.3). The long retention time assists in reducing faecal coliform and biological oxygen demand (BOD). Some ponds are mechanically aerated while others utilize natural oxygen from the wind and also take advantage of the dissolved oxygen produced as a result of the symbiotic relationship between the algae and bacteria existing in the pond for a continuous aerobic condition. Algae grow using energy from the sun and carbon dioxide (Mohammed and Hayder, 2013).

Figure 2.3: Oxidation ponds with different facultative and stabilization ponds (www.biosystemssa.co.za).

2.3.2. Activated sludge

This technology operates by mixing wastewater with recycled activated sludge under aeration followed by separation of the treated water from the sludge (Figures 2.4 and 2.5). This is achieved in two main compartments. The first is the aeration basin, where a favourable condition is created for bacterial growth in the wastewater under continuous aeration to keep the biomass in suspension and to circulate it through the different reactor components. The separation of the sludge from the treated water is completed in the secondary clarifier (Jansen, 2007; Emilia *et al.*, 2013).

Figure 2.4: Activated sludge wastewater treatment technology (www.constructionphotogrphy.com).

Figure 2.5: A single tank type of activated sludge wastewater treatment technology (www.pbwatertech.com).

2.3.3. Trickling filter (Biofiltration)

The trickling filter is composed of a filter with medium grains (e.g. sand, granular activated carbon) usually covered with biofilms that capture unwanted contaminants in the wastewater as they pass through them as well as break down nutrients (nitrogen and phosphorus containing compounds (Figure 2.6) (Emilia *et al.*, 2013; Vigueras-Cortés *et al.*, 2013).

Figure 2.6: Trickling filter wastewater treatment plant (www.igreenion.com).

2.3.4. Fate of PAEs and phenolic compounds in WWTP

The design of the WWTP is to remove, among other things, carbon and nitrogen from wastewater mostly through biological treatment which has limited influence on the removal of micro-pollutants (Hamid and Eskicioglu, 2012; Gani and Kazmi, 2016). As a result, EDCs are partially removed from the wastewater. The remains escape from the treatment plants and continually released as effluent into surface water and eventually into drinking and ground water (He *et al.*, 2011; Gao *et al.*, 2014). The removal rate of these organic pollutants from the effluent of WWTPs varies greatly, based on plant location and the physico-chemical parameters of the contaminants (Leusch *et al.*, 2006; Olujimi *et al.*, 2012). However, the bulk of removal usually occurs in the primary treatment stage through adsorption and settling. The micro-pollutants are sorbed during the retention period in the grit chamber and settle in the primary settling tank. Marttinen *et al.* (2003) therefore suggested a strengthened solid separation mechanism in the primary treatment stage to enhance sorption of the micro-pollutants on solids for better removal. According to Dargnat *et al.* (2009), the amount of PAEs removed during this stage varied from 38% (DEHP) - 81.4% (BBP). PAEs that are suspected of being most effectively removed in this stage are those that have an octanol-water partitioning factor (logK_{ow}) value greater than four. This is because such organic compounds are removed mainly by adsorption (Langford *et al.*, 2007; Dargnat *et al.*, 2009; Gani and Kazmi, 2016). The removal efficiencies are directly proportional to the log K_{ow} value. Dargnat *et al.* (2009) also reported a higher percentage removal of DEHP (log K_{ow} of 8.71) over DBP (log K_{ow} 5.00) in the primary settling tank.

The next stage involves the biological degradation of some of the organic pollutants in the secondary treatment usually under aeration treatment. Variation in the removal capacities of some EDCs at this stage also depends on the physico-chemical properties of the contaminants which permit resistance to biodegradation for some organic pollutants (Vogelsang *et al.*, 2006; Kong *et al.*, 2008). From the literature search, large molecular weight phthalates are more resistant to biodegradation than low molecular weight ones (Gani and Kazmi, 2016). The large molecular weights phthalates are usually removed by adsorption, which explain their prevalence in the sludge (Clara *et al.*, 2010). In a sludge sample, an increase in microbial diversity or microbial metabolism activities and sludge retention time (SRT) may increase biodegradation and adsorption of micro-pollutants such as phthalate and phenolic compounds (Semblante *et al.*, 2015). Langford *et al.* (2007) also suggest an enhanced growth for slow growing bacterial with longer SRT in the biological reactor for increased biodegradation. Huang *et al.* (2008) reported an increased biodegradation of DEHP (69 - 74%) and DBP (68 - 77%) with an increase in SRT (from 10 - 25 days) in a lab-scale anaerobic-anoxic-oxic (A/A/O) experiment.

2.4. Method of extractions of EDCs in water samples

The commonest steps in the determination of organic micro-pollutants or semi-volatile organics in water samples include the extraction method, which may sometimes be followed by an extensive clean-up and fractionation of the extract before an instrumental analysis, usually chromatographic (GC) techniques with an array of detectors (Cai *et al.*, 2003; Ling *et al.*, 2007; Damas *et al.*, 2009). Liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase micro-extraction (SPME), supercritical fluid extraction (SFE), subcritical water extraction (SCWE), and liquid-phase micro extraction (LPME) are different methods in place today for the extraction of micro pollutants from liquid samples (Abdel daiem *et al.*, 2012). LLE is a well-defined, precise and efficient method, which is more cost effective than other extraction methods for liquid samples but, it is time-consuming and uses a large amount of inflammable and toxic organic solvents such as dichloromethane. Possible losses during clean-up and formation of emulsions are still a challenge in the use of LLE (Cortazar *et al.*, 2005; Zhou *et al.*, 2005; Kayali *et al.*, 2006; Santana *et al.*, 2009).

However, the introduction of the SPE technique, which among other advantages, permits a high recovery rate, uses small amounts of organic solvent, offers a higher concentration factor, faster and easier manipulation, is now becoming the most widely used in the extraction of trace EDCs in a liquid environmental sample (Zhou, *et al.*, 2005; Zhao *et al.*, 2007; Eberlin and De Saliva, 2008). SPE cartridges also come with different sorbent materials which play a major role in determining the reproducibility and recovery levels of analytes in environmental samples. Polarity of the eluent is another factor that influences the recovery of organic compounds. Most sorbents that are used in SPE are either hydrophobic alkyl groups such as styrene-divinylbenzene or porous silica particles bounded with C-18 (Patrolecco *et al.*, 2004).

2.5. Human exposure to EDCs and health effect

There are several routes by which humans are exposed to EDCs. These may be through inhalation, ingestion, drinking and use of personal care products especially those having to do with oral contact (Clark *et al.*, 2011). The most common routes are drinking fresh water contaminated with EDCs-laden effluent from WWTPs, or groundwater contaminated through the use of EDCs sorbed sludge without adequate treatment to improve soil conditions. Another exposure route is inhaling air that is saturated with these organic contaminants, although, exposures so far are below the oral reference doses (Rfd) reported for some EDCs by US EPA (Gani and Kazmi, 2016). For example, Singh and Li, (2012) reported phthalate metabolites (mono-phthalate) in human blood, serum and urine as a result of oxidation of phthalates to which humans are exposed. The concentrations of mono-ethyl phthalate (MEP), mono-ethylhexyl phthalate and mono-butyl phthalate metabolites found in human urine are 71.42 mg/L, 15.37 mg/L, and 71.42 mg/L respectively (Gani and Kazmi, 2016). Figure 2.7 shows a schematic exposure route of phthalates to human. Young infants and children are exposed to EDCs usually through the oral route of direct ingestion of the chemicals, breast milk, infant formula, contaminated water, food, toys, surface and carpet dust, and medical devices (Huang *et al.*, 2008; Olujimi *et al.*, 2010). In aquatic animals, EDCs bio-accumulate in the animals' fat tissues because they are fat soluble and as a result, concentrations are usually 100 times greater in body tissues than in the surrounding water (Huang *et al.*, 2008; Mckinlay *et al.*, 2008). Marine animals are also included in this bioaccumulation effect. However, endocrine disruption in humans may take a longer time or be selective in certain stages of development or manifest in later generations (Falconer *et al.*, 2006; Juvancz *et al.*, 2008). An investigation on exposure of EDCs to a number of experimental animals revealed health problems such as; cancer, reproductive and fertility problems and problem on development of new born and kids (Behnke *et al.*, 1987; Gani and Kazmi, 2016). In the aquatic environment, the presence of EDCs has been linked to feminization of male fish and changes in immunologic system of marine mammals (Cesaro and Belgiorno, 2015). There are also problems of the breaking of eggs in birds, fishes, and turtles and problems with their reproductive system as well as those of mammals (Esplugas *et al.*, 2007; Cesaro and Belgiorno, 2015).

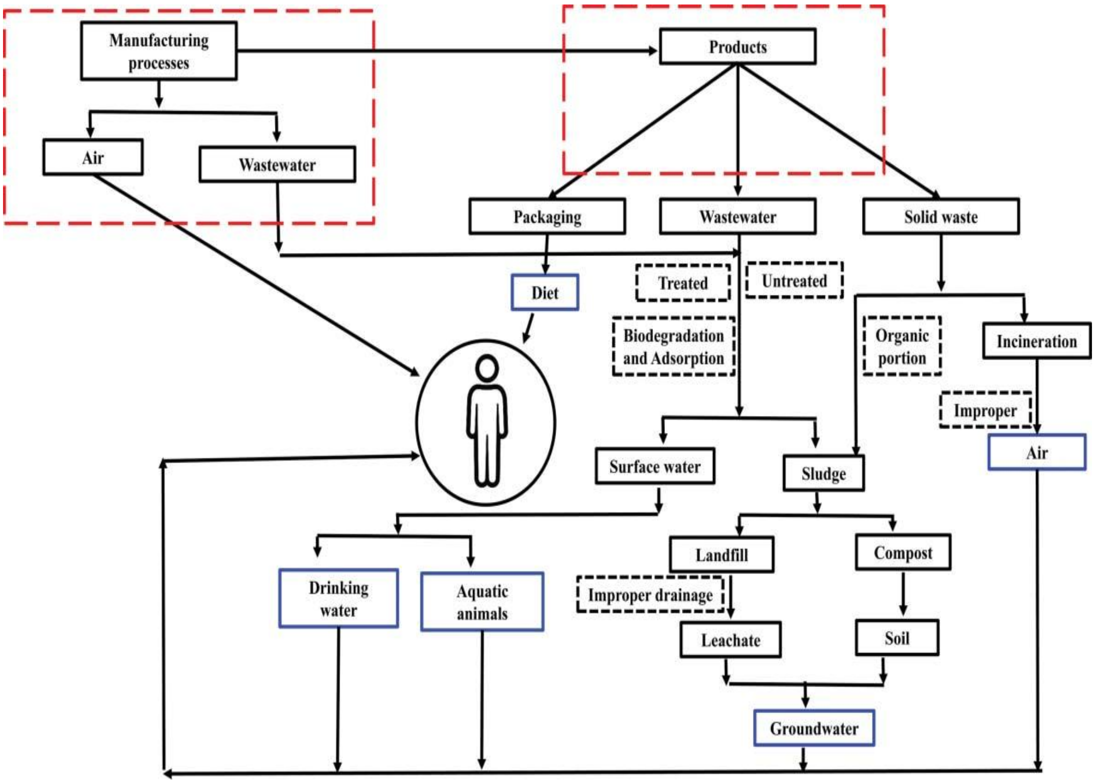


Figure 2.7: Phthalate exposure routes to humans in the environment. Source: (Gani and Kazmi, 2016).

2.6. Conclusions

The continuous release of these health challenging micro-pollutants into surface water such as rivers, streams, and estuaries from wastewater treatment plant effluents and the possible contamination of groundwater and drinking water through the use of sludge with the same WWTPs calls for a global approach to finding solutions. From the literature search, there is still limited evidence to show the mechanisms of operation of these EDCs in relation to different human health related diseases that are attributed to them. For a more efficient approach to combating the global challenge posed by these chemicals in South Africa, there is need for continuous monitoring of their occurrence and distribution in the environment. This should provide a better understanding of what is needed for a more efficient remedial approach. The challenge of the extraction and detection of these micro-pollutants in different environmental matrices also calls for future research in the development of more convenient methods of extraction that are less toxic to health and not too expensive. In the immediate future, incorporation of additional treatment processes for specific degradation of these EDCs is worthy of consideration.

References

Abdel daiem, M. M., Rivera-Utrilla, J., Ocampo-Pérez, R., Méndez-Díaz, J. D. and Sánchez-Polo, M. (2012) ‘Environmental impact of phthalic acid esters and their removal from water and sediments by different technologies - A review’, *Journal of Environmental Management*, 109, 164–178. doi: 10.1016/j.jenvman.2012.05.014.

Adeniyi, A., Dayomi, M., Siebe, P. and Okedeyi, O. (2008) ‘An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo Province, South Africa.’, *Chemistry Central Journal*, 2, 9. doi: 10.1186/1752-153X-2-9.

ATSDR, (Agency for Toxic Substances and Disease Registry). (2006) *U.S. Department of Health and Human Services: Atlanta, Georgia*.

Baker, V. A. (2001) ‘Endocrine disrupters - testing strategies to assess human hazard.’, *Toxicology in Vitro.*, 15, 413–419.

Behnke, W., Nolting, F., and Zetzsch, C. (1987) ‘An aerosol smog chamber for testing abiotic degradation In R. Greenhalgh and T. R. Roberts (Eds.).’, in *Pesticide science and biotechnology, proceedings of the International Congress of Pesticide Chemistry*, 401–404 Oxford, England: Blackwell.

Berkett, J. and Lester, J. (2003) *Endocrine disrupters in Wastewater and sludge treatment processes. IWA publishing, Lewis Publishers CRC press LLC.ISBN: 1843390310*.

Boyd, G. R., Reemtsma, H., Grimm, D. A. and Mitra, S. (2003) ‘Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada.’, *Science of the Total Environment*, 311, 135–149.

Cai, Y. ., Jang, G., and Liu, J. . (2003) ‘Solid-phase extraction of sev- eral phthalate esters from environmental water samples on col- umn packed with polytetrafluoroethylene turning’, *Analytical Sciences*, 19, 1491–1494.

Castillo, M. and Barceló, D. (2001) 'Characterisation of organic pollutants in textile wastewaters and landfill leachate by using toxicity-based fractionation methods followed by liquid and gas chromatography coupled to mass spec- trometric detection.', *Analytica Chimica Acta*, 426, 253–264.

Cesaro, A. and Belgiorno, V. (2015) 'Removal of endocrine disruptors from urban wastewater by advanced oxidation processes (AOPs): a review', *The Open Biotechnology Journal*, 10(1), 1–28. doi: 10.2174/1874070720151112E012.

Chen, L., Zhao, Y., Li, L., Chen, B. and Zhang, Y. (2012) 'Exposure assessment of phthalates in non-occupational populations in China.', *Science of the Total EnvironmentTotal Environ.*, 427–428, 60–69.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C. and Chovanec, A. (2010) 'Occurrence of phthalates in surface runoff , untreated and treated wastewater and fate during wastewater treatment', *Chemosphere* 78(9), 1078–1084. doi: 10.1016/j.chemosphere.2009.12.052.

Clark, K. E., David, R. M., Guinn, R., Kramarz, K. W., Lampi, M. A. and Staples, C. A. (2011) 'Modeling human exposure to phthalate esters: a comparison of in- direct and biomonitoring estimation methods.', *Human and Ecological Risk Assessment*, 17, 923–965.

Cortazar, E., Bartolome, L., Delgado, A., Etxebarria, N., Fern´andez, L.A., Usobiaga, A. and Zuloaga, O. (2005) 'Optimisation of microwave-assisted extraction for the determination of nonylphenols and phthalate esters in sediment samples and comparison with pressurised solvent extraction.', *Analytica Chimica Acta*, 534, 247–254.

Damas, E. Y. C., Medina, M. O. C., Clemente, A. C. N., Diaz, M. A. D., Bravo, L. G., Ramada, R. M. and Porto, R. M. D. (2009) 'Validation of an analytical methodology for the quantitative analysis of petroleum hydrocarbons in marine sediment samples.', *Quimica Nova*, 32(4), 855–860.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R. J. and Van der Kraak, G. (2002) *Global assessment of the state-of-the-science of endocrine disruptors, WHO/PCS/EDC/02.2, International Programme on Chemical Safety, World Health Organisation.*

Dargnat, C., Teil, M. J., Chevreuil, M. and Blanchard, M. (2009) 'Phthalate removal throughout wastewater treatment plant. Case study of Marne Aval station (France)', *Science of the Total Environment* 407 1235–1244. doi: 10.1016/j.scitotenv.2008.10.027.

Dean, J. R., Tomlinson, W. R., and Comber, M. (1995) 'Octanol-water partition coefficients of substituted phenols and their correlation with molecular descriptors', *Analytica Chimica Acta*, 315(95), 193–200.

Eberlin, M. and De Saliva, C. R. (2008) 'Faster and simpler determination chlorophenols in water by fiber introduction mass spectrometry.', *Analytica Chimica Acta*, 620, 97–102.

EC, (European Community) (2001) 'The list of priority substances in the field of water policy and amending directive, Council directive 2455/2001/ECC.', *Official Journal,* 331, 1–5.

Emilia, P., Tamara, R., Carmen, G. and Blaj, I. (2013) 'Comparison of efficiency of different type systems for wastewater treatment', 21, 665–674.

Esplugas, S., Bila, D., Krause, L. and Dezotti, M. (2007) 'Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. *J Hazard Mater*, 149, 631–42. [http://dx.doi.org/10.1016/j.jhazmat.2007.07.073] [22991565]'.

EU-RAR. (2007) *European Union Risk Assessment Report on Benzyl Butyl Phthalate (BBP)., Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau, 3rd Priority List, vol. 76. <http://ecb.jrc.ec.europa.eu/DOCUMENTS/ Existing-Chemicals/RISK_ASSESSMENT/RE* (accessed 11/01/2017).

EU-RAR. (2008) *European Union Risk Assessment Report on Bis(2-ethylhexyl) Phthalate (DEHP)., Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau,* *2nd Priority List, vol. 80. <http://ecb.jrc. Ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/ Dehpreport042.pdf>*(accessed 11/01/2017).

Falconer, I., Chapman, H., Moore, M. and Ranmuthugala, G. (2006) ‘Endocrine-disrupting compounds: A review of their challenge to sustainable and safe water supply and water reuse.’, *Environmental Toxicology*, 21, 181–191.

Fatoki, O. S., Bornman, M., Ravandhalala, L., Chimuka, L., Genthe, B. and Adeniyi, A. (2010) ‘Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects’, *Water SA*, 36(1), 117–126. doi: 10.4314/wsa.v36i1.50916.

Fent, K., Weston, A., and Carminada, D. (2006) ‘Ecotoxicology of human pharmaceuticals.’, *Aquatic Toxicology*, 76, 122–59.

Fierens, T., Servaes, K., Van Holderbeke, M., Geerts, L., De Henauw, S., Sioen, I. and Vanermen, G. (2012) ‘Analysis of phthalates in food products and packaging materials sold on the Belgian market.’, *Food and Chemical Toxicology*, 50, 2575–2583.

Gani, K. M. and Kazmi, A. A. (2016) ‘Phthalate contamination in aquatic environment: A critical review of the process factors that influence their removal in conventional and advanced wastewater treatment’, *Critical Reviews in Environmental Science and Technology* 46(17), 1402–1439. doi: 10.1080/10643389.2016.1245552.

Gao, D., Li, Z., Wen, Z. and Ren, N. (2014) ‘Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China’, *Chemospher*. 95, 24–32. doi: 10.1016/j.chemosphere.2013.08.009.

Gao, D. W. and Wen, Z. D. (2016) ‘Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes’, *Science of the Total Environment*, 541, 986–1001. doi: 10.1016/j.scitotenv.2015.09.148.

Gasperi, J., Garnaud, S., Rocher, V. and R., M. (2008) ‘Priority pollutants in wastewater and combined sewer overflow’, *Science of the Total Environment*, 407(1), 263–272.

Guedes, S. and Leitao, A. (2012) ‘Effect of phenolic compounds on osmotic stress on the expression of penicillin biosynthetic genes from penicillium chrysogenum var. halophenolicum strain.’, *Journal of xenobiotics*, 2, 7–12.

Hamid, H. and Eskicioglu, C. (2012) ‘Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. doi:10.1016/j.watres.2012.08.002’, *Water Research*, 46, 5813–5833.

He, H., Hu, G.-J., Sun, C., Chen, S.-L., Yang, M.-N., Li, J., Zhao, Y. and Wang, H. (2011) ‘Trace analysis of persistent toxic substances in the main stream of Jiangsu section of the Yangtze River, China. doi:10.1007/s11356-010-0414-z’, *Environmental Science and Pollution Research*, 18, 638–648.

Hogberg, J., Hanberg, A., Berglund, M., Skerfving, S., Remberger, M., Calafat, A. M., Filipsson, A. F., Jansson, B., Johansson, N. and Appelgren, M. (2008) ‘Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable Populations.’, *Environmental Health Perspectives.*, 116, 334–339.

Huang, M., Li, Y., and Gu, G. (2008) ‘The effects of hydraulic retention time and sludge retention time on the fate of di-(2-ethylhexyl) phthalate in a laboratory-scale anaerobic-anoxicaerobic activated sludge system. doi:10.1016/j. biortech.2008.03.031’, *Bioresource Technology*, 99, 8107–8111.

IARC (2000) *Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Industrial Chemicals. International Agency for Research on Cancer, Lyon, France*.

Jansen, C. (2007) ‘Water treatment technology Earth ’ s n atural water resources’, (17), 207–214.

Jelic, A., Gros, M., Petrović, M., Ginebreda, A. and Barceló, D. (2012) ‘Occurrence and elimination of phar- maceuticals during conventional wastewater treatment. In: Guasch H, Ginebreda A, Geiszinger A, editors. Emerging and priority pollutants in rivers. Berlin', *Springer*, 1–24.

Jiao, B. and Cheng, C. (2008) 'Disruption actions of bisphenol A and malachite green on growth hormone receptor gene expression and signal transduction in seabream. DOI: 10.1007/s10695-008-9227-0.', *Fish Physiology and Biochemistry,* 36(2), 251–261.

Jung, E., An, B., Choi, K. and Jeung, E. (2012) 'Potential estrogenic activity of triclosan in the uterus of immature rats and rat pituitary GH3 cells.', *Toxicology Letters*, 208, 142–148.

Juvancz, Z., Barna, S., Gyarmathy, D. and Konorot, F. (2008) 'Study of endocrine disrupting chemicals in the environment. Acta Polytech.', *Hungarica,* 5(3), 49–58.

Kang, Y., Man, Y. B., Cheung, K. C. and Wong, M. H. (2012) 'Risk assessment of human Pearl, exposure to bioaccessible phthalate esters via indoor dust around the River Delta.', *Environmental Science & Technology.,* 46, 8422–8430.

Kasprzyk-Hordern, B., Dinsdale, R., and Guwy, A. (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 Research.,* 43, 363–80.

Kayali, N., Tamayo, F. G., and Polo-Diez, L. M. (2006) 'Determination of dimethylhexyl phthalate in water by solid phase microextraction coupled to high performance liquid chromatography.', *Talanta,* 69, 1095–1099.

Koch, H., Drexler, H., and Angerer, J. (2003) 'An estimation of the daily intake of di(2ethylhexyl)phthalate(DEHP)andotherphthalatesinthegeneralpopulation.', *International Journal of Hygiene and Environmental Health,* 206, 77–83.

Kong, X., Cao, L. D., Zhang, L., Zhao, X. and Lv, Y. et al (2008) 'Evaluation of municipal sewage treatment systems for pollutant removal efficiency by measuring levels of micropollutants', *Chemosphere,* 72, 59–66.

Kookana, R., Williams, M., and Waller, N. (2007) *Endocrine- disrupting chemicals in the*

Australian environment, Land & Water Australia.

Kotowska, U., Garbowska, K., and Isidorov, V. (2006) 'Distribution coefficients of phthalates between absorption fiber and water and its using in quantitative analysis.', *Analytica Chimica Acta*, 560, 110–117.

Kumar, B., Verma, V. K., Sharma, C. S. and Akolkar, A. B. (2014) 'Quick and easy method for determination of priority phenolic compounds in water and wastewater', *Journal of Xenobiotics*, 4(1), 46–52. doi: 10.4081/xeno.2014.4680.

Langford, K., Scrimshaw, M., and Lester, J. (2007) 'The impact of process variables on the removal of PBDEs and NPEOs during simulated activated sludge treatment. doi:10.1007/s00244-006-0052-0', *Archives of Environmental Contamination and. Toxicology.*, (53), 1–7.

Lapworth, D., Baran, N., Stuart, M. and Ward, R. (2012) 'Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. [http://dx.doi.org/10.1016/j.envpol.2011.12.034]', *Environmental Pollution.*, 163, 287–303.

Latini, G. (2005) 'Monitoring phthalate exposure in humans.', *Clinica Chimica Acta*, 361, 20–29.

Leusch, F. D. ., Chapman, H. F., Heuvel, M. R. van den, Tan, B. L. ., Gooneratne, S. . and Tremblay, L. . (2006) 'Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand.', *Ecotoxicology and Environmental Safety*, 65, 403–411.

Ling, W., Gui-biin, J., Ya-qi, C., Bin, H., Ya-wei, W. and Da-zhong, S. (2007) 'Cloud point extraction coupled with HPLC-UV for the determination of phthalate esters in environmental water samples.', *Journal of Environmental Sciences*, 19, 874–878.

Liu, X., Shi, J., Bo, T., Zhang, H., Wu, W., Chen, Q. and Zhan, X. (2014) 'Occurrence of phthalic acid esters in source waters: A nationwide survey in China during the period of 2009-2012', *Environmental Pollution*. 184, 262–270. doi: 10.1016/j.envpol.2013.08.035.

Llompart, M., Lourido, M., Landin, P., Garcia-Jares, C., and R. Cela, J. (2002) ‘Optimization of a derivatization-solid-phase microextraction method for the analysis of thirty phenolic pollutants in water samples.’, Journal of *Chromatography. A*, 963(1–2), pp. 137–148.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S. and Wang, X. C. (2014) ‘A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment’, *Science of the Total Environment*. 473–474, 619–641. doi: 10.1016/j.scitotenv.2013.12.065.

Marttinen, S. K., Kettunen, R. H., Sormunen, K. M. and Rintala, J. A. (2003) ‘Removal of bis (2ethylhexyl) phthalate at a sewage treatment plant.’, *Water Research.*, 37(6), 1385–1393.

Matsui, S. (2008) ‘Endocrine Disruptors’, *Encyclopedia of Toxicology,* (May), 1259–1260. doi: http://dx.doi.org/10.1016/B978-008045405-4.00402-X.

Mckinlay, R., Plant, J., Bell, J. and Voulvoulis, N. (2008) ‘Calculating human exposure to endocrine disrupting pesticides via agricultural and non-agricultural exposure routes.’, *Science of the Total Environment.*, 398, 1–12.

Michalowicz, J. and Duda, W. (2007) ‘Phenols-Sources and toxicity.’, *Polish Journal of Environmental Studies*, 16, 347–62.

Mohammed, A. I. A.-H. and Hayder, T. H. (2013) ‘Stabilization pond for wastewater treatment’, *European Scientific Journal* 9(14), 1857–7881.

Ogunfowokan, A. O., Torto, N., Adenuga, A. A. and Okoh, E. K. (2006) ‘Survey of levels of phthalate ester plasticizers in a sewage lagoon effluent and a receiving stream’, *Environmental Monitoring and Assessment*, 118(1–3), 457–480. doi: 10.1007/s10661-006-1500-z.

Olujimi, O., Fatoki, S., Odendaal, J., and Daso, A. P. (2012) ‘Chemical monitoring and temporal variation in levels of endocrine disrupting chemicals (priority phenols and phthalate esters) from selected wastewater treatment plant and freshwater systems in Republic of South

Africa', *Microchemical Journal*. 101, 11–23. doi: 10.1016/j.microc.2011.09.011.

Olujimi, O. O., Fatoki, O. S., Odendaal, J. P. and Okonkwo, J. O. (2010) 'Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: A need for more monitoring', *Water SA*, 36(5), 671–682. doi: 10.4314/wsa.v36i5.62001.

Padilla-Sanchez, J., Plaza-Bolanos, P., Romero-Gonzalez, R., Garrido-Frenich, A. and Vidal, J. (2010) 'Application of a quick, easy, cheap, effective, rugged and safe-based method for the simultaneous extraction of chlorophenols, alkylphenols, nitrophenols and cresols in agricultural soils, analyzed by using gas chromatography–triple quadrupole-mass spectr', *Journal of Chromatography A.*, 1217, 5724–5731.

Park, M. A., Hwang, K. A., Lee, H. R., Yi, B. R., Jeung, E. B. and Choi, K. C. (2012) 'Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. http://dx.doi.org/10.3892/mmr.2011.712.', *Molecular Medicine Reports*, 5(3), 761–766.

Patrolecco, I., Capri, S., De Angelis, S., Polesello S, A. and Valsecchi, S. (2004) 'Determination of endocrine disrupting chemicals in environmental solid matrices by extraction with a non-ionic surfactant (Tween 80).', *Journal of Chromatography A.*, 1022, 1–7.

Petrovic, M., de Alda, M., Diaz-Cruz, S., Postigo, C., Radjenovic, J. and Gros, M. (2009) 'Fate and re- moval of pharmaceuticals and illicit drugs in conventional and membrane bioreactor wastewater treatment plants and by riverbank filtration.', *Philosophical Transactions of the Royal Society A.*, 367, 3979–4003.

Pruden, A., Pei, R., Storteboom, H. and Carlson, K. (2006) 'Antibiotic resistance genes as emerging con- taminants: studies in Northern Colorado.', *Environmental Science & Technology*, 40, 7445–50.

Ravi, K. P., Liza, B. P., and Somashekar, R. K. (2010) ‘Assessment of the efficiency of sewage treatment plants: a comparative study between Nagasandra and Mailasandra sewage treatment plants’, *Kathmandu University Journal of Science, Engineering and Technology*, 6(2), 115–125. doi: 10.3126/kuset.v6i2.4020.

Romero-Franco, M., Hernandez-Ramírez, R. U., Calafat, A. M., Cebrian, M. E., Needham, L. L., Teitelbaum, S., Wolff, M. S. and Lopez-Carrillo, L. (2011) ‘Personal care product use and urinary levels of phthalate metabolites in Mexican women.’, *Environment International.*, 37, 867 − 871.

Roslev, P., Vorkamp, K., Aarup, J., Frederiksen, K. and Nielsen, P. H. (2007) ‘Degradation of phthalate esters in an activated sludge wastewater treatment plant’, *Water Research*, 41(5), 969–976. doi: 10.1016/j.watres.2006.11.049.

Safe, S. H. and Gaido, K. (1998) ‘Phytoestrogens and anthropogenic estrogenic compounds.’, *Environmental Toxicology and Chemistry.*, 17, 119–126.

Santana, C., Ferrera, Z., Padron, M. and Rodrigues, J. (2009) ‘Methodologies for the extraction of phenolic compounds from environmental samples: new approaches.’, *Molecules*, 14, 298–320.

Schiliro, T., Pignata, C., Rovere, R., Fea, E. and Gilli, G. (2009) ‘The endocrine disrupting activity of surface waters and of wastewater treatment plant effluents in relation to chlorination’, *Chemosphere*. 75(3), 335–340. doi: 10.1016/j.chemosphere.2008.12.028.

Semblante, G. U., Faisal, I., Hai, X. H., Ball, A. S., Price, W. E. and Nghiem, L. D. (2015) ‘Trace organic contaminants in biosolids: Impact of conventional wastewater and sludge processing technologies and emerging alternatives.’, *Journal of Hazardous Materials.*, 300, 1–17.

Sibali, L. L., Okonkwo, J. O., and McCrindle, R. I. (2013) ‘Determination of selected phthalate esters compounds in water and sediments by capillary gas chromatography and flame ionization detector.', *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering*, 48(11), 1365–77. doi: 10.1080/10934529.2013.781884.

Singh, S. and Li, S. S.-L. (2012) 'Epigenetic effects of environmental chemicals bisphenol A and phthalates.', *International Journal of Molecular Sciiences*, 13, 10143–10153.

Sprynskyy, M., Lebedynets, M., Namieśnik, J. and Buszewski, B. (2007) 'Phenolics occurrence in surface water of the Dniester river basin (West Ukraine): natural background and industrial pollution', *Environmental Geology*, 53, 67–75.

Suliman, F. E. O., Al-kindi, S., Al-kindy, S. and Al-lawat, H. (2006) 'Analysis of phenols in water by high-performance liquid chromatography using coumarin-6-sulfonyl chloride as a fluorogenic precolumn label.', *Journal of Chromatography A*, 1101, 179–184.

Sumpter, J. (2005) 'Endocrine disruptor in the aquatic environment: A review.', *Acta Hydrochim. Hydrobiol.*, 33(1), 9–16.

Sun, Q., Deng, S., Huang, J., Shen, G. and Yu, G. (2008) 'Contributors to estrogenic activity in wastewater from a large waterwater treatment plant in Beijing, China', *Environmental Toxicology and Pharmacology*, 25, 20–26.

USEPA, (United States Environmental Protection Agency). (1982) *Appendix A to 40 CFR, Part 423--126 Priority Pollutants. Available from: https://www.epa.gov/eg/toxic-and-priority-pollutants-under-clean-water-act. Accessed 19.01.2017.*

Vigueras-Cortés, J. M., Villanueva-Fierro, Ignacio, GarzónZúñiga, M. A. and José de Jesús Návar-Cháidez, I. C.-H. and C. H.-R. (2013) 'Performance of a biofilter system with agave fiber filter media for municipal wastewater treatment', *Water Science and Technology*, 68(3), 599–607.

Vikelsøe, J., Thomsen, M., and L, C. (2002) 'Phthalates and nonylphenols in profiles of differently dressed', *Science of the Total Environment*, 296, 105–116.

Vogelsang, C., Grung, M., Jantsch, T., Tollefsen, K. and Liltved, H. (2006) ‘Occurrence and removal of selected organic micropollutants at mechanical, chemical and advanced wastewater treatment plants in Norway.’, *Water Research*, 40, 3559–3570.

WERF, (Water Environment Research Foundation) (2006) *Final report on Removal of Endocrine Disrupting Compounds in water reclamation processes, IWA publishing, IWAP ISBN; 184339-758-7. Water SA 35 (3): 289-299.*

WHO (2009) *World Health Statistics 2009. Geneva: WHO Press*.

WHO-UNEP (2013) *State of the Science: Endocrine Disrupting Chemicals - 2012, Summary for Decision Makers*. Available at: http://apps.who.int/iris/bitstream/10665/78102/1/WHO_HSE_PHE_IHE_2013.1_eng.pdf?ua=1(accessed 17/02/2017).

Xue, W. C., Wu, C. Y., Xiao, K., Huang, X., Zhou, H. D., Tsuno, H. and Tanaka, H. (2010) ‘Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation’, *Water Research.*, 44, 5999–6010.

Yang, M., Park, M. S., and Lee, H. (2006) ‘Endocrine Disrupting Chemicals: Human Exposure and Health Risks’, *Journal of Environmental Science and Health, Part C*, 24(2), 183–224. doi: 10.1080/10590500600936474.

Yuan, S. Y., Liu, C., Liao, C. S. and Chang, B. V. (2002) ‘Occurrence and microbial degradation of phthalate esters in Taiwan river sediments’, *Chemosphere*, 49(10), 1295–1299. doi: 10.1016/S0045-6535(02)00495-2.

Zhao, X., Li, J., Shi, Y., Cai, Y., Mou, S. and Jiang, G. (2007) ‘Determination of perfluorinated compounds in wastewater and river water samples by mixed hemimicelle-based solid-phase extraction before liquid chromatography electrospray tandem mass spectrometry detection.’, *Journal of Chromatography A*, 1154, 52–59.

Zheng, Z., He, P.-J., Shao, L.-M. and Lee, D.-J. (2007) ‘Phthalic acid esters in dissolved fractions of landfill leachates. doi:10.1016/j.watres.2007.06.040’, *Water Res.,* 41, 4696–4702.

Zhou, F., Li, X.., and Zeng, Z. (2005) ‘Determination of phenolic com- pounds in wastewater samples using a novel fiber by solid-phase microextraction coupled to gas chromatography.’, *Analitica Chimica Acta*, 538, 63–70.

CHAPTER THREE

Method validation for the quantitative analysis of phthalates and phenolic compounds in wastewater samples

Abstract

Validation of analytical method for routine extraction and quantification of selected phthalates esters and phenolic compounds in wastewater samples from Alice treatment plant was carried out. Spiked water samples and wastewater were extracted using solid-phase extraction (SPE) C_{18}-U (strata) 1000 mg/6mL and extracts analysed for targeted compounds using gas chromatography-mass spectrometer (GC-MS). A combination of different organic solvents for analyte elution and different SPE sorbent sizes were also optimized. The analysis of the spiked water gave good recoveries for phthalates and phenolic compounds which varied in percentage from 72.56 ± 2.60 (di-n-octylphthalate) to 128.04 ± 6.20 (dimethyl phthalate) and 77.38 ± 0.36 (2-chlorophenol) to 111.64 ± 0.51 (2,4- di-chlorophenol), respectively. Only the unsubstituted phenol gave a lower percentage recovery of 47.59 ± 0.12. The estimated limit of detection (LOD) varied between 0.55 µg/L (dimethyl phthalate) and 1.25 µg/L (di-n-octyl phthalate). The phenolic compounds which were derivatized using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) before analysis also gave an LOD that ranged from 0.10 µg/L (2,4,6-trichlorohenol) to 0.59 µg/L (pentachlorophenol). The recoveries of the spiked matrix samples (wastewater) were above 60% for most analytes. The precision and accuracy of the method showed that the proposed method is reliable, selective and reproducible for the intended analytical investigation. The SPE method of extraction was convenient, reliable but expensive. Therefore, it is important that future research be focused on a less expensive but efficient extraction methods especially for the convenience of developing countries.

Key-words: Method validation, endocrine disrupting chemicals, wastewater, SPE and GC-MS

3.1. Introduction

The spread of a wide variety of chemicals substances in the world today has been driven by modern industrial civilization and approximately 300 million tons of these chemicals are produced annually worldwide (Schwarzenbach *et al.*, 2006). Significant amounts of these chemicals are released into the environment through the continuous production of manufactured products, and in the course of their usage and disposal (Matsui, 2008; Jinya, 2013). In some cases, trace amounts of these chemical pollutants may exceed the tolerable thresholds for humans and in wildlife. To effectively monitor and control these chemicals, a very reliable and efficient analytical method is needed (Jinya, 2013).

Since the mid-1990s, a wide range of adverse effects in humans and in wildlife have been associated with these chemicals which may also manifest in subsequent generations (Matsui, 2008). This poses a threat to human as these effects may be irreversible (Ballesteros *et al.*, 2006; Matsui, 2008). These compounds are man-made organic chemicals, readily released into the aquatic environment especially receiving water bodies through various human activities (Cesaro and Belgiorno, 2015). For future protection, the need to assess the level and effect of these chemical compounds has been identified. European Union and the US Environmental Protection Agency (EPA) have considered further investigations into the roles of a “priority” list of chemicals in endocrine disrupting activities. Phthalate esters (PAEs), alkylphenols and bisphenol A (BPA) are considered potential active endocrine disrupting chemicals (EDCs), among others (Ballesteros *et al.*, 2006). Their continuous and frequent detection in the effluent of wastewater treatment plants (WWTPs) as a result of their persistence has adverse effects on public health and the aquatic environment (Cesaro and Belgiorno, 2015).

The procedure for the determination of trace organics in complex matrices is usually accomplished in several analytical steps. The common steps basically include extraction, which is sometimes followed by an extensive clean-up and fractionation of the extract and finally further separation by instrumental analysis of the fractions usually by chromatographic techniques, high performance liquid chromatography (HPLC) or gas chromatography (GC) with an array of detectors (Cai *et al.*, 2003; Ling *et al.*, 2007; Damas *et al.*, 2009). Liquid-liquid extraction (LLE) with dichloromethane has been the norm in the extraction of semi-volatile organics from water. However, previous studies have shown that it is time-consuming and uses large amounts of inflammable and toxic organic solvent such as dichloromethane. In addition, possible losses during clean-up and the formation of emulsions are still challenges in the use of LLE (Cortazar *et al.*, 2005; Zhou *et al.*, 2005; Kayali *et al.*, 2006; Santana *et al.*, 2009). In recent times, the use of solid phase extraction (SPE), which is faster, easily manipulated and requiring the use of small amounts of solvent for the simultaneous analyses of separate groups of EDCs such as phenols, phthalates and pesticides, has been reported (Zhou *et al.*, 2005; Eberlin and De Saliva, 2008; Jinya, 2013). The polarity of the eluent is an important factor that influences the recovery of organic compounds. Most sorbents that are being used in SPE are either hydrophobic alkyl groups such as styrene-divinylbenzene or porous silica particles bounded with C-18 (Patrolecco *et al.*, 2004).

Due to the insensitivity of some of these compounds to GC, especially the alkylphenol and its derivatives, several derivatization procedures have also been developed (Kumar *et al.*, 2014b). From previous studies, commonly used derivatizing agents include; acetic anhydride, pentafluorobenzyl (PFB), N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) or N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) which forms trimethylsilyl (TMS) or tributylsilyl (TBS) derivatives. These derivatizing agents are more thermally stable and enhance sensitivity to the GC (Rodrıguez *et al.*, 1997; Ballesteros *et al.*, 2006; Santana *et al.*, 2009).

Method validation in analytical works gives the assurance that the intended method is specific, accurate, reproducible and robust over the specified range of analysis. It provides a quality control system and documented evidence that the method adopted is reliable for routine use and can be referenced (Olujimi *et al.*, 2011b). In analytical work, there are usually no standard methods that may be conveniently applied directly to the analysis of a particular analyte in different matrices without optimization. This is to ensure the suitability or reliability of the method for the intended analytical work with full compliance with national and international regulations (Thompson *et al.*, 2002; Kumar *et al.*, 2014a). Despite the well-established institutional frameworks for the analysis of EDCs compounds, continuous validation and modification of the existing methods are still a norm for a better and more suitable instrumental technique. In different countries such as South Africa, some of the instrumental methods of analysis used at present to determine the occurrence, distribution and characterisation of EDCs in different environmental matrices and clinical/biological samples are GC-MS, gas chromatography-flame ionization detector (GC-FID), gas chromatography-electron captured detector (GC-ECD).

This study therefore aimed at validating a suitable and reproducible method for the extraction of trace phthalates and phenolic compounds in wastewater and receiving water bodies. In addition, different SPE sorbent sizes under different solvent combination for convenient and accurate extractions were also investigated.

3.2. Materials and Methods

3.2.1. Chemicals and reagents

Standards of dimethyl phthalate (DMP) 98.0%, diethyl phthalate (DEP) 99.9%, di-n-butyl phthalate (DBP) 96.8%, benzyl butyl phthalate (BBP) 99.0%, di(2-ethyl hexyl) phthalate (DEHP) 99.6% and di-n-octyl phthalate (DOP) 99.1%, in 100 mg each and, 2000 µg/mL standard mixture of 4-chloro-3-methylphenol (4C-3MP), (99.7%), 2-chlorophenol (2-CP), (99.8%), 2,4-dichlorophenol (2,4-DCP), (100%), 2,4-dimethylphenol (2,4-DMP), (100%), 2,4-dinitrophenol (2,4-DNP), (100%), 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), (100%), 2-nitrophenol (2-NP), (100%), 4-nitrophenol (4-NP), (100%), pentachlorophenol (PCP), (99%), phenol (99%) and 2,4,6-trichlorophenol (2,4,6-TCP) (100%) were all sourced from Accu- standard, Inc. USA. The selected surrogate, 2-fluorobiphenyl (2FB) was purchased from Restek, USA. A working mixture of 100 µg/mL including the surrogate was prepared from the 2000 µg/mL stock solution separately for phthalates and phenols and stored below 4 ^{o}C in an amber bottle. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and chlorotrimethylsilane were purchased from Macherey-Nagel and Darmstadt respectively, both from Germany. Double-distilled water was produced in the Department of Chemistry laboratory, University of Fort Hare. Sodium thiosulphate pentahydrate and anhydrous sodium sulphate (which was heated in a muffle furnace at 450 ^{o}C for 2 h) were sourced from Merck, South Africa. Pure nitrogen gas (99.99%) supplied by Afrox gas, South Africa, was used for the drying of sample extracts. High performance liquid chromatography (HPLC) grade methanol, n-hexane, dichloromethane and acetone, were all purchased from Merck, Germany.

3.2.2. Solid-phase materials

Three different SPE cartridges were examined for efficient recovery; C18-U (strata) 1000 mg/6 mL and C-18X (strata) 500 mg/6 mL both from Phenomenex supplied by Separations, and C18 500 mg/6 mL with a more compacted sorbent disc was from Restek, USA.

3.2.3. Cleaning of glassware

All glass-ware was thoroughly washed with detergent, rinsed with tap water followed by distilled water before finally being soaked in 10% nitric acid overnight (24 h). This was followed by soaking in acetone (300 mL) for at least 1 h before it was oven dried at 200 ^{o}C for 4 h. A routine rinse of the glass-ware with HPLC grade acetone or dichloromethane was carried out before use to remove any form of phthalate contaminants. All apparatus used were made of glass equipped with Polytetrafluoroethylene (PTFE) lined caps.

3.2.4. Sample collection and preparation

Double-distilled water was used to run all recovery experiments to validate the analytical method. To evaluate the efficiency of the method, influent and final effluent samples including receiving river (Tyhume) were collected in pre-cleaned 1 L amber bottles from a nearby wastewater treatment plant (WWTP) serving Alice, in the Amathole District Municipality, Eastern Cape, South Africa. All water samples were adjusted to a pH ≤ 2 with HCl (aq) (1:1 v/v) after they had been dechlorinated with 20 - 40 mg of sodium thiosulphate and stored in a refrigerator at 4 ^{o}C before extraction (Munch, 2000). The same treatment was carried out on all the laboratory fortified blank (LFB) samples. Wastewater samples were allowed to settle overnight (24 h) and all filtered through stocked glass wool to remove all solid particles that may clog the cartridge. Surrogate standard (2-fluorobiphenyl) (25 or 50 µL) was spiked to 500 mL of water samples to make 5 or 10 µgL^{-1} prior to extraction. This also provided a means to monitor the efficiency of the extraction procedure for subsequent correction.

3.2.4.2. Solid-phase extraction procedure and derivatization

Three different cartridges: C18-U (strata) 1000 mg/6 mL, C-18X (strata) 500 mg/6 mL and C18 500 mg/6 mL were used to analyse LFBs fortified at two different concentrations (5 and 10 µg/L) with phthalate standard mixtures to compare extraction efficiency. The cartridges fitted to a vacuum manifold connected to a pump were first conditioned with organic solvents and washed with double-distilled water before the loading of the spiked water samples. This was followed by solvent elution of the analytes after drying the cartridges under vacuum. Three different solvent combinations (Hexane, dichloromethane and acetone) were also examined for better extraction efficiency. To confirm the effectiveness of extraction and quantification of the desired analytes, actual samples were subjected to C18-U 1000 mg/L SPE cartridge and GC-MS analysis. An organic solvent combination of method A was adopted for the other water analysis. One litre influent, effluent and receiving water samples collected from Alice WWTP were divided into two equal halves (500 mL each). One half (500 mL) of each sampling point (influent, effluent and receiving water) was spiked with 25 µL of phthalate esters and surrogate standard before extraction (LFM) while the other half was spiked with surrogate standard only.

3.2.4.3. Solid-phase extraction (SPE) (Method A)

Extraction method A was developed from the reference method of Sánchez-Avila *et al.,* (2009). Cartridges were successively conditioned with 7 mL each of n-hexane, dichloromethane and methanol, respectively at about 3 mL/min and later washed with 10 mL of double-distilled water. The respective sorbent beds were not allowed to go dry from the time the conditioning commenced until the drying stage. The separate water samples (500 mL) connected to the cartridges by PTFE tubes, were run through the conditioned cartridges at a flow rate of about 10 mL/min. The sample bottles were separately rinsed with 5 mL of double-distilled water and used to rinse the cartridges before allowing them to dry under vacuum for 1 h. Cartridge elution was carried out with 7 mL mixture of dichloromethane: hexane (1:1) followed by 7 mL dichloromethane: acetone (1:1) and the eluents collected in a 60 mL amber glass vial. The eluent was later reduced to about 2 mL in a rotary evaporator and finally blown to 1 mL at 30 ^{o}C under a stream of dry nitrogen gas, ready for GC analysis.

3.2.4.4. SPE (Method B)

This extraction method followed modified guidelines for the UCT SPE system for the US EPA method 8270, (U C T, 2000, 2013). The cartridge conditioning was carried out with 10 mL each of dichloromethane, methanol and double-distilled water successively in that sequence and finally with 10 mL of 0.05 N HCl. Other routine SPE extraction procedures were followed as enumerated above. Sample analytes were eluted with 5 mL each of acetone, dichloromethane, 1 mL ammonium hydroxide (25%) and finally by 2 × 5 mL dichloromethane. Eluent drying was conducted with 20g anhydrous sodium sulphate (Na_2SO_4) and treated as mentioned in method A above.

3.2.4.5. SPE (Method C)

Extraction method C was a modified reference method of Olujimi *et al.*, (2011a) in which, cartridges were conditioned with 5 mL each of n-hexane:acetone (1:1 v/v) and methanol, then washed with 10 mL double-distilled water. The basic extraction steps enumerated above were followed. Analytes elution was carried out with 5 mL each of methanol and n-hexane: acetone (1:1 v/v).

3.2.4.6. Derivatization

Derivatization was carried out for phenolic compounds only. Sample extracts were blown to almost dryness under a gentle flow of dry nitrogen for better recovery. Two hundred microliters (200 µL) of a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and chlorotrimethylsilane as catalyst (99:1 v/v) was added to the respective extract residues in the 5 mL glass vial and left in the oven at 70 ^{o}C for 30 min to achieve complete derivatization (Ballesteros *et al.*, 2006).

3.2.5. Instrumental analysis

For phthalates the GC-MS analysis was performed using Agilent technology 7890B gas chromatograph coupled with the mass selective detector (MSD) 5977A. The capillary column HP-5 MS (30 m × 250 µm I.D. × 0.25 µm) film thick was used for extract separation with helium as carrier gas at 90 kPa pressure and a constant flow rate of 1 mL/min. The mass spectrometry detector (MSD) was operated in full-scan mode from m/z 50-750 amu and the mass spectrometer calibrated with perfluorotributylamine (PFTBA) before use. Data acquisition and integration analysis were carried out by Chemstation software. Samples were injected automatically using paused splitless mode with injection pulse pressure of 90 kPa for 1 min and purge flow to split vent 50 mL/min at 2 min. The transfer line and injection port were set at 280 ^oC and 300 ^oC respectively with ionization energy of 70eV. The GC oven temperature was initially set at 50 ^oC and held for 1 min, then increased to 310 ^oC at a rate of 10 ^oC/min and was maintained at 5 min. A solvent delay of 4 min was allowed to protect the ion multiplier of the MS instrument.

A different temperature programme was set for phenol compounds using the same GC-MS instrument explained above. One microliter (1 µL) of sample extract was injected in splitless mode at an initial temperature of 70 ^oC which was held for 1 min. The temperature was further increased to 150 ^oC and later to 290 ^oC at the rate of 14 ^oC/min and 6 ^oC/min, respectively.

3.2.6. Quality assurance and quality control

The procedure blank fortified with the surrogate and calibrated standard mixture, as well as the laboratory reagent blank (LRB) were always included in every batch of 10 samples analysed and subjected to the same analytical procedure using the same reagents. This was conducted to determine the background contaminants of phthalate or any other interfering compounds and subsequent background subtraction. All samples were also fortified with surrogate (2FB) at 5 µg/L prior to extraction to verify the method performance. The method precision was verified by analysing four replicates of LFBs fortified at different concentrations (5 and 10 µg/L) which gave a relative standard deviation of 15%. A continuous calibration check (CCC) standard (using one of the calibration standards) was always injected into the instrument before and after a batch analysis to test for instrument sensitivity and reproducibility.

An external standard calibration was adopted with five points for each analyte and the concentration was determined within the linear range of the calibration curve. Two replicate analyses of calibration standards ranging from 0.1-15 µg/mL for each analyte including the surrogate standard were performed to generate the calibration curve. The regression coefficient between the peak area and the injected concentration for each of the six PAEs and surrogate varied between 9.93E-01 (BBP) – 1.00 E+00 (DBP). The recoveries for each of the PAEs spiked in water at 5 and 10 µgL^{-1} including the surrogate were also determined.

To determine the sensitivity of the method, the U.S. EPA guideline was assessed for the determination of LOD and LOQ. This was achieved by calculating the standard deviation of the responses from seven replicate injections of a recovery made for phthalate esters at 5 µg/L and four replicate injections for phenolic compounds at the same concentrations. The values obtained were multiplied by the "t" value at 99% confidence level (Caruso and Santoro, 2014; Kumar *et al.*, 2014b).

$$LOD = Standard\ deviation \times t_{student}$$

(Where the $t_{student}$ at n-1 degree of freedom and n = number of observation) t value was obtained from t-test probability tables.

The LOQ is a measured value above which specified a degree of confidence is given to the analytical results obtained. This was mathematically determined by multiplying the standard deviation obtained from series of replicate analysis used in the determination of LOD by ten (10) at a signal to noise >10:1. (WDNR, 1996; Kumar *et al.*, 2014a)

$$LOQ = Standard\ deviation \times 10.$$

3.3. Results and discussion

3.3.1. Pre-concentration procedure

The results of the analyte recoveries carried out on LFB fortified at 5 µg/L using different SPE cartridges are presented in Table 3.1. The percentage recovery for the phthalate using C18-U (strata) 1000 mg/6mL ranged between 73 and 119%. C-18X (strata) 500 mg/6mL and C18 500 mg/6mL also varied between 79 - 103% and 77 - 106% respectively. This falls within the standard permissible range for semi volatile compounds like EDCs (70 - 130%) (Munch, 2000). This suggested that the three different SPE sorbent sizes were all suitable for the pre-concentration of the six phthalates mixture. Perhaps, the non-turbid nature of the double-distilled water permitted the easy flow of the water samples and retention capacity of the respective SPEs. However, it was observed that the C18-U 1000 mg/L showed more retention capacity for analytes in real wastewater samples especially the ones with high turbidity (influent) Table 3.7 (Under recovery for Laboratory fortified matrix for the influent and effluent)

The analytical procedure for the determination of analytes in trace amounts requires a careful selection of an extraction method that is capable of isolating the trace analytes and pre-concentrating them to a level detectable by the analytical instrument. The solid-phase extraction method was adopted in this study owing to its easy adaptation to adjustment and efficient use of organic solvents.

Table 3.1 Recovery studies for phthalate in different SPE sorbent sizes

		SPE1		SPE2		SPE3	
Analyte	**Spiked (µg/L)**	**Mean ± SD (µg/L)**	**Recovery (%)**	**Mean ± SD (µg/L)**	**Recovery (%)**	**Mean ± SD (µg/L)**	**Recovery (%)**
DMP	5	4.43 ± 0.83	89	4.59 ± 0.71	92	4.40 ± 0.28	88
DEP	5	5.95 ± 0.29	119	5.11 ± 0.33	102	5.23 ± 0.17	105
DBP	5	4.95 ± 0.33	99	3.91 ± 0.27	78	4.27 ± 0.24	85
BBP	5	5.92 ± 0.23	118	5.14 ± 0.43	103	5.30 ± 0.45	106
DEHP	5	5.28 ± 0.34	106	3.97 ± 0.30	79	4.14 ± 0.22	83
DOP	5	3.66 ± 0.22	73	3.93 ± 0.32	79	3.85 ± 0.28	77

*Dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate, (BBP), di (2-ethyl hexyl) phthalate, (DEHP), di-n-octyl phthalate (DOP), C18-U (strata) 1000 mg/6ML (SPE1), C-18X (strata) 500 mg/6ML (SPE2), C18 500 mg/6ML (SPE3)

The role of the different organic solvent (eluent) combinations dichloromethane, n-hexane, methanol and acetone for desorption of the analytes was also investigated. There was no notable difference in the recovery results obtained for methods A and B (Table 3.2). The percentage recovery results varied between 71 - 118% and 68 - 124% for methods A and B, respectively. These were within the acceptable range of 70-130% for semi-volatile organic compounds in aqueous samples according to U.S. EPA method 528 (Munch, 2000). The recovery for method C was relatively lower than the acceptable range as it varied between 46 and 87%. The solvent combinations of polar and non-polar solvents (dichloromethane, n-hexane, methanol and acetone) for elution were relatively similar for all the methods. However, the low recovery recorded for method C may be attributed to the variation in elution volume. The 2 × 5 mL volume used in method C may not be sufficient to completely desorb the anaytes compared to 2 × 7 mL and 2 × 10 mL used, respectively for methods A and B. The disparity in the breakthrough volume, the blocking of the sorbent pores (depending on turbidity of water) and sometimes, the low percentage recovery are some of the challenges in the SPE method (Olujimi *et al.*, 2011b). Solvent combination for methods A and C18-U (strata) 1000 mg/6mL were eventually adopted for the subsequent analysis of phthalate and phenolic compounds in the wastewater samples for good analyte elution and high extraction yield. This was because, apart from other things, the eluent from this method required no additional drying in as much as the cartridges were allowed to dry unlike those in method B.

Table 3.2 Recovery study results for different solvent extraction methods.

		Method A		**Method B**		**Method C**	
Analyte	**Spiked (µg/L)**	**Mean ± SD (µg/L)**	**Recovery (%)**	**Mean ± SD (µg/L)**	**Recovery (%)**	**Mean ± SD (µg/L)**	**Recovery (%)**
DMP	5	4.7 ± 0.47	88 – 101	4.8 ± 0.04	97 - 98	2.48 ± 0.24	46 - 53
DEP	5	4.5 ± 0.59	82 – 99	4.82 ± 0.10	95 - 98	2.7 ± 0.20	52 - 58
DBP	5	4.31 ± 0.27	82 – 90	4.39 ± 0.02	87 - 88	2.61 ± 0.19	50 - 55
BBP	2.5	2.81 ± 0.27	106 – 118	3.05 ± 0.08	119 - 124	2.16 ± 0.01	86 - 87
DEHP	2.5	2.85 ± 0.28	106 – 122	2.52 ± 0.29	92 - 109	1.72 ± 0.13	65 - 73
DOP	2.5	1.85 ± 0.11	71 - 77	1.83 ± 0.12	68 - 78	1.43 ± 0.09	54 - 59

*Dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate, (BBP), di (2-ethyl hexyl) phthalate, (DEHP), di-n-octyl phthalate (DOP)

3.3.2. Analytical method performance and validation

The quantification of each of the analysed compounds was performed within the linear range of the calibration curves. The retention time, regression equation and linearity (R^2) separately for phthalate and phenolic compounds are presented in Tables 3.3 and 3.4, respectively.

Table 3.3 Performance parameters for quantification of phthalate esters

Analytes	**Retention time**	**Regression Equation**	**Linearity ($R^{2)}$**	**LOD µg/L**	**LOQ (µg/L)**
Dimethyl phthalate	13.239	y = 2.16E+07x – 3.49E+06	9.99E-01	0.55	1.75
Diethyl phthalate	14.938	y= 2.16E+07x + 2.73E+06	9.98E-01	0.68	2.16
Di-n-butyl phthalate	18.862	y = 2.50E+07x + 8.22E+05	1.00E+00	0.85	2.71
Benzyl butyl phthalate	22.404	y = 1.69E+07x + 3.98E+06	9.93E-01	1.01	3.23
Di(2-ethyl hexyl) phthalate	23.91	y = 2.80E+07x – 2.51E+06	9.98E-01	0.88	2.81
Di-n-octyl phthalate	25.294	y = 2.13E+07x – 3.59E+06	9.98E-01	1.25	3.99

*LOD = Limit of detection, LOQ = Limit of quantification

Table 3.4 Performance parameters for quantification of phenolic compounds

Analytes	Retention Time	Regression Equation	Linearity	LOD (µg/L)	LOQ(µg/L)
Phenol	4.745	y = 1.93E+08x + 3.81E+08	9.95E-01	0.163	0.358
2-Chlorophenol	6.293	y = 9.14E+07x – 1.57E+07	9.99E-01	0.257	0.565
2,4-Dimethylphenol	6.483	y = 1.17E+08x – 3.89E+07	9.99E-01	0.137	0.302
4-Chloro-3-methylphenol	7.508	y = 1.07E+08x – 1.87E+07	9.99E-01	0.279	0.614
2,4-Dichlorophenol	7.984	y = 1.65E+08x – 1.17E+08	9.98E-01	0.222	0.488
2-Nitrophenol	8.337	y = 6.85E+07x – 4.25E+07	9.99E-01	0.242	0.534
2,4,6-Trichlorophenol	9.47	y = 9.35E+07x – 4.78E+07	9.99E-01	0.102	0.225
4-Nitrophenol	9.681	y = 9.14E+07x – 7.31E+07	9.96E-01	0.33	0.726
Pentachlorophenol	14.962	y = 5.49E+07x – 1.80E+07	9.99E-01	0.586	1.29

*LOD = Limit of detection, LOQ = Limit of quantification

3.3.2.1. Linearity

Linearity is the ability of an analytical method within a given range to produce results that are proportional to the analyte concentration in respective samples. This was determined from the calibration curve built from the peak area versus different standard concentrations in the range of 0.1 - 15 µg/mL for all the analytes. The straight lines obtained showed a good and acceptable linearity (R^2) ranging from 9.93E-01 to 1.00E+00 and 9.94E-01 - 9.99E-01 for phthalates and phenolic compounds, respectively (Tables 3.3 and 3.4). The linear regression equation has a non-zero intercept which is an indication of no effect on the method accuracy (Kumar *et al.*, 2014a). Figures 3.1 and 3.2 show the chromatograms of all the analytes generated from Mass-Hunter software which were separately resolved under the chromatographic conditions explained above, within 31 min and 30 min analysis time for phthalates and phenolic compounds, respectively.

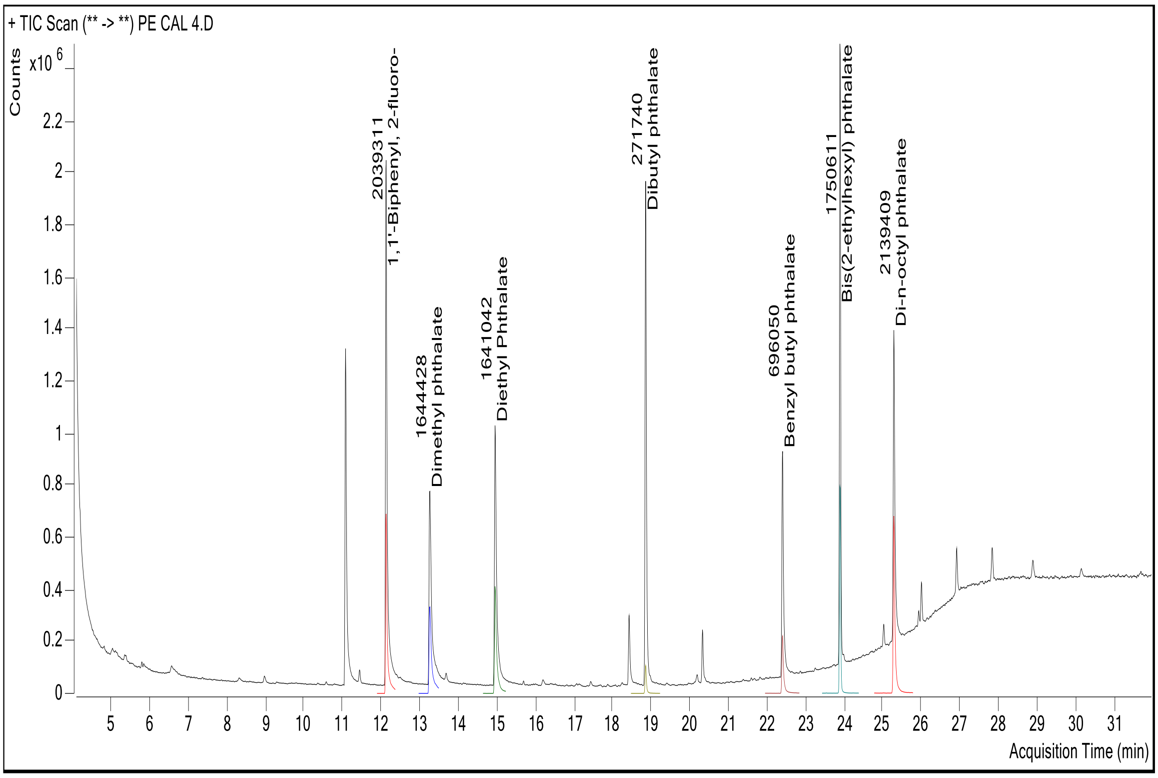


Figure 3.1: Chromatogram of phthalates and surrogate at 2 µg/mL.

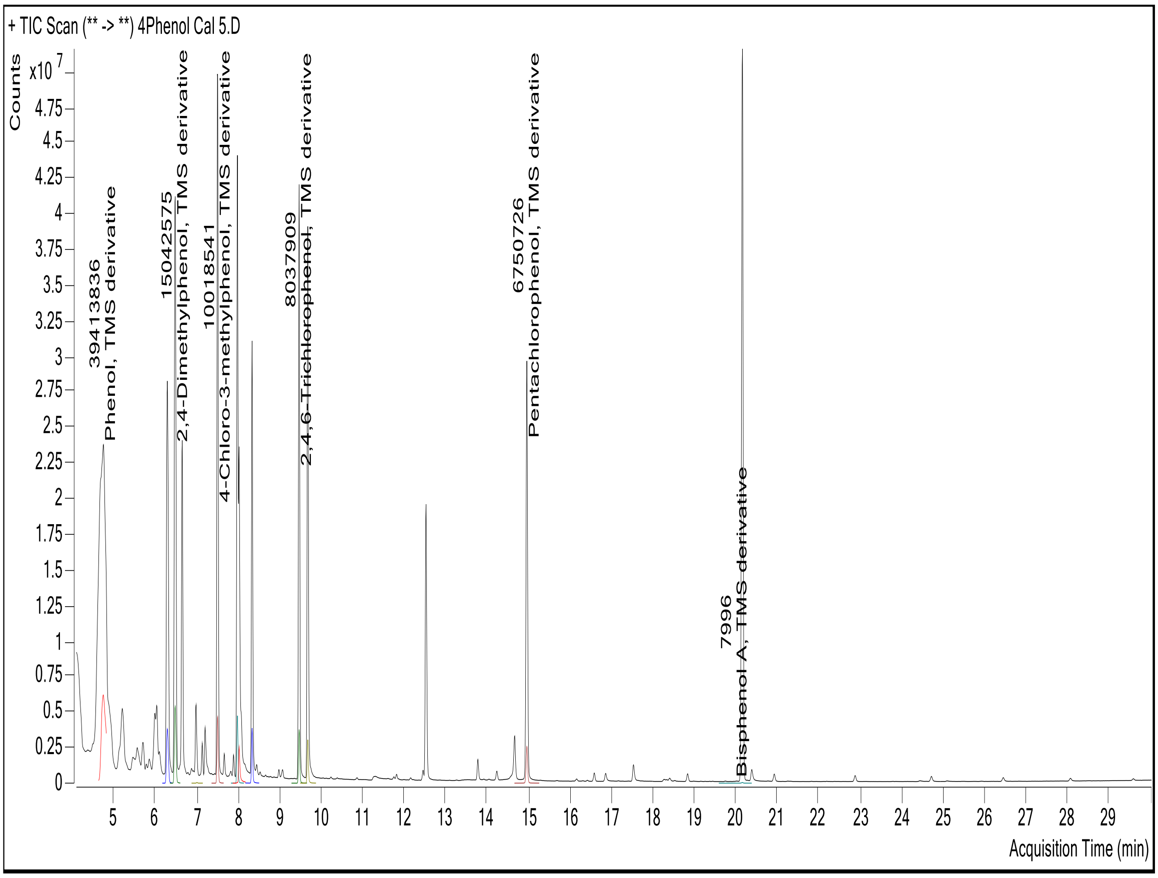


Figure 3.2: Chromatogram of phenolic compounds at10 µg/mL generated from Mass-Hunter software.

3.3.2.2. Accuracy and Precision

The accuracy of the analytical method for all the analytes under investigation was determined by measuring the recoverable amount of known standard analytes spiked in a predetermined amount of double-distilled water under different chromatographic conditions for phthalate and phenol. Four replicate analysis of 500 mL volume of double-distilled water spiked at two different concentrations with phthalate standards (5 and 10 µg/L) and 10 µg/L for phenol were used for the recovery studies as shown in Tables 3.5 and 3.6. The chromatogram in Figures 3.1 and 3.2 show no significant matrix interference and the respective percentage recoveries were all within the acceptable range (70 - 130%). The recovery of the normal unsubstituted phenol yielded a lower value (48%) which falls outside the acceptable recovery range of 50 - 150% for phenol only, because of its higher solubility in water. This suggested its possible breakthrough from the cartridge during the extraction process. The results were similar in the order of magnitude to some previous reports (Olujimi *et al.*, 2011b; Jinya, 2013). This clearly indicated that the desired analytes could be quantitatively extracted using this method.

The method precision expressed as relative standard deviation (RSD) measures the closeness of the results carried out independently under the same validated condition. The replicate analysis results (four times) used for method accuracy were also used to calculate the RSD. The RSD results for phthalates at two different spiked concentrations ranged between 1.02-16.11% and 1.42-10.43% for phenol respectively Tables 3.5 and 3.6 above. The RSD results which were less than 17% respectively suggested that the optimized analytical method is repeatable and reproducible.

Table 3.5 Recovery of phthalate esters in double-distilled water.

		Concentration (µg/L)	
Analyte	**Spiked Conc (µg/L)**	**Mean ± SD (% RSD)**	**(Recovery (%)**
Dimethyl phthalate	5	5.27 ± 0.27 (5.02)	105.37 ± 5.29
Diethyl phthalate	5	5.41 ± 0.35 (6.43)	108.17 ± 6.95
Di-n-butyl phthalate	5	5.21 ± 0.30 (5.21)	115.64 ± 6.03
Benzyl butyl phthalate	5	6.15 ± 0.27 (4.43)	123.06 ± 5.45
Di(2-ethyl hexyl) phthalate	5	3.78 ± 0.61 (16.11)	75.63 ± 12.18
Di-n-octyl phthalate	5	3.76 ± 0.56 (14.87)	75.25 ± 11.19
Dimethyl phthalate	10	12.80 ± 0.62 (4.84)	128.04 ± 6.20
Diethyl phthalate	10	12.61 ± 0.65 (5.16)	126.06 ± 6.50
Di-n-butyl phthalate	10	11.47 ± 0.12 (1.02)	114.77 ± 1.17
Benzyl butyl phthalate	10	8.52 ± 0.11 (1.31)	85.18 ± 1.11
Di(2-ethyl hexyl) phthalate	10	9.41 ± 1.05 (11.17)	91.48 ± 2.34
Di-n-octyl phthalate	10	7.26 ± 0.26 (3.58)	72.56 ± 2.60

*Conc = Concentrations, RSD = Relative standard deviation, SD = standard deviation

Table 3.6 Recovery of phenolic compounds in double-distilled water

		Concentration (µg/L)	
Analyte	**Spiked Conc. (µg/L)**	**Mean ± SD (RSD %)**	**Recovery (%)**
Phenol	10	4.76 ± 0.12 (2.55)	47.59 ± 0.12
2-Chlorophenol	10	10.77 ± 0.53 (4.94)	107.72 ± 0.53
2,4-Dimethylphenol	10	7.74 ± 0.36 (4.60)	77.38 ± 0.36
4-Chloro-3-methylphenol	10	10.53 ± 0.45 (4.31)	105.33 ± 0.45
2,4-Dichlorophenol	10	11.16 ± 0.51 (4.54)	111.64 ± 0.51
2-Nitrophenol	10	10.47 ± 0.18 (1.75)	104.71 ± 0.18
2,4,6-Trichlorophenol	10	11.20 ± 0.30 (2.72)	112.01 ± 0.30
4-Nitrophenol	10	9.32 ± 0.97 (10.43)	93.19 ± 0.97
Pentachlorophenol	10	9.534 ± 0.76 (8.00)	95.38 ± 0.76

*Conc = Concentrations, RSD = Relative standard deviation, SD = standard deviation

3.3.2.3. Method sensitivity: LOD and LOQ

There are different validated procedures that have been reported in the literature for the determination of limit of the detection (LOD) and the limit of quantification (LOQ). The resultant LOD and LOQ values are as shown in Tables 3.7 and 3.8

The calculated LOD and LOQ for the phthalate varied between 0.55 - 1.25 µg/L and 1.75 - 3.99 µg/L, respectively while phenol and its derivatives ranged between 0.10 – 0.586 µg/L and 0.23 - 1.29 µg/L (Tables 3.7 and 3.8). The values for LOD and LOQ were low enough for the method to accommodate trace amounts of these analytes in the water samples.

Table 3.7 Results of LOD and LOQ for phthalate esters

PAE	STDEV	No of replicate	t(7-1)	LOD	LOQ
Dimethyl phthalate	0.1752	7	3.143	0.5505	1.751634
Diethyl phthalate	0.2161	7	3.143	0.6792	2.160836
Di-n-butyl phthalate	0.2713	7	3.143	0.8526	2.712609
Benzyl butyl phthalate	0.3232	7	3.143	1.0157	3.231768
Di(2-ethyl hexyl) phthalate	0.2813	7	3.143	0.8841	2.813069
Di-n-octyl phthalate	0.3991	7	3.143	1.2544	3.99094

*STDEV-Standard deviation, LOD – Limit of detection, LOQ – Limit of quantification

Table 3.8 Results of LOD and LOQ for phenol compounds

Compounds	No of Replicate	STDEV	t value	LOD	LOQ
Phenol	4	0.035777	4.541	0.162466	0.357775
2-Chlorophenol	4	0.056522	4.541	0.256665	0.565218
2,4-Dimethylphenol	4	0.030194	4.541	0.137111	0.301941
4-Chloro-3-methylphenol	4	0.061377	4.541	0.278713	0.61377
2,4-Dichlorophenol	4	0.048841	4.541	0.221786	0.488409
2-Nitrophenol	4	0.053357	4.541	0.242293	0.533568
2,4,6-Trichlorophenol	4	0.02252	4.541	0.102264	0.2252
4-Nitrophenol	4	0.072614	4.541	0.329739	0.726137
Pentachlorophenol	4	0.129027	4.541	0.585914	1.290275

*STDEV-Standard deviation, LOD – Limit of detection, LOQ – Limit of quantification

3.3.3. Application of method A on surface water and wastewater samples from treatment plants in Alice.

Method A was applied on the extraction of surface and real wastewater samples collected from Alice WWTP for validation. The results of the analysis are presented in Tables 3.9 and 3.10. All the selected phthalates were detected in the influent, effluent and the receiving water except DOP. DBP was the most abundant analyte in the influent sample with an average concentration of 518 ± 24.60 µg/L in the sample matrix (SM), followed by BBP and DEHP with a concentration of 17.22 ± 2.73 µg/L and 5.34 ± 1.31 µg/L respectively. The concentrations of these analytes were significantly reduced in the final effluent suggesting a good removal capacity of the WWTP. The concentrations for all PAEs except DEHP in the downstream sample of the receiving water (Table 3.10) were generally below 3 µg/L recommended by US-EPA for DEHP in surface water for aquatic life.

The percentage recovery (R) for each of the analytes in the laboratory fortified matrix (LFM) was calculated by subtracting the concentration in SM from LFM divided by the spiked concentration (5 µg/L) and multiplied by 100.

$$R = \{LFM - SM\} / \text{spiked concentration} \times 100. \text{ (Laboratory fortified matrix =LFM).}$$

Table 3.9 Laboratory fortified matrix recovery for the influent and effluent (Method validation).

	Influent			**Effluent**		
	LFM	**SM**	**Rec. (%)**	**LFM**	**SM**	**Rec. (%)**
	Mean ±SE (µg/L)	**Mean ± SE (µg/L)**		**Mean ± SE (µg/L)**	**Mean ± SE (µg/L)**	
DMP	3.85 ± 0.53	1.15 ± 1.02	54	4.34 ± 0.02	0.61 ± 0.07	75
DEP	4.21 ± 0.60	2.00 ± 0.75	45	6.35 ± 0.20	1.87 ± 0.06	90
DBP	564.2 ± 34.43	518 ± 24.60	920	6.91 ± 0.48	1.80 ± 0.28	102
BBP	19.87 ± 2.00	17.22 ± 2.73	53	3.71 ± 0.67	1.37 ± 0.01	47
DEHP	7.13 ± 1.26	5.34 ± 1.31	36	4.57 ± 0.73	1.76 ± 0.25	56
DOP	3.26 ± 2.24	ND	65	2.54 ± 0.88	ND	51

LFM = Laboratory fortified matrix, SM = Sample matrix (Influent and effluent), ND = Not detected (below LOD) SE = Standard error.

Table 3.10 Laboratory fortified matrix recovery for the receiving river water (Method validation).

Upstream				Downstream		
	LFM	SM	Rec. (%)	LFM	SM	Rec. (%)
	Mean ± SE (µg/L)	Mean ± SE (µg/L)		Mean ± SE (µg/L)	Mean ± SE (µg/L)	
DMP	4.09 ± 0.12	0.59 ± 0.03	70	4.74 ± 0.30	0.58 ± 0.01	83
DEP	6.10 ± 0.85	0.84 ± 0.01	105	7.01 ± 0.18	0.94 ± 0.03	121
DBP	7.80 ± 0.39	2.82 ± 0.05	100	8.19 ± 0.333	2.63 ± 0.83	111
BBP	3.46 ± 0.36	0.41 ± 0.07	61	3.83 ± 0.32	0.46 ± 0.01	67
DEHP	6.57 ± 0.51	2.43 ± 0.84	83	4.82 ± 0.27	4.51 ± 0.15	6
DOP	2.58 ± 0.63	ND	52	4.98 ± 1.58	ND	100

LFM = Laboratory fortified matrix, SM = Sample matrix (Upstream and downstream), ND = Not detected (below LOD) SE = Standard error.

The percentage recoveries for phthalates in the influent samples varied from 36% (DEHP) to 65% (DOP) except for DBP (920%) Table 3.9 where the amount detected in the LFM was in excess of SM. This means that the amount of the compound (DBP) in the LFM (564.2 ± 34.43) was significantly higher than what was detected in the SM (518 ± 24.60) coupled with the additional effect of the spiked concentration (5 µg/L). This probably suggests the possibility of matrix interference in the recovery method. The recoveries for the effluent samples appeared to be better than the influent. It varied from 47 % (BBP) to 102% (DBP). This is an indication of the likelihood of matrix effects lowering the recovery of the analytes. The matrix of the WWTP's influent is much more complex than that of the effluent and may have been responsible for the low recovery. Also, the recovery of the samples (both influent and effluent) were lower than the recovery results obtained from spiked double distilled water which also indicate the role of matrix effect on analysis and quantification of organic pollutants. For the receiving water, the upstream and downstream percentage recoveries were in the range 52 - 105% and 67 - 121 %, respectively. Another matrix effect probably occurred with DEHP in the downstream where the concentration of the unfortified matrix was almost equal the LFM. The recovery results, to a large extent, suggest that the laboratory performance was good for most of the analytes. However, the tendency of matrix effects on some analytes especially in the influent samples cannot be completely ruled out as witnessed in the recovery studies. Recoveries may exhibit matrix dependence if the fortified concentration is at or above the native concentration of that analyte in the sample. In a situation where the possible problem cannot be figured out, the concentration of the analyte in the unfortified samples will be labelled as suspect (Munch, 2000).

3.4. Conclusions

The desired phthalates in the real wastewater samples were successfully extracted and quantified with the SPE and modified analytical method. The results of the recovery of phthalates and phenolic compounds from the spiked double distilled water (73 - 128%) and real wastewater sample analysis results suggest that the method was sensitive, precise, reproducible and reliable. However, the analysis of the laboratory fortified matrix in every batch of analysis will serve as a good means to identify a possible matrix effect especially with influent samples. It is also important that the amount of organic solvent required to desorb analytes completely from SPE cartridges be adequate and optimized for good recovery. A larger size of SPE sorbent cartridge (100 mg/6mL) provided retention capacity for more analytes in very turbid water samples. It is important that a less expensive and convenient extraction method be developed in future research to encourage routine determination of environmental pollutants in developing countries, especially in Africa.

References

Ballesteros, O., Zafra, A., Navalón, A. and Vílchez, J. L. (2006) 'Sensitive gas chromatographic-mass spectrometric method for the determination of phthalate esters, alkylphenols, bisphenol A and their chlorinated derivatives in wastewater samples', *Journal of Chromatography A*, 1121(2), 154–162. doi: 10.1016/j.chroma.2006.04.014.

Cai, Y. ., Jang, G., and Liu, J. . (2003) 'Solid-phase extraction of sev- eral phthalate esters from environmental water samples on col- umn packed with polytetrafluoroethylene turning', *Analytical Sciences*, 19, 1491–1494.

Caruso, A. and Santoro, M. (2014) 'Detection of Organochlorine Pesticides by GC-ECD Following U.S. EPA Method 8081', 4.

Cesaro, A. and Belgiorno, V. (2015) 'Removal of endocrine disruptors from urban wastewater by advanced oxidation processes (AOPs): a review', *The Open Biotechnology Journal*, 10(1), 151-172. doi: 10.2174/1874070720151112E012.

Cortazar, E., Bartolome, L., Delgado, A., Etxebarria, N., Fern´andez, L.A., Usobiaga, A. and Zuloaga, O. (2005) 'Optimisation of microwave-assisted extraction for the determination of nonylphenols and phthalate esters in sediment samples and comparison with pressurised solvent extraction.', *Analytica Chimica Acta*, 534, 247–254.

Damas, E. Y. C., Medina, M. O. C., Clemente, A. C. N., Diaz, M. A. D., Bravo, L. G., Ramada, R. M. and Porto, R. M. D. (2009) 'Validation of an analytical methodology for the quantitative analysis of petroleum hydrocarbons in marine sediment samples.', *Quimica Nova*, 32(4), 855–860. Available at: wos:000267331400006.

Eberlin, M. and De Saliva, C. R. (2008) 'Faster and simpler determination chlorophenols in water by fiber introduction mass spectrometry.', *Analytica Chimica Acta*, 620, 97–102.

Jinya, D. (2013) *Report Development of Solid-Phase Extraction Method for Simultaneous Analysis of Semi-Volatile Organic Compounds Using a GC-MS Database System*.

Kayali, N., Tamayo, F. G., and Polo-Diez, L. M. (2006) ‘Determination of dimethylhexyl phthalate in water by solid phase microextraction coupled to high performance liquid chromatography.’, *Talanta*, 69, 1095–1099.

Kumar, B., Verma, V., Gaur, R., Kumar, S., Sharma, C. and Akolkar, A. (2014a) ‘Validation of HPLC method for determination of priority polycyclic aromatic hydrocarbons (PAHS) in waste water and sediments.’, *Advances in Applied Science Research*, 5(1), 201–209.

Kumar, B., Verma, V. K., Sharma, C. S. and Akolkar, A. B. (2014b) ‘Quick and easy method for determination of priority phenolic compounds in water and wastewater’, *Journal of Xenobiotics*, 4(1), 46–52. doi: 10.4081/xeno.2014.4680.

Ling, W., Gui-biin, J., Ya-qi, C., Bin, H., Ya-wei, W. and Da-zhong, S. (2007) ‘Cloud point extraction coupled with HPLC-UV for the determination of phthalate esters in environmental water samples.’, *Journal of Environmental Sciences*, 19, 874–878.

Matsui, S. (2008) ‘Endocrine Disruptors’, *Encyclopedia of Toxicology*, (May), 1259–1260. doi: http://dx.doi.org/10.1016/B978-008045405-4.00402-X.

Munch, J. W. (US. E. (2000) *Determination of Phenols in drinking water by solid phase extraction and capillary column gas chromatography/mass spectrometry (GC/MS).*, *U.S. Environmental Protection Agency, Washington D.C. EPA/815/R-00/014*.

Olujimi, O., Fatoki, O. S., and Odendaal, J. P. (2011a) ‘Method development for simultaneous determination of phthalate and eleven priority phenols as tert-butyldimethylsilyl derivatives in grab samples from wastewater treatment plants using GC-MS in Cape Town, South Africa’, *Fresenius Environmental Bulletin*, 20(1), 69–77.

Olujimi, O., Fatoki, S., Odendaal, J., and Okonkwo, J. (2011b) ‘Solid-Phase Extraction Method for the Analysis of Eleven Phenolic Pollutants in Water Samples’, *Asian Journal of chemistry.*, 23(2), 657–662.

Patrolecco, I., Capri, S., De Angelis, S., Polesello S, A. and Valsecchi, S. (2004)

‘Determination of endocrine disrupting chemicals in environmental solid matrices by extraction with a non-ionic surfactant (Tween 80).’, *Journal of Chromatography A.*, 1022, 1–7.

Rodrıguez, I., Quintana, J. B., Carpinteiro, J., Carro, A. M., Lorenzo, R. A., and R., C. (1997) ‘Determination of acidic drugs in sewage water by gas chromatography–mass spectrometry as tert.-butyldimethylsilyl derivatives.’, *Journal of Chromatography A,* 985, 265–274.

Sánchez-Avila, J., Bonet, J., Velasco, G. and Lacorte, S. (2009) ‘Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant’, *Science of the Total Environment.,* 407(13), 4157–4167. doi: 10.1016/j.scitotenv.2009.03.016.

Santana, C., Ferrera, Z., Padron, M. and Rodriguez, J. (2009) ‘Methodologies for the extraction of phenolic compounds from environmental samples: New approaches.’, *Molecules,* 14, 298–320.

Schwarzenbach, R. ., Escher, B. ., Fenner, K., Hofstetter, T. ., Johnson, C. ., Gunten, U. and Wehrli, B. (2006) ‘The challenge of Micropollutants in Aquatic systems’, *Science*, 313, 1072–1077.

Thompson, M., Ellison, S., and Wood, E. (2002) ‘Harmonized guidelines for single laboratory validation of methods of analysis (IUPAC Technical Report).’, *Pure and Applied Chemistry*, 74, 835–855.

U C T (2000) *EPA Method 8270 : Determination of Semi-volatile Organic Compounds in Water using Solid Phase Extraction and GC / MS SPE Procedure :*

U C T (2013) ‘Solid Phase Extraction of EPA 8270 Compounds in Water Procedure ’:, 15.

WDNR, (Wisconsin Department of Natural Resources) (1996) ‘Analytical Detection Limit Guidance & Laboratory Guide for Determining Method Detection Limits’, *Laboratory Certification Program,* (April), 1–33.

Zhou, F., Li, X. ., and Zeng, Z. (2005) ‘Determination of phenolic com- pounds in wastewater samples using a novel fiber by solid-phase microextraction coupled to gas chromatography.’, *Analitica Chimica Acta*, 538, 63–70.

CHAPTER FOUR

Performance assessment of wastewater treatment plant using physicochemical qualities of discharged effluents and removal of selected phenolic endocrine disruptors as criteria

Abstract

This study investigated the performance of five different wastewater treatment plants (WWTPs) in some rural areas in the Eastern Cape, South Africa through an assessment of the physicochemical qualities of the effluents and their removal capacity for selected phenolic compounds. Water samples were collected on a monthly basis for six months from February to July, 2016. The physicochemical parameters which were measured *on-site* using standard methods included temperature, pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS), electrical conductivity (EC) and dissolved oxygen (DO). Phenolic compounds were determined using the solid-phase extraction (SPE) technique followed by derivatization and gas chromatography-mass spectrometry (GC-MS) analysis. Recorded temperatures for all the WWTPs effluents were 29-30 ^{o}C in summer, 20-27 ^{o}C in autumn and 12-17 ^{o}C in winter and the pH was between 6.8 and 9.1. The treatment processes increased only the DO of the final effluents while TDS, EC, turbidity and TSS were reduced. Three out of the five WWTPs effluents mostly meet the South African guidelines for water meant for domestic use. The nine phenolic compounds investigated were detected in the influent, effluents and the receiving water bodies. However, 2,4-dimethylphenol (2,4-DMP), 4-chloro-3-methylphenol (4-C-3MP), 2-nitrophenol (2-NP) and pentachlorophenol (PCP) were prominent in the influent (17.3 to 42.1 µgL^{-1}) while their concentrations in the effluents and receiving water bodies were below the tolerable limits of 5 µgL^{-1} set by US Environmental Protection Agency (USEPA) for domestic use. Generally, the WWTPs removal capacities range between 33-96%, however, the wastewater generated in these areas appeared not as polluted with phenolic compounds when compared with industrialised areas.

Key words: Physicochemical parameters, phenolic compounds, wastewater treatment plants, SPE and GC-MS.

4.1. Introduction

The relative ease of removal of contaminants of health concern such as pathogens, chemical waste including heavy metals in the WWTPs remained a strong yard-stick for measuring the efficiency of WWTPs (Coskuner and Ozdemir, 2006). Developing an integrated approach in assessing the efficiency of wastewater treatment plants (WWTPs) is a welcome idea in this direction (Sukumaran *et al.*, 2015). Efficient WWTPs are those that produce effluents that meet the desired standard criteria in chemical and biological quality at the minimum operational cost and maintenance (Arar, 1988; Sukumaran *et al.*, 2015).

In South Africa for instance, many water quality studies conducted identified poor operation and maintenance of wastewater and sewage treatment facilities as contributors to pollution of surface water on which many rural communities depend for their domestic, agricultural and other purposes. Consequently, this has direct negative impacts on human health and the environment (Mema, 2010). Williums (2008), stated that the water quality of a combined sewer is dependent on the different lifestyles of the inhabitants of the area and the temporal flow pattern. The numerous pollution problems that have contributed to major health hazards in most parts of the country, especially in poor rural communities, have been linked to the deteriorating state of municipal wastewater and WWTPs in South Africa. Different disease outbreaks: diarrhoea, typhoid fever, cholera, reported in some parts of the country (Delmas, Mpumalanga Province, KwaZulu-Natal, Limpopo Transkei and Eastern Cape) have led to death of many children (Coovadia *et al.*, 1992; Addendum., 2008; Mema, 2010). These outbreaks were linked to town water supplies which were suspected to have been contaminated with human faeces, because of poor microbiological water quality linked to sewage spills from catchment based land activities and spillage of untreated sewage. A research conducted on groundwater pollution in the Western Cape revealed seepage from wastewater treatment plants as the cause of the increase in the levels of toxic minerals in groundwater samples (Momba *et al.*, 2006).

The monitoring of physicochemical parameters in water resources in order to assess water quality and any potential risk is a necessary tool in the protection of the environment and of public health (Okoh *et al.*, 2007). Temperature, pH, odour, electrical conductivity (EC), total dissolved solids (TDS), turbidity, radioactivity, chloride, phosphate, nitrate-nitrogen and biochemical oxygen demand are among physicochemical parameters listed by Tebbut, (1998) that need to be tested for in different water sources (river, raw sewage, drinking water and sewage effluent). In view of their significance to health, these parameters are recognized by international standards for the evaluation of surface water quality (WHO, 1996; Tebbut, 1998; Chigor *et al.*, 2013).

In the Eastern Cape of South Africa, many households have no access to pipe-borne water especially in the rural areas according to the South African Department of Environmental Affairs and Tourism (DEAT) (Chigor *et al.*, 2013). This has made the use of water from alternative sources such as surface water inevitable. However, the inefficiency of most treatment plants in this region has been reported, along with their negative impact on the physicochemical qualities of the receiving water bodies (Fatoki *et al.*, 2003; Igbinosa and Okoh, 2009; Odjadjare and Okoh, 2010).

Phenol and phenolic compounds are ubiquitous contaminants in the environment. Industrial, domestic and vehicle emissions are the various sources through which these contaminants are introduced to the environment (Kumar *et al.*, 2014). They are produced in industry for various uses which include plant protection (pentachlorophenol and tetrachlorophenol), and wood preservation because of their fungicidal and antiseptic properties (Schummer *et al.*, 2009; Olujimi *et al.*, 2011; Kumar *et al.*, 2014). These compounds are dominant chemicals in polymers, pharmaceuticals, textiles, plasticizers, pulp and paper, pesticide manufacturing, the wood industry, detergent application and metallurgic industries (Grynkiewicz *et al.*, 2002; Santana *et al.*, 2009). Phenolic compounds, especially chlorophenols have been reported to be highly toxic, mutagenic, estrogenic and carcinogenic (Guedes and Leitao, 2012). They also inhibit biological activities during wastewater treatment (Michalowicz and Duda, 2007; Olujimi *et al.*, 2010; Guedes and Leitao, 2012). They occur as by-products of the natural degradation of humic substances such as tannin and lignin in the aquatic environment (Sim *et al.*, 2009; Kumar *et al.*, 2014).

The potential toxicity and negative impacts of these compounds in the environment has garnered increased public attention in recent years, so much so that some phenolic compounds such as chlorophenols and nitrophenols have been classified as priority pollutants by World Health Organisation (WHO), U.S. Environmental Protection Agency (USEPA) and the European Community (EC) (USEPA, 1982; WHO., 1989; EC, 2001). These same bodies have classified some of these compounds as endocrine disrupting chemicals (EDC) due to their potential to interact with hormone receptors. The USEPA and EC have set the minimum permissible level for phenolic compounds in water intended for drinking at 0.5 μgL^{-1} for total compounds and 0.1 μgL^{-1} for individual compounds, and 5 μgL^{-1} for bathing water (Llompart *et al.*, 2002; Czaplicka, 2004; Fattahi *et al.*, 2007; Silva *et al.*, 2009; Kumar *et al.*, 2014).

This present study focuses on evaluating the performance of five different WWTPs in two Municipalities in the Eastern Cape, South Africa using the physicochemical qualities of their discharged effluents and their removal capacity for phenolic compounds. Effluent compliance with set standards of physicochemical quality will also be assessed for the purpose of determining their impact on the quality of the receiving water for domestic and agriculture uses, as well as for the survival of the aquatic life.

4.2. Materials and Methods

4.2.1. Study area

The Eastern Cape Province, where this study was conducted, is located on the east coast of South Africa and is the second-largest province in the country (area 168,966km^2), with the third-largest population (about 7 million) and where subsistence agriculture predominates. Out of the five selected WWTPs for this study, four are located in the Amathole District Municipality and they include; WWTPs serving Alice, Adelaide, Bedford, and Seymour while the Berlin WWTP is in the Buffalo District Municipality (Table 1). The global positioning system was used to generate the coordinates of the treatment plants during sampling and the estimate of their population was based on the South Africa population census results for 2011 (Bay *et al.*, 2011).

Table 4.1 Brief descriptions of selected wastewater treatment plants.

Plants	**Adelaide**	**Alice**	**Bedford**	**Berlin**	**Seymour**
Technology	Activated Sludge	Activated Sludge	Oxidation Pond	Trickling filters	Activated Sludge
Design capacity (ML/d)	0.5	0.5-2	0.5-2	0.5-2	0.25
Geographical location	32^{o}42.343'S 26^{o}18.790'E	32^{0}47.566'S 26^{0} 50.958'E	32^{0}41.15S 26^{0} 05'E	32^{0}50.700'S 27^{0} 37'E	32^{0}47.566'S 26^{0} 50.958'E
Treatment process	Scr→Gr→Se→ AS→ Sc→ Chl	Scr→Gr→Sed → AS→ Sc→ Chl	Scr→Gr→Ap →Sed	Scr→Gr→Sed→Tf→ Sc→ Chl	Scr→Gr→Sed→ AS→ Chl
Population	12,191	15,143	8,770	3,048	2,467
Total area km^2	40	9.65	14.6	38.22	2.59
Receiving River	Cobra	Tyume	No discharge	Tributaryof Nahoon	Kat

Abbreviations; Scr. = Screening; Gr.= Grit removal; Sed.= Sedimentation; AS.= Activated Sludge; Sc.=Secondary Clarifier; Chl.=Chlorination; Ap.= Aeration pond; Tf. = Trickling filter.

The five selected treatment facilities are classified as micro or small size plants depending on their designed capacity. The micro size plants which include Adelaide and Seymour had a design capacity of < 0.5 ML/day while the other three are classified as small size, with a design capacity of 0.5-2 ML/day. The final effluent at Bedford was not discharged into any river, but was used as irrigation water to serve the Golf Course adjacent the treatment plant. Sometimes it was collected by Construction Companies for site work. At Berlin, the final effluent is a tributary of the Nahoon River which flows a long distance before it gets to the final destination. The river could not be assessed for the upstream, but sample was taken about 600 m away from the discharging point.

4.2.2. Chemicals and Materials

A standard mixture of phenolic compounds (2000 µgmL^{-1}), containing phenol (PH) 99%, 2-chlorophenol (2-CP) 99.8%, 2,4-dimethylphenol (2,4-DMP) 100%, 2,4-dichlorophenol (2,4-DCP) 100%, 4-chloro-3-methylphenol (4-C-3MP) 99,7%, 2-nitrophenol (2-NP)100%, 4-nitrophenol (4-NP) 100%, pentachlorophenol (PCP) 99%, 2,4,6-trichlorophenol (2,4,6-TCP) 100% was sourced from Accu-standard, Inc. USA. Surrogate standard, 2-Fluorobiphenyl (2FB) was bought from Restek, USA, while Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and Chlorotrimethylsilane used for derivatization were purchased from Macherey-Nagel and Darmstadt respectively, both from Germany. A working mixture of phenolic compounds and surrogate at100 µgmL^{-1} was prepared from the stock standard solution in methanol and stored under 4^{o}C in an amber bottle.

Hach instrument 2100P Turbidimeter was used to measure turbidity, Hanna multi-parameter probe (HI 98195) and Hach DR 900 colorimeter were used for the measurement of other physicochemical parameters *on-site*, including temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), turbidity and total suspended solids (TSS).

Solid-phase extraction cartridges (SPE) C18-U (strata) 1000 mg/6mL, used for the extraction of wastewater were from Phenomenex. HPLC grade dichloromethane, n-hexane, methanol and acetone and glass wool, were sourced from Darmstadt, Germany. Double-distilled water was produced in the Department of Chemistry's laboratory, University of Fort Hare. Sodium thiosulphate pentahydrate and anhydrous sodium sulphate (which was heated in a muffle furnace at 450^oC for 2 h) were from Merck, South Africa. Pure nitrogen gas (99.99%) bottled by Afrox gas, South Africa was used for drying.

4.2.3.1. Sample collection and preparation

A monthly sampling exercise was conducted for six months between February and July, 2016 for physicochemical parameters and four months (April- July) for phenolic compounds. Wastewater samples were collected from two different sampling points at the respective WWTPs; the influent, and final effluent while samples from receiving water bodies (upstream and downstream) were collected about 500 m away from the discharging point. Pre-cleaned 1 L amber glass bottles with PTFE lined polypropylene screw cap (to prevent phthalate contamination) were used for sample collection. Sampling bottles were first rinsed with the wastewater samples before composite samples were collected and immediately preserved with 20- 40 mg sodium thiosuphate and $HCl_{(aq)}$ to a pH of ≤ 2 according to standard procedure. The sodium thiosulphate was used to dechlorinate the water sample while the acid ensures chemical preservation. Samples were preserved in ice and transported to the Chemistry laboratory at the University of Fort Hare, South Africa where samples were kept at 4 ^oC in the refrigerator until the time of analysis, which was not more than three days after the collection.

4.2.3.2. Physicochemical measurement

The Hach equipment was appropriately checked and calibrated according to manufacturer's instructions before its use *on-site* for the physicochemical parameters. Readings were carried out in triplicate and enough time was allowed for the Hannan multi-parameter probe to stabilise before readings were taken. The probe was regularly rinsed with distilled water before and after use. The reduction capacity of WWTPs for some physicochemical parameters (Turbidity, TSS and TDS) was calculated in percentage as the ratio of the difference between average influent values and final effluent value to the average influent value multiplied by 100.

100×{(Inf-Ef)/Inf}.

Where Inf = Average influent value and Ef = Average effluent values

The WWTPs evaluation was based on its compliance to standard practice as contained in South African water quality guidelines for domestic and recreational water (Dwaf, 1996; DWAF, 1996a, 1996b).

4.2.3.3. Sample extraction and derivatization

All water samples collected were extracted following the modified method of Sánchez-Avila *et al.*, (2009) and Munch, (2000). Wastewater samples were first filtered through glass wool to remove all suspended particles. Extraction was carried out with 500 mL samples of the filtrate which was spiked with 25 µL of the standard surrogate (2FB) to monitor method efficiency. The wastewater samples were passed at the rate of 10 mL/min through the C-18-U cartridges previously conditioned, with 7 mL each of n-hexane, dichloromethane, methanol and 10 mL distilled water at the rate of 3 mL/min. Sample bottles were rinsed into the cartridges and allowed to dry under vacuum for 1 h. Elution was carried out with 7 mL each of a mixture of dichloromethane:n-hexane (1:1 v/v) and dichloromethane:acetone (1:1 v/v) into 60 mL amber glass bottles. Eluents were reduced to about 2 mL using a rotary evaporator and blown to almost dryness before derivatization. The respective residues were re-constituted with 200 µL of a mixture of N,O-Bis(trimethylsilyl)trfluoroacetamide (BSTFA) and chlorotrimethylsilane (99:1 v/v) in vials and put in the oven at 70 ^{o}C for 30 min for complete derivatization.

4.2.4. Instrumental analysis

Extracted samples were analysed using gas chromatography-mass spectrometer (GC-MS) Agilent technology 7890B gas chromatograph coupled with mass selective detector (MSD) 5977A. The dimension of the capillary column HP-5 MS was 30 m × 250 µm i.d. × 0.25 µm film thick. Helium gas at 90 kPa pressure with a constant flow of 1 mL/min was used as the carrier gas. The transfer line and injection port were set at 280 ^{o}C and 300 ^{o}C, respectively and the volume of the sample extract (1 µL) was injected by auto-sampler in a splitless mode. The GC oven temperature was initially set at 70 ^{o}C held for 1min before it was increased to 150 ^{o}C and 290 ^{o}C at the rate of 14 ^{o}C/min and 6 ^{o}C/min, respectively.

4.2.5. Quality assurance and quality control

Fortified procedural blanks and a solvent blank (laboratory reagent blank LRB) were subjected to the same analytical procedure in every batch of 10 samples under analysis to detect any possible background contaminant during extraction. Every sample analysed was fortified with the surrogate standard for evaluation of method performance and the replicate analysis of four laboratory fortified blanks (LFBs) gave a standard deviation of below 15%. Instrument sensitivity and reproducibility was always verified by injecting one calibration standard before and after every batch analysis. A calibration standard of phenol mixture and surrogate with a concentration ranging from 0.1 - 15 µg/mL was analysed to generate a calibration curve. An external standard calibration was adopted with at least five points for each of the phenol analytes and concentrations were determined within the linear range of the calibration curve. The correlation coefficient for each of the nine selected phenol compounds varied between 9.95 E-01 – 9.99 E-01.

4.2.6. Statistical analysis

The mean values of data obtained were compared using SPSS (IBM SPSS Statistics 20), one-way analysis of variance and Tukey's multiple range tests. Significance for statistical analysis was set at p values < 0.05).

4.3. Results and discussion

Every WWTP operation is usually preceded by a physical process referred to as primary treatment to improve the biodegradation of the wastewater during the secondary treatment process which is usually a biological process. This initial stage which usually involves removal of grit and settling at the primary clarifier influences some physicochemical parameters of the wastewater more than others. For example, TSS and turbidity are greatly affected. However, this treatment alone cannot produce the desired effluent with an acceptable residual organic concentration. Organic materials are usually metabolised by biological process (bacterial) in virtually all the treatment technologies (Emilia *et al.*, 2013; Choksi *et al.*, 2015).

4.3.1. Temperature variation in WWTPs processes and receiving water bodies.

The results of the temperature variation at the five selected WWTPs that serve Adelaide, Alice, Bedford, Berlin and Seymour in the Eastern Cape, South Africa at different sampling points are presented in Table 4.2. The sampling periods cut across three weather seasons; summer, autumn and winter. For all the WWTPs, the temperature varied as 29 - 36 ^{o}C, 20 - 27 ^{o}C and 12 - 17 ^{o}C for summer, autumn and winter respectively. There was no significant difference (p>0.05) in temperature among the respective sampling points (influent, effluent and receiving water). The temperatures for all the treatment plants in the influent, effluent, upstream and downstream were in the range of 21- 25 ^{o}C, 14 - 23 ^{o}C, 18 - 22 ^{o}C and 18 - 25

^{o}C respectively. A significant difference in temperature was observed across the three seasons ($p<0.05$). Temperatures were observed to be generally lower at Seymour than other areas; it varied on the average from 14 - 21 ^{o}C and a little higher at Bedford (Table 4.2). This might be connected to the time of sampling during the day. All sampling exercise at Seymour was carried out earlier in the day while at Bedford; it was usually in the afternoon. Temperature is a significant physicochemical parameter that affects other parameters such as DO in water, the toxicity of some chemicals and the consequent sensitivity of living organisms to toxic substances (Mayer and Ellersieck, 1988; Akan *et al.*, 2008; Odjadjare and Okoh, 2010). The impact of temperature on a variety of parameters makes it a vital tool in water quality determination (Fondriest Environmental, 2014c).

Table 4.2 Temperature (^{o}C) variance at different points in different WWTPs. (South Africa maximum permissible limit; ≤ 25 ^{o}C).

WWTP Name	Influent	Range	Effluent	Range	Upstream	Range	Downstream	Range
Adelaide	23 ± 3	15 - 35	22 ± 3	15 - 33	22 ± 3	15 - 34	22 ± 3	16 - 34
Alice	22 ± 2	16 - 30	22 ± 3	13 - 31	22 ± 4	11 - 30	22 ± 3	14 - 29
Bedford	25 ± 4	15 - 36	21 ± 3	13 – 35	NS	NS	NS	NS
Berlin	23 ± 2	17 - 28	23 ± 4	15 – 31	NS	NS	25 ± 3	17 - 31
Seymour	21 ± 2	17 - 24	14 ± 7	18 - 25	18 ± 2	13 - 23	18 ± 1	16 - 23

*NS = No sample

The relatively similar temperature ranges between the sampling points and the receiving water suggests that temperature was independent of WWTP processes and as such, the final effluent may not impact negatively on the receiving water. However, a possible temperature change may have occurred during microbial activities during the secondary treatment, (especially with AS). Such an effect probably could have been neutralised at the secondary clarifier where the treated water was exposed to atmospheric temperature. By inference, the temperature across the sampling points was significantly influenced by seasonal change. These results are similar to other studies reported by Igbinosa and Okoh, (2009) and Odjadjare and Okoh, (2010) in South Africa but those previous studies both observed a significant difference between sampling points (final effluent and receiving water). Although, temperature differs among sampling points in this present study, the difference was not statistically significant. The average temperature across the weather seasons also fell within the acceptable limit of ≤ 25 ^{o}C for domestic water set by South Africa (DWAF, 1996b) except during summer where temperatures reached their climax and were above 30 ^{o}C for all the plants except at Seymour.

This peak in temperature might be caused by a sudden increase in the intensity of sunlight during the day which is expected to normalise within a short, especially at nights. If this temperature rise remained extended for a long period of time, it may have a detrimental effect on the aquatic metabolic rate, biological activities and some other physicochemical parameters (DO, EC, pH) (Fondriest Environmental, 2014c).

4.3.2. pH in different WWTP processes and receiving water bodies.

Another important indicator of water quality is pH. It is a parameter that influences biological operations in the treatment plant. The average pH values for the six months of sampling from the five WWTPs and their respective receiving water bodies are presented in Table 4.3.

Table 4.3 Six Months' sample pH characteristics from February to July, 2016. (South Africa maximum permissible limit; 6.0 – 9.0).

WWTP Name	Influent	Range	Effluent	Range	Upstream	Range	Downstream	Range
Adelaide	7.9 ± 0.2	7.2 - 8.5	7.8 ± 0.2	7.2 - 8.7	8.4 ± 0.3	7.7 - 9.4	8.2 ± 0.2	7.7 - 9.2
Alice	7.6 ± 0.2	6.8 - 8.5	7.3 ± 0.4	6.5 - 8.8	7.6 ± 0.3	6.7 - 8.7	7.0 ± 0.3	6.8 - 8.7
Bedford	7.8 ± 0.3	6.9 - 8.6	7.9 ± 0.3	6.8 - 9.1	NS	NS	NS	NS
Berlin	7.8 ± 0.2	7.1 - 8.8	8.3 ± 0.3	7.6 - 8.9	NS	NS	8.0 ± 0.2	7.6 - 8.8
Seymour	7.9 ± 0.3	7.3 - 8.8	7.4 ± 2.5	7.3 - 7.5	7.5 ± 0.1	7.3 - 8.0	7.4 ± 0.2	7.3 - 8.0

*NS = No Sample

The pH values in the influent and the final effluent samples in all the treatment plants varied at 6.8 - 8.8 and 6.5 - 9.1, respectively. A similar trend was observed in the receiving water bodies at 6.7-9.4 and 6.8-9.2 for the upstream and downstream, respectively. There was no significant difference in pH across the sampling points, and also among the five treatment plants ($p>0.05$). At Adelaide (AS), the pH values at both ends of the receiving water were sometimes as high as 9.0 compared with others just as we observed similar high values in the effluent at the Berlin (TF) plant. This suggests the dependence of effluent composition on the type of household, business, industries and the level of treatment received by the wastewater and public facilities discharging into the water system (Odjadjare and Okoh, 2010). The average pH for all WWTPs fell within the permissible limit of 6.0 – 9.0 for domestic and recreational water in South Africa (DWAF, 1996a, 1996b), as well as the European Union tolerance limit for fisheries and aquatic life (Chapman, 1996). These values were also similar to pH values reported by some researchers in the previous studies for Rivers and wastewater effluent in South Africa (Fatoki *et al.*, 2003; Igbinosa and Okoh, 2009; Odjadjare and Okoh, 2010). This suggests that effluent from WWTPs mainly from domestic wastes may not be acidic after all. However, industrial effluent may bring about fluctuation in pH characteristics. Decomposition of organic matter, interactions of water with rocks and acid rain are other factors that may greatly influence pH (Fondriest Environmental, 2013; Choksi *et al.*, 2015). Similar pH fluctuations have been reported by some authors (Ogunfowokan *et al.*, 2005; Akan *et al.*, 2008; Chigor *et al.*, 2013) both in south west Nigeria and the Eastern Cape, South Africa.

4.3.3 Electrical conductivity (EC) in different WWTPs processes and receiving water bodies.

Electrical conductivity (EC) measures the amount of dissolved ions or total salt content in water, and is a useful indicator of salinity (Morrison *et al.*, 2001; Odjadjare and Okoh, 2010; Fondriest Environmental, 2014a). The values of EC measured across the sampling points for all the treatment plants are presented in Table 4.4. The EC of the final effluents varied at 218-587 µS/cm for all the treatment plants except for TF (Berlin) with appreciably higher values 557-781 µS/cm. On the average, there was a notable decrease in EC values as the wastewater passed through the treatment processes indicating a significant effect on the wastewater. Likewise, a significant spatial difference was noticed across the sampling points, especially in the final effluent and among the treatment plants ($p<0.05$), but the variation observed across the seasons were not statistically significant. The relative difference in EC among WWTPs may suggests geochemical effects; (as clay soils contribute to EC unlike granite) agricultural runoffs and constituents of the sewage that are being released (Fondriest Environmental, 2014a).

Moreover, the relative amount of chlorine disinfectant received by the respective effluents could have also increased the dissolved ions in the final effluents (Mamba *et al.*, 2009). The average EC in the final effluent at Adelaide, Alice and Seymour WWTPs that operated AS technology, are relatively higher than the EC values in their respective upstream. This suggests a notable influence on the downstream water quality. The biodegradation of the organic matter load in the wastewater by microorganisms could probably have increased the amount of total dissolved solids (TDS); which has a strong correlation with EC, in addition to other possible effects. Generally, the final effluent increased EC in the downstream of the receiving water for the entire WWTPs. However, there are possibilities that the upstream water could also have found other pollution point source for dissolved ions apart from effluent discharge. These values are nevertheless, within the acceptable limit recommended by DWAF for domestic water supply of 700 µS/cm (DWAF, 1996b). These values compare favourably with those reported by Igbinosa and Okoh, (2009) as well as Chigor *et al.*, (2013) but are lower than those reported by Fatoki *et al.*, (2003) and Odjadjare and Okoh (2010) obtained from Keiskamma River water and from urban areas of South Africa.

Table 4.4 Electrical Conductivity (µS/cm) at different points in different WWTPs. (South Africa maximum permissible limit; 700 µS/cm).

WWTP	Influent	Range	Effluent	Range	Upstream	Range	Downstream	Range
Adelaide	530.3 ± 50.7	357 - 664	454.3 ± 38.5	357 - 587	442.1 ± 76.2	143 - 637	354.4 ± 45.7	143.3 - 480.3
Alice	365.8 ± 54.8	218 - 619	257.7 ± 18.4	218.3 - 318	194.6 ± 20.2	155 - 260	216.7 ± 29.9	142.5 - 319
Bedford	436.8 ± 81.6	258 - 772	323.5 ± 22.3	279.3 – 413	NS	NS	NS	NS
Berlin	648.1 ± 84.7	471 - 1030	646.1 ± 48.3	556.7 – 781	NS	NS	689.4 ± 61.9	492.3 - 827.3
Seymour	372.8 ± 32.7	290 - 449.7	204.4 ± 102.6	292.3 - 321	152.6 ± 31.7	23 -219	181.1 ± 41.2	24.7 - 312.7

*NS = No Sample

4.3.4. Dissolved oxygen (DO) in different WWTPs processes and receiving water.

Table 4.5 shows the values of DO in mg/L in the influent, effluent of the WWTPs and the receiving water. The DO in the influent and effluent of all the WWTPs generally varied between 0.2 - 6.6 mg/L (1.6 ± 0.7-4.4 ± 0.4 mg/L) and from 4.6 - 14.1 mg/L (mean 6.8 ± 1.0 - 8.0 ± 0.9) respectively. There was no significant difference in DO between the final effluent and the receiving water for any of the WWTPs ($p>0.05$). The respective DO for upstream and downstream also varied between 7.6 - 10.5 mg/L on the average. This probably suggested that the WWTPs did not in any way impact additional organic load. There was a notable increase in DO as the wastewater passed through the treatment plants suggesting possibility of increased dissolution of atmospheric oxygen during influent agitation during treatment. The microbial activities and other treatment processes that may have reduced the organic matter in the wastewater would have, as a consequence, increased availability of DO. The green algal growth that is usually observed in the final effluent during storage with OP at Bedford may probably have been as a result of increased DO (which was as high as 14.1 mg/L) in comparison to effluent from other plants. However, the final effluent at this WWTP is usually not released to any stream or river water body.

It was also observed during the sampling periods that the organic matter (in terms of water turbidity) of the wastewater entering the TF in Berlin was lower than what we observed with other WWTPs. This may, however, be attributed to sampling mostly conducted outside peak periods of influent flow. Throughout the sampling periods, the DO in the receiving water both at the upstream and downstream fell within the acceptable standard limits of (5-9 mg/L) for aquaculture in South Africa (DWAF, 1996a) and 8-10 mg/L in unpolluted water for the survival of aquatic lives (DFID, 1999). The DO values for the final effluent of all the selected WWTPs were also above the minimum benchmark of 5 mg/L. DO is an important indicator for water quality as reduction below 2 mg/L may lead to death of many organisms (Chapman, 1996; Fatoki *et al.*, 2003). This may affect fishes more by impacting negatively on their feeding regime, reproductive behaviour, swimming ability and may lead to death if persistent (Fondriest Environmental, 2014c). The results for DO are similar to values reported by other authors (Igbinosa and Okoh, 2009; Chigor *et al.*, 2013).

Table 4.5 Dissolved Oxygen (mg/L) at different sampling points in different WWTPs. (South Africa maximum permissible limit; 5-9 mg/L).

WWTP Name	Influent	Range	Effluent	Range	Upstream	Range	Downstream	Range
Adelaide	1.6 ± 0.7	0.2 - 4.5	7.9 ± 0.2	7.2 - 8.5	10.5 ± 0.7	8.7 - 13.1	8.9 ± 0.2	8.3 - 9.9
Alice	1.9 ± 0.90	0.6 - 6.6	6.8 ± 1.0	4.6 - 11.2	8.5 ± 0.3	7.5 - 10.0	7.6 ± 0.6	5.4 - 9.4
Bedford	1.5 ± 0.4	0.2 - 3.0	6.8 ± 1.7	6.8 - 14.1	NS	NS	NS	NS
Berlin	4.4 ± 0.4	3.0 -5.3	8.0 ± 0.9	6.0 - 9.5	NS	NS	8.3 ± 0.3	6.9 - 9.0
Seymour	3.6 ± 1.1	0.7 - 6.1	6.8 ± 2.3	6.7 - 7.0	8.8 ± 0.2	8.3 - 9.5	8.3 ± 0.5	6.0 - 9.05

*NS = No Sample

4.3.5 Total dissolved solids (TDS) in different WWTPs processes and receiving water.

TDS and EC are two parameters that correlate positively and are also indicators of water salinity. TDS is a measure of ion particles smaller than 2-microns, which includes organic solutes such as hydrocarbon and urea apart from salt ions, and also helps aquatic life to balance their cell density (Fondriest Environmental, 2014a). The average TDS in the influent for Adelaide, Alice and Bedford are 309 ± 20 mg/L, 197 ± 12 mg/L, 343 ± 70 mg/L, respectively while Berlin recorded 381 ± 42.5 mg/L and Seymour 228 ± 30 mg/L (Table 4.6).

It varied generally from 145 to 650 mg/L for the five WWTPs. There was a significant reduction ($p<0.05$) in TDS as the influent was passed through the treatment processes to the final effluent for all the WWTPs, suggesting a notable influence of the treatment processes on the wastewater. This observation was different in TF at Berlin, where a slight increase in the average TDS was observed, ranging from 381 mg/L in the influent to 385 mg/L in the final effluent. The geological difference and variation in influent constituents may be attributed to the significant difference observed in TDS among the plants under study ($p<0.01$). The average values of TDS downstream of the receiving water bodies were also lower than what was being discharged from the WWTPs except in TF at Berlin where the result was different. The downstream values at Berlin on the average were higher than the effluent at the discharge point. This may suggest other soluble particles (like clay ions) along its path as the effluent flows for a longer distance before it finally reaches the receiving water, unlike other effluents, that would have been diluted by the receiving river water. We could not assess the receiving water due to this reason. Generally, none of the TDS values in the final effluent and receiving water recorded in this work exceeded the South African stipulated standard of 0-450 mg/L for domestic water (DWAF, 1996b) or the USEPA maximum contaminant level of 500 mg/L (USEPA, 2009). Igbinosa and Okoh, (2009) submitted that elevated TDS (above recommended values) could be injurious to freshwater animals through the obstruction of the osmoregulation ability of the organism.

Table 4.6 Total dissolved solids (TDS) mg/L) at different points in different WWTPs. (South Africa maximum permissible limit; 0-450 mg/L).

WWTP	Influent	Range	Effluent	Range	Upstream	Range	Downstream	Range
Adelaide	308.8 ± 20.3	228 -368	265.2 ± 9.9	229 -- 296	293.7 ± 55.8	90 - 487	209.9 ± 29.9	89.6 - 307
Alice	196.6 ± 12.3	184 - 227	147.2 ± 5.1	136 -- 166	107.9 ± 4.9	99.5 - 131	122.2 ± 12.3	91 - 171
Bedford	342.7 ± 70.2	181- 650	188.6 ± 4.1	179 – 206	NS	NS	NS	NS
Berlin	380.6 ± 42.5	231 - 523	384.9 ± 12.4	352 -- 412	NS	NS	466.4 ± 35.3	314 - 531
Seymour	227.8 ± 30.0	145 - 288	131.5 ± 65.9	189 -- 205	103.5 ± 15.0	64 - 142	126.9 ± 14.0	77 -- 156

*NS = No Sample

4.3.6. Turbidity (NTU) in different WWTPs processes and receiving water.

Turbidity correlates significantly with microbial growth in water (Fondriest Environmental, 2014b). It is one parameter that provides a platform to measure the ability of a WWTP to reduce suspended organic and inorganic loads through its processes. The values of turbidity in the influent, effluent, and receiving waters including the percentage reduction in all the WWTPs are presented in Table 4.7.

Table 4.7 Turbidity (NTU) at different sampling points of different WWTPs. (South Africa maximum permissible limit; 0 - 1 NTU).

WWTP	Influent	Range	Effluent	Range	Red. (%)	Upstream	Range	Downstream	Range
Adelaide	560.3 ± 134.7	165 -- 988	14.6 ± 5.5	1.9 -- 29.1	97	183.2 ± 56.1	51 - 417	150.5 ± 55.3	48 - 417
Alice	546.7 ± 136.2	169 -- 980	17.8 ± 6.9	2.2 -- 38	97	111 ± 23.3	43 - 189	106.6 ± 23.6	34 - 181
Bedford	637.7 ± 134.0	165 -- 997	119.1 ± 18.9	50 -- 174	81	NS	NS	NS	NS
Berlin	129.4 ± 36.2	30 -- 241	44.8 ± 21.3	3 -- 88	83	NS	NS	129.6 ± 29.7	58 - 241
Seymour	266.9 ± 62.4	149 -- 414	72.5 ± 45.2	46 -- 183	73	136.4 ± 41.7	6 - 286	230.3 ± 72.3	74 - 551

*NS = No Sample

The influent values varied between 165 - 988 NTU, at Adelaide and 169 - 980 NTU in Alice, while the results in Bedford, Berlin and Seymour were in the range of 165 - 997 NTU, 30 - 241 NTU, and 149 - 414 NTU, respectively. The effluent values varied as 1.9 - 38 NTU for Alice, Adelaide and Berlin plants respectively and this was reflected in their removal efficiencies of 97%, 97%, and 83% (Figure 4.1). The effluent values in Seymour (AS) and Bedford (OP) were notably higher (46-183 NTU) and respectively recorded 73 and 81%

removal rate (Figure 4.1). This shows a significant reduction of $p<0.01$ between turbidity in the influent and the final effluent for all the WWTPs, but there was less influence of seasonal variation ($p<0.05$).

The nature and chemistry of the wastewater discharge catchment from which Berlin influent is sourced is likely more of dissolved component than the light scattering suspended component measured in turbidity hence, the reason for the lowest values recorded in this area. In addition, this suggestion was evidently supported by the highest values of EC, TDS and DO recorded at Berlin. However, there was a relative difference in the turbidity of the daily inflow for all the WWTPs. It was mostly very high at Bedford, Alice and Adelaide probably due to the high levels of anthropogenic activities and the large populations. The turbidity values for the final effluent reported in this study, were similar to those in other works reported in South Africa (Igbinosa and Okoh, 2009; Odjadjare and Okoh, 2010; Chigor *et al.*, 2013).

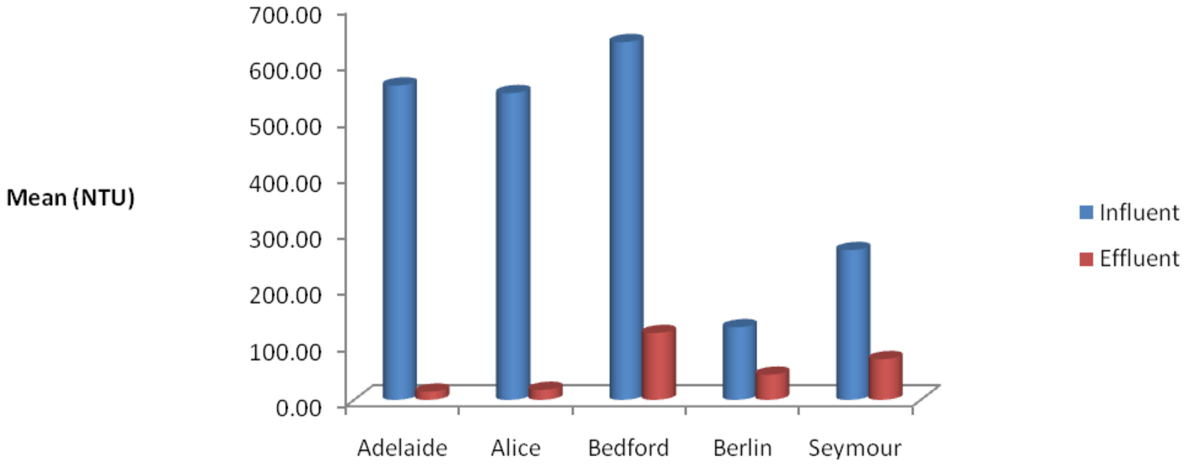


Figure 4.1 Different removal capacities for removal of turbidity in WWTPs.

It is worthy of note that high turbidity values usually observed in the final effluent in OP at Bedford suggested a cumulative effect of algae growth and other microbial activities that prevailed during effluent storage. The lower efficient capacity also observed with AS technology at Seymour as compared to other AS may be attributed to the single tank technology which was used and which limited the numbers of clarification tanks at this WWTP. The turbidity values in the receiving water (upstream and downstream) for all the WWTPs were notably higher than the effluent. This may be attributed to other anthropogenic activities and agricultural runoff which also influenced the turbidity of the river. Highly turbid water tends to aid the growth of pathogens thereby increasing the chances of infection (Obi *et al.*, 2007). The values in the receiving water exceeded both the South African target range of 0 - 1 NTU and the World Health Organization value of ≤ 5 NTU (DWAF, 1996b; WHO, 2004). However, the effluent discharged at Adelaide, Alice (using AS) and Berlin (using TF) sometimes fell within the permissible range (0 - 1 NTU). This may naturally not have qualified the surface water as fit for domestic use because the values could increase the chances of disease transmission through microorganisms associated to the particulate matter. A highly turbid effluent reduces disinfection potential of chlorine (DWAF, 1996b) which also increases the chances of trihalomethane (THM) formation, a by-product of the reaction of chlorine and organic matter. This is carcinogenic and may be injurious to aquatic and human lives (Fatoki *et al.*, 2003).

4.3.7. Total suspended solids (TSS) in different WWTPs processes and receiving water.

Total suspended solid (TSS) is a parameter that is very similar to turbidity. It is a parameter that is most influenced by the primary treatment (grit removal) of WWTPs processes followed by their respective clarifiers. TSS values for all the WWTPs in the influent, effluent, and receiving water with the percentage reduction achieved by the WWTPs are presented in Table, 4.8. The TSS values at Adelaide, Alice, and Bedford in the influent, varied from 134-303 mg/L, 106-311 mg/L, 122-221 mg/L, respectively. While in Berlin, it varied between 10 and 91 mg/L, and in Seymour between 66 and 106 mg/L. Relatively low TSS was observed in the influent at Berlin (TF) and Seymour (AS). Generally, there was a significant removal of TSS in the final effluent of all the WWTPs (Figure 4.2).

Table 4.8 Total suspended solids (mg/L) at different points in different WWTPs. (South Africa maximum permissible limit; 25 mg/L).

WWTP Name	Influent	Range	Effluent	Range	Red. (%)	Upstream	Range	Downstream	Range
Adelaide	195.1 ± 27.3	134 - 303	4.4 ± 1.0	2.3 - 7	98	48.5 - 19.9	11 - 115	41.8 ± 19.1	11 - 115
Alice	179.9 ± 36.5	106 - 311	6.7 ± 2.6	3 - 15.3	96	27.5 ± 9.5	5 - 49	21.3 ± 6.6	6 - 41
Bedford	184.9 ± 18.8	122 - 221	57.4 ± 10.8	22 - 87	69	NS	NS	NS	NS
Berlin	49.1 ± 17.5	10 - 91	20.3 ± 11.1	7 - 43	75	NS	NS	40.2 ± 11.5	17 - 79
Seymour	79.7 ± 13.0	66 - 106	15.5 ± 6.4	15 - 16	87	35.3 ± 13.1	3 - 69	49.1 ± 14.	17 - 83

*NS = No Sample

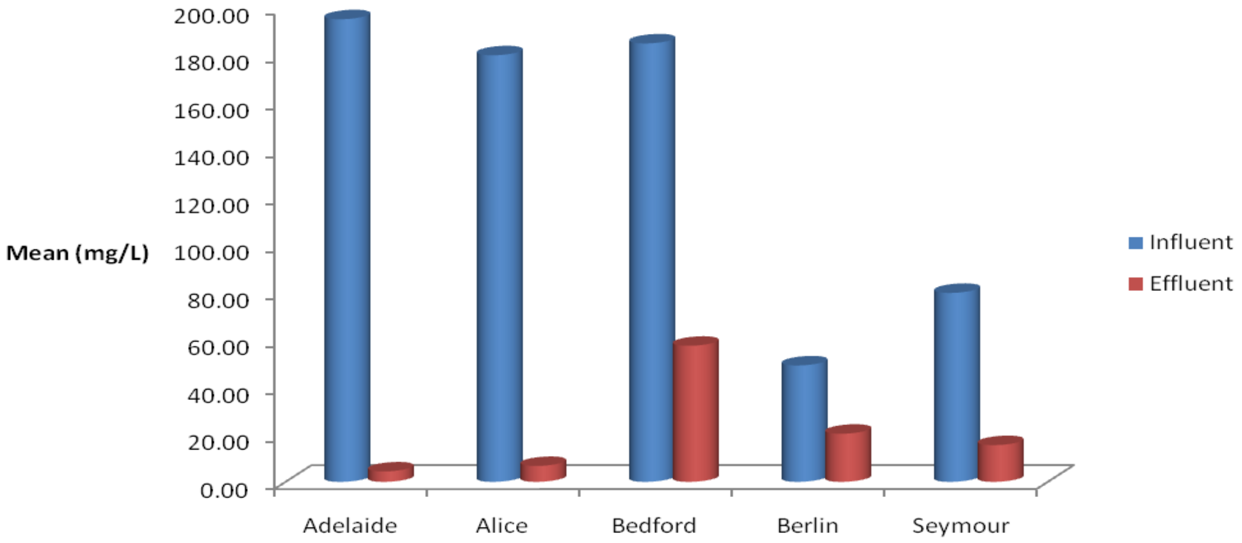


Figure 4.2 Average removal capacities of WWTPs for TSS

Adelaide and Alice operating AS recorded over 95% reduction while Seymour (AS), Berlin (TF) and Bedford (OP) had 87%, 75% and 69%, respectively. The high TSS values observed both in the upstream and downstream of the receiving water varied between 3 and 115 mg/L for all the WWTPs. This also suggests other anthropogenic activities along the course of the river and surface runoff that may be occasioned by rain. The variations of TSS values across the treatment processes were statistically significant (p<0.01). However, the final effluent showed no additional impact on the receiving water. TSS is a parameter that indicates water clarity and usually is constituted by inorganic materials but may include bacterial and algae solids found in the water column larger than 2 microns (Fondriest Environmental, 2014b). The South African acceptable limit for TSS in domestic water is 25 mg/L. The WWTP effluent values fall within the purview of the acceptable standard but the same cannot be said of Bedford (OP) and the receiving water. The reason for the observed increase in TSS in the effluent at Bedford, Berlin and Seymour has been explained under turbidity and TDS.

4.3.8.1. Occurrence and fate of phenolic compounds in wastewater treatment plant in Adelaide.

The concentrations of detected phenolic compounds, the frequency of detection (FD) and the percentage reduction at different sampling points (Influent and effluent) at the Adelaide treatment plant and receiving water body are presented in Table, 4.9. For the four-month period of sampling, all the phenolic compounds were detected in the influent sampled, however, only 4-C-3MP, 2,4DCP, 2-NP and PCP out of the nine under investigation were found higher than 10 μgL^{-1}. The most abundant compound was 4-C-3MP with a concentration of 42.1 ± 17.7 μgL^{-1} followed by 2-NP and 2,4-DCP with a concentration of 18.0 ± 7.1 μgL^{-1} and 13.8 ± 8.3 μgL^{-1}, respectively. The concentration in the effluent varied between not detected (ND) and 3.7 μgL^{-1}. The frequency of detection of all the compound was 100% in the influent except for phenol. The final effluent concentrations of these compounds were considerably lower than what were observed in the influent, indicating good removal capacity of the WWTP. The removal capacity of the treatment plant varied between 34% for 2,4,6-TCP and 96% for 4-C-3MP.

Table 4.9 Concentrations of phenolic compounds in the wastewater from WWTP in Adelaide (Values are means ± SE µg/L).

Compounds	Influent	Range	FD (%)	Effluent	Range	Red. (%)	Upstream	Range	Downstream	Range
PH	3.2 ± 2.1	ND - 9.5	75	1.8 ± 1.0	ND - 3.9	42	1.5 ± 0.6	ND - 2.5	1.2 ± 0.6	ND - 2.4
2-CP	6.7 ± 1.4	3.8-10.2	100	2.5 ± 0.5	1.0 - 3.6	63	2.4 ± 0.6	0.8 - 3.9	2.2 ± 0.5	0.8 - 3.2
2,4-DMP	7.5 ± 1.8	4.2 - 12.5	100	2.7 ± 0.3	2.2 - 3.2	82	4.0 ± 0.6	3.2 - 4.8	2.5 ± 0.6	1.6 - 3.4
4-C-3MP	42.1±17.7	9.8 - 83.0	100	2.5 ± 0.7	ND - 3.9	96	2.8 ± 0.7	ND - 4.0	2.1 ± 1.1	ND -4.3
2,4-DCP	13.8 ± 8.3	2.1 - 37.3	100	2.3 ± 0.5	1.6 - 3.0	92	0.8 ± 0.4	ND -1.7	1.1 ± 0.4	ND -1.7
2-NP	18.0 ± 7.1	6.5 - 36.8	100	3.7 ± 1.5	1.8 - 8.3	79	2.9 ± 0.4	1.7 - 3.6	3.2 ± 0.8	1.3 - 5.1
2,4,6-TCP	9.3 ± 2.0	5.2 - 13.7	100	6.2 ± 4.3	1.0- 19.1	34	4.3 ± 2.2	1.1. ±10.7	4.7 ± 2.9	0.9- 13.1
4-NP	7.4 ± 3.2	1.3 - 14.0	100	0.9 ± 0.2	ND - 1.1	91	1.6 ± 0.9	0.5 - 4.1	6.4 ± 3.6	1.3- 11.5
PCP	12.8 ± 1.9	10.4- 18.5	100	2.4 ± 0.7	ND - 3.7	86	2.4 ± 0.7	ND - 3.1	4.5 ± 1.6	ND - 6.7

*PH=Phenol , 2-CP=2-Chlorophenol, 2,4-DMP=2,4-Dimethylphenol, 4-C-3MP=4-Chloro-3-methylphenol, 2,4-DCP=2,4-Dichlorophenol, 2-NP=2- Nitro-phenol, 2,4,6-TCP=2,4,6-Trichlorophenol, 4-NP=4-Nitrophenol, PCP= Pentachlorophenol, RED. =Reduction, FD=Frequency of detection, ND= Not detected (Below limit of detection 0.102 – 0.586 µg/L), SE = Standard error

4.3.8.2. Occurrence and fate of phenolic compounds in wastewater treatment plant in Alice.

Alice is a town popularly known for being the base of University of Fort Hare. The wastewater from this area is largely domestic wastes and agricultural runoffs. Values of phenolic samples taken from the influent, effluent, upstream and downstream of the receiving water body at Alice WWTP are presented in Table 4.10. All the nine selected phenolic compounds were detected in the influent except for phenol. The most abundant compound in the influent here also include; 4-C-3MP, 2,4,6-TCP, 2,4-DMP, PCP and 2-NP in that order with a concentration of 35.3 ± 10.6, 25.4 ± 7.3, 22.7 ± 12.0, 20.3 ± 8.9 and 11.6 ± 3.7 μgL^{-1}, respectively. Others were of minor importance in the influent with concentrations below 10 μgL^{-1}. The respective concentrations of these congeners in the final effluent were also inconsequential. They varied between ND and 5.3 μgL^{-1} on the average. The removal capacity of the WWTP varied between 55% for 2-NP and 93% for 2,4-DMP. There was no significant difference in the concentrations of any of the congeners (P>0.05) between the final effluent and the receiving water bodies, suggesting no significant impact by the effluent on the river.

Table 4.10 Concentrations of phenolic compounds in the wastewater from WWTP in Alice (Means ± SE µg/L).

Compounds	Influent	Range	FD (%)	Effluent	Range	Red. (%)	Upstream	Range	Downstream	Range
PH	ND			ND			1.0 ± 0.5	ND - 1.9	1.3 ± 0.7	ND- 2.7
2-CP	3.6 ± 1.3	1.8 - 6.1	100	1.1 ± 0.3	0.2 - 1.7	60	2.0 ± 0.3	1.2 - 2.8	2.6 ± 0.4	1.6- 3.2
2,4-DMP	22.7±12	3.3- 44.6	100	1.1 ± 0.5	0.2 - 2.4	93	1.3 ± 0.6	0.2 - 2.8	1.6 ± 0.7	0.5 - 3.1
4-C-3MP	35.3±11	22.1-56	100	2.7 ± 0.6	ND- 3.9	92	3.6 ± 1.1	ND - 5.6	4.3 ± 0.8	ND - 5.8
2,4-DCP	4.0 ± 1.4	2.3 -5.7	100	1.1 ± 0.1	0.9 - 1.3	74	1.1 ± 0.1	0.9 - 1.1	1.5 ± 0.1	1.3 - 1.6
2-NP	11.6±3.7	7.0- 18.6	100	4.0 ± 0.7	2.8 - 5.9	55	3.8 ±0.8	2.0 - 5.7	6.7 ± 2.2	3.3 - 13.2
2,4,6-TCP	25.4±7.3	ND-34.3	75	1.6 ± 0.5	0.6 - 3.0	87	2.3 ± 0.4	1.4 - 3.3	4.9 ± 2.3	1.3 - 11.5
4-NP	4.2 ± 0.4	3.4 - 4.9	100	0.8 ± 0.2	ND- 1.0	82	2.28±1.0	1.6 - 5.0	2.7 ± 1.2	1.3 - 5.5
PCP	20.3±8.9	6.1- 36.7	100	5.3 ± 1.3	ND- 7.5	74	2.4 ± 0.5	1.5 - 3.7	4.9 ± 8.3	ND - 8.3

PH=Phenol , 2-CP=2-Chlorophenol, 2,4-DMP=2,4-Dimethylphenol, 4-C-3MP=4-Chloro-3-methylphenol, 2,4-DCP=2,4-Dichlorophenol, 2-NP=2- Nitrophenol, 2,4,6-TCP=2,4,6-Trichlorophenol, 4-NP=4-Nitrophenol, PCP= Pentachlorophenol, RED.=Reduction, FD=Frequency of detection,

SE = Standard error, ND= Not detected (Below limit of detection 0.102 – 0.586 **µg/L**).

4.3.8.3. Phenolic compounds in wastewater from WWTP in Bedford.

Bedford, unlike other selected areas, adopted the oxidation pond technology in the WWTP that serves the rural dwellers, where domestic wastes and agricultural runoffs are the main sources of wastewater generated. The summary of phenolic compounds detected both in the influent and effluent of the treatment plant are presented in Table 4.11. All the selected congeners were detected in the influent with 100% frequency of detection except for phenol and 2-CP.

Table 4.11 Concentrations of phenolic compounds in wastewater from WWTP in Bedford (Means ± SE µg/L).

Compounds	Influent	Range	FD (%)	Effluent	Range	Reduction (%)
PH	2.2 ± 1.6	ND - 5.5	75	1.1 ± 0.6	ND - 2.4	49
2-CP	4.5 ± 0.6	ND - 5.6	75	1.6 ± 0.7	ND - 2.7	65
2,4-DMP	8.2 ± 1.5	5.6 - 12.2	100	2.7 ± 0.4	1.8 - 3.3	75
4-C-3MP	22.5 ± 11.1	6.4 - 55.1	100	2.4 ± 1.6	ND - 5.5	92
2,4-DCP	7.0 ± 3.6	1.8 - 15.2	100	1.5 ± 0.6	ND - 2.4	78
2-NP	17.3 ± 5.6	5.7 - 32.3	100	2.9 ± 0.9	ND - 4.8	87
2,4,6-TCP	7.6 ± 0.7	6.3 - 9.4	100	6.8 ± 4.4	0.9 - 19.5	11
4-NP	6.5 ± 2.1	2.9 - 11.7	100	8.7 ± 5.9	1.7 - 22.6	-0.5
PCP	8.2 ± 1.3	4.9 - 10.6	100	5.3 ± 1.2	ND - 6.5	68

PH= Phenol, 2-CP= 2-Chlorophenol, 2,4-DMP= 2,4-Dimethylphenol, 4-C-3MP= 4-Chloro-3-methylphenol, 2,4-DCP = 2,4-Dichlorophenol, 2-NP =2 - Nitrophenol , 2,4,6-TCP = 2,4,6-Trichlorophenol, 4-NP = 4-Nitrophenol, PCP = Pentachlorophenol, RED.= Reduction, FD = Frequency of detection., ND= Not detected (Below limit of detection 0.102 – 0.586 **µg/L**), SE = Standard error

The most abundant compound in the influent was 4-C-3MP followed by 2-NP with concentration ranging from 6.4 to 55.1 µgL^{-1} (22.5 mean) and 5.7 to 32.3 µgL^{-1} (mean 17.3 µgL^{-1}), respectively. Other compounds were also of minor importance with concentration below 10 µgL^{-1}. There was a notable reduction of the compounds in the final effluent suggesting high removal capacity of the WWTP. The percentage removal varied between 65% for 2-CP to 92% for 4-C-3MP. There was poor removal performance for 2,4,6-TCP and phenol with 11% and 49%, respectively although, the respective concentrations of these compounds both in the influent and effluent were less important. The concentration of 4-NP detected in the final effluent was higher than what was obtained in the influent suggesting that some compounds are not degraded by microorganisms or converted to biomass (sludge) hence, they may be released with the effluent (Wang *et al.*, 2005).

4.3.8.4. Phenolic compounds in wastewater from WWTP in Berlin.

Berlin is the only town out of the five investigated that is in the Buffalo District Municipality instead of the Amathole District Municipality. The WWTP in operation in this area, which is the biggest among the selected treatment plants, adopted the trickling filter (TF) technology. All the nine phenolic congeners were detected in the influent, effluent and the downstream

Table 4.12. Like other WWTPs, 2-NP was the most abundant compounds in the influent with an average of 40.3 µg/L^{-1} followed by PCP, 4-C-3MP with similar means of 31.8 ± 22 and 18.6 ± 10 µg/L^{-1} in that order. Others apart from 2,4,6-TCP and 2,4-DMP were of minor importance with concentrations below 10 µg/L^{-1}. These congeners were frequently detected in the influent, effluent and the downstream except phenol. The WWTP appreciably reduced all the phenolic compounds in the influent with the most abundant compounds being the ones that are most efficiently removed (>80%). 2-CP was the compound least removed (33%) as calculated but it was already in very low concentrations across the WWTPs.

Table 4.12 Concentrations of phenolic compounds in wastewater from WWTP in Berlin (means ± SE µg/L).

Compounds	Influent	Range	FD (%)	Effluent	Range	Reduction (%)	Downstream	Range
PH	1.5 ± 0.5	ND - 2.1	67	ND		100	ND	
2-CP	2.0 ± 1.2	0.2 - 4.3	100	1.4 ± 0.5	0.4 - 2.2	33	1.7 ± 0.8	0.3 - 2.9
2,4-DMP	11.5 ± 6.5	1.1 - 23.3	100	1.0 ± 0.8	0.1 - 2.5	91	1.0 ± 0.7	0.2 - 2.4
4-C-3MP	18.6 ± 9.6	2.8 - 36.0	100	2.6 ± 1.0	1.1 - 4.5	86	2.3 ± 0.7	1.5 - 3.7
2,4-DCP	2.8 ± 1.5	1.0 - 5.8	100	1.3 ± 0.0	ND -1.3	85	0.5 ± 0.3	0.1 - 1.1
2-NP	40.3 ± 21.1	1.7 - 74.5	100	2.4 ± 0.9	1.1 - 4.1	94	2.8 ± 0.8	1.3 - 4.1
2,4,6-TCP	12.0 ± 6.5	2.5 - 24.4	100	2.1 ± 0.6	1.2 - 3.2	83	2.2 ± 0.6	1.1 3.2
4-NP	2.5 ± 1.4	0.8 - 5.1	100	0.8 ± 0.3	0.3 - 1.3	67	1.3 ± 0.5	0.3 - 1.8
PCP	31.8 ± 21.7	1.7 - 73.9	100	3.1 ± 1.2	0.8 - 4.8	90	3.3 ± 1.0	1.3 - 4.5

*PH= Phenol, 2-CP= 2-Chlorophenol, 2,4-DMP= 2,4-Dimethylphenol, 4-C-3MP= 4-Chloro-3-methylphenol, 2,4-DCP = 2,4-hlorophenol, 2-NP- Nitrophenol , 2,4,6-TCP = 2,4,6-Trichlorophenol, 4-NP = 4-Nitrophenol, PCP = Pentachlorophenol, RED.= Reduction,,, FD = Frequency of detection., ND= Not detected (Below limit of detection 0.102 – 0.586 **µg/L**), SE = Standard error

The concentration of phenolic compounds both in the final effluent and receiving water for all the treatment plants were below the tolerable limits of 5µgL^{-1}set by USEPA and the European Union for bathing water (domestic use) (Llompart *et al.*, 2002; Czaplicka, 2004; Fattahi *et al.*, 2007; Petrovic *et al.*, 2009; Silva *et al.*, 2009). It is worthy to note that the concentrations of 2,4,6-TCP and 2-NP, 4-NP were occasionally above this limit in the effluent and receiving water. The prominent phenolic compounds detected in these areas were similar to compounds detected in Cape Town, South Africa include 2-NP, 4-C-3MP, PCP, and 2,4-DMP (Olujimi *et al.*, 2012). Michalowicz and Duda, (2007) identified nitro-phenols and methyl-phenols as common products of vehicle emissions that find their way to the environment. The relatively low amounts of phenolic compounds detected in this study can in no way be compared to similar investigation carried out in Cape Town by Olujimi *et al.*, (2012) from sewers receiving effluents from industries that use these chemicals. The phenolic compounds reported in the final effluent and downstream of the investigated WWTPs in Cape Town were in the range of 30-200 µg/L^{-1}. Although, the high population reported in these areas (133,000 – 900,000) would have increased the anthropogenic activities in the area apart from the presence of industries. This suggests that these compounds are more likely to be introduced to surface water from industries that use these chemicals than from domestic and agricultural runoff. It could therefore be inferred that these meagre amounts in the influent and receiving water bodies could probably be the result of decomposition or leachate from the use of products containing these compounds, for example, plastics, dyes, drugs, pesticides, paper and petrochemical products (Zhou, *et al.*, 2005). Pesticides containing phenolic compounds and vehicle emissions are probably the commonest contributors of these compounds in these areas (Michalowicz and Duda, 2007). Sim *et al.*, (2009), also suggests that phenolic compounds might occur naturally through degradation of lignin, tannin and humic substances.

There was a notable reduction of the compounds in the final effluent suggesting high removal capacity of the WWTP. The concentration in the final effluent varied between not detected (ND) and 5.3 µgL^{-1}. Though, this concentration is usually exceeded in OP at Bedford. The removal capacities of all the treatment plant which varied between 33% and 96% suggest adsorption of the compounds on settling particles as we found a strong correlation between TSS, Turbidity and removal of phenolic compounds (especially the ones in abundance) Table 8. We may therefore conclude that the relative amounts of phenolic compounds that are generated in these parts of the rural areas were not above the removal capacity of the existing WWTPs.

Table 4.13 Pearson correlations of physicochemical properties with phenolic compounds

	pH	TEMP	E.C	DO	TDS	TUR	TSS	PH	2-CP	2,4-DMP	4-C-3MP	2,4-DCP	2-NP	2,4,6-TCP	4-NP	PCP
pH	1															
TEMP	-0.198	1														
E.C	0.230	-0.077	1													
DO	0.113	-0.364	-0.415	1												
TDS	0.218	-0.095	0.973**	-0.402	1											
TUR	0.126	0.052	0.237	-0.293	0.158	1										
TSS	0.153	-0.034	-0.178	-0.181	-0.258	0.872**	1									
PH	-0.256	0.303	-0.618	-0.135	-0.578	-0.189	0.562	1								
2-CP	-0.042	0.156	0.103	-0.298	0.130	0.846**	0.708*	-0.161	1							
2,4-DMP	-0.027	0.249	-0.319	0.316	-0.361	-0.192	-0.083	0.028	-0.201	1						
4-C-3MP	0.146	0.105	0.055	-0.543	0.036	0.555	0.705*	0.707	0.607*	-0.218	1					
2,4-DCP	0.226	0.186	-0.256	-0.177	-0.222	-0.237	-0.021	0.641	-0.355	0.268	0.389	1				
2-NP	-0.228	-0.104	-0.172	-0.050	-0.165	0.573	0.408	0.604	0.501	-0.007	0.531	0.049	1			
2,4,6-TCP	-0.154	0.109	-0.376	0.305	-0.362	-0.057	0.066	-0.112	0.116	0.765**	-0.323	-0.201	-0.071	1		
4-NP	0.007	0.224	-0.248	-0.028	-0.269	-0.005	0.219	-0.183	0.199	0.356	-0.100	-0.249	-0.394	0.673*	1	
PCP	-0.242	0.121	-0.074	-0.369	-0.060	0.760**	0.648*	0.787*	0.735**	-0.245	0.630*	-0.081	0.744**	-0.027	-0.148	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

^{h}TUR = Turbidity

4.4. Conclusions

The five selected WWTPs processes from different technologies, impacted considerably on the raw wastewater to produce a final effluent that met acceptable South Africa standards in terms of EC, DO, TDS, turbidity and TSS. However, standard limits for discharged effluents were occasionally exceeded especially with some WWTPs (Bedford and Seymour) hence, there is need for concerned authorities to ensure compliance of these WWTPs to standard practices and carry out regular monitoring to ensure safe environment for aquatic life and domestic use. The effect of temperature was largely determined by the seasons and the level of pH was dependent on the type of household waste, public facilities and agricultural runoff that are released to the sewer. The discharged effluent may not have an impact on the suspended solids (TSS) in the receiving water; nevertheless, there is evidence of the negative impact of total organic load measured in terms of TDS, EC, and turbidity depending on the geological difference and the constituents of the wastewater. This poses health challenge to rural communities that rely heavily on surface water for domestic use. Four phenolic compounds 4-C-3MP, 2-NP, 2,4-DCP, and PCP were the most prominent phenolic compounds detected in virtually all the WWTPs. Although, all the phenolic compounds were notably reduced by the WWTPs, it is not enough reason to overestimate the performance of the plants judging by the meagre amounts that were detected entering the WWTPs. A routine monitoring of the performance of these WWTPs is therefore vital so that abnormal fluctuations can easily be detected and addressed promptly. Also, the evaluation of these compounds in sediment (sludge) is suggested for further study. Sediments are sinks of pollutants and they measure the effect of pollution discharge over a period of time. They can be useful in studying the pollution history of an area.

Reference

Addendum., (Ukhahlamba District Municipality) (2008) *Infant deaths: Barkly East. Eastern Cape : Preliminary Epidemological Investigation Report*.

Akan, J. C., Abdulrahman, F. I., Dimari, G. A., and Ogugbuaja, V. O. (2008) 'Physicochemical determination of pollutants in wastewater and vegetable samples along the Jakara wastewater channel in Kano Metropolis, Kano State, Nigeria.', *European Journal of Scientific Research*, 23(1), 122–133.

Arar, A. (1988) *Background to treatment and use of sewage effluent.Treatment and Use of Sewage Effluent for Irrigation 1960. doi:10.1016/B978-0-408-02622-2.50005-5*.

Bay, N. M., Clinic, P. and William, K. (2011) 'The population of the Eastern Cape Province by census years Https://www.citypopulation.de/php/southafrica-easterncape.php accessed 17/02/2017', 40.

Chapman, D. (1996) *Water quality assessments: A guide to the use of biota, sediments and water in environmental monitoring (2nd ed.). London: UNESCO, World Health Organization, United Nations Environment Programme.*

Chigor, V. N., Sibanda, T., and Okoh, A. I. (2013) 'Variations in the physicochemical characteristics of the Buffalo River in the Eastern Cape Province of South Africa', *Environmental Monitoring and Assessment*, 185(10), 8733–8747. doi: 10.1007/s10661-013-3208-1.

Choksi, K. N., Sheth, M. A., And and Mehta, D. (2015) 'To assess the performance of Sewage Treatment Plant : A Case study of Surat city', *International Research Journal of Engineering and Technology*, 2(8), 1071–1075.

Coskuner, G. and Ozdemir, N. . (2006) 'Performance assessment of a wastewater treatment plant treating weak campus wastewater', *International Journal of Environment and Pollution,* 28(1/2), 185–197.

Coskuner, G. and Ozdemir, N. . (2006) ‘Performance assessment of a wastewater treatment plant treating weak campus wastewater’, *Int. J. Environment and Pollution*, 28(1/2), 185–197.

Czaplicka, M. (2004) ‘Sources and transformations of chlorophenols in the natural environment’, *Science of the Total Environment.*, 322(1–3), 21–39.

DFID (1999) *A Simple Methodology for Water Quality Monitoring. G. R. Pearce, M. R. Chaudhry and S. Ghulum (Eds.), Department for International Development Wallingford.*

DWAF (1996a) *South African Water Quality Guidelines, (Volume 2), Recreational Water Use (2nd edn.). Department of Water Affairs & Forestry, Pretoria.*

DWAF (1996b) *South African water quality guidelines (Vol.1), domesticuses(2nded.)Pretoria:Department of Water Affairs and Forestry.*

EC, (European Community) (2001) ‘The list of priority substances in the field of water policy and amending directive, Council directive 2455/2001/ECC.’, *Official Journal*, 331, 1–5.

Emilia, P. V., Tamara, R., Carmen, G. and Blaj, I. (2013) *Comparison of Efficiency of Different Type Systems for Wastewater Treatment (November 17, 2014). Analele Universităţii din Oradea, Fascicula Protecţia Mediului - Available at SSRN: https://ssrn.com/abstract=2692007 XXI, 665–674.*

Fatoki, O. S., Gogwana, P., and Ogunfowokan, A. O. (2003) ‘Pollution assessment in the Keiskamma River and in the impoundment downstream’, *Water SA*, 29(2), 183–187. doi: 10.4314/wsa.v29i2.4854.

Fattahi, N., Assadi, Y., Hosseini, M. R. M., and Jahromi, E. Z. (2007) ‘Determination of chlorophenols in water samples using simultaneous dispersive liquid-liquid microextraction and derivatization followed by gas chromatography-electron-capture detection.’, *Journal of Chromatography A*, 1157(1–2), 23–9.

Fondriest Environmental, I. (2013) *'pH of Water.' Fundamentals of Environmental Measurements. 19 Nov. 2013. Web. < http://www.fondriest.com/environmental-measurements/parameters/water-quality/ph/ >.Assessed 02/02/2017.*

Fondriest Environmental, I. (2014a) *'Conductivity, Salinity and Total Dissolved Solids.' Fundamentals of Environmental Measurements. 3 Mar 2014. Web. < http://www.fondriest.com/environmental-measurements/parameters/water-quality/conductivity-salinity-tds/ >.Assessed 02/02/2017.*

Fondriest Environmental, I. (2014b) *'Turbidity, Total Suspended Solids and Water Clarity.' Fundamentals of Environmental Measurements. Web. < http://www.fondriest.com/environmental-measurements/parameters/water-quality/turbidity-total-suspended-solids-water-clarity/ >.13 Jun. 2014.*

Fondriest Environmental, I. (2014c) *'Water Temperature.' Fundamentals of Environmental Measurements. 7 Feb. 2014. Web. < http://www.fondriest.com/environmental-measurements/parameters/water-quality/water-temperature/ >.Assessed 02/02/2017.*

Grynkiewicz, M., Polkowska, Z., Kot-Wasik, A. and Namiesnik, J. (2002) 'Determination of phenols in runoff.', *Polish J Environ Stud*, 11, 85–89.

Guedes, S. and Leitao, A. (2012) 'Effect of phenolic compounds on osmotic stress on the expression of penicillin biosynthetic genes from penicillium chrysogenum var. halophenolicum strain.', *Journal of xenobiotics*, 2, 7–12.

Igbinosa, E. O. and Okoh, A. I. (2009) 'Impact of discharge wastewater effluents on the physico-chemical qualities of a receiving watershed in a typical rural community.', *International Journal of Environmental Science and Technology*, 6, 175–182.

Kumar, B., Verma, V. K., Sharma, C. S. and Akolkar, A. B. (2014) 'Quick and easy method for determination of priority phenolic compounds in water and wastewater', *Journal of Xenobiotics*, 4(1), 46–52. doi: 10.4081/xeno.2014.4680.

Llompart, M., Lourido, M., Landin, P., Garcia-Jares, C., and R. Cela, J. (2002) ‘Optimization of a derivatization-solid-phase microextraction method for the analysis of thirty phenolic pollutants in water samples.’, *Journal of Chromatogr. A*, 963(1–2), 137–148.

Mamba, B. B., Krause, R. W., Matsebula, B., and Haarhof, J. (2009) ‘Monitoring natural organic matter and disinfection by-products at different stages in two South African water treatment plants.’, *Water SA*, 35(1), 121–127.

Mayer, J. F. L. and Ellersieck, M. R. (1988) ‘Experiences with single-species tests for acute toxic effects in freshwater animals.’, *Ambio*, 17, 367–375.

Mema, V. (2010) ‘Impact of Poorly Maintained Wastewater and Sewage Treatment Plants : Lessons From South Africa’, *ReSource*, 12(3), 60–61, 63, 65.

Michalowicz, J. and Duda, W. (2007) ‘Phenols-Sources and toxicity.’, *Polish Journal of Environmental Studies*, 16, 347–62.

Momba, M. N. B., Osode, A. N., and Sibewu, M. (2006) ‘The impact of inadequate wastewater treatment on the receiving watebodies - Case study: Buffalo City and Nkonkobe Municipalities of the Eastern Cape Province.’, in *Durban, South Africa : Water SA, Water Institute of South Africa (WISA) Bennial Conference.*, 0378–4738.

Morrison, G., Fatoki, O. S., Persson, L. and Ekberg, A. (2001) ‘Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River - pH , electrical conductivity , oxygen- demanding substance (COD) and nutrients’, *Water SA*, 27(4), 475–480.

Munch, J. W. (US. E. (2000) *Determination of Phenols in drinking water by solid phase extraction and capillary column gas chromatography/mass spectrometry (GC/MS)., U.S. Environmental Protection Agency, Washington D.C. EPA/815/R-00/014*.

Obi, C. L., Momba, M. N. B., Samie, A., Igumbor, J. O., Green, E., and Musie, E. (2007) ‘Microbiological, physicochemical and management parameters impinging on the efficiency of small water treatment plants in Limpopo and Mpumalanga Provinces of SouthAfrica.', *Water SA*, 33(2), 229–237.

Odjadjare, E. E. O. and Okoh, A. I. (2010) 'Physicochemical quality of an urban municipal wastewater effluent and its impact on the receiving environment', *Environmental Monitoring and Assessment*, 170(1–4), 383–394. doi: 10.1007/s10661-009-1240-y.

Ogunfowokan, A. O., Okoh, E. K., Adenuga, A. A., A. and Asubiojo, O. I. (2005) 'Assessment of the impact of point source pollution from a university sewage treatment oxidation pond on the receiving stream—A preliminary study.', *Journal of Applied Sciences*, 6(1), 36–43.

Okoh, A. I., Odjadjare, E. E., Igbinosa, E. O., and Osode, A. N. (2007) 'Wastewater treatment plants as a source of microbial pathogens in the receiving watershed.', *Africa Journal of Biotechnology*, 6, 2932–2944.

Olujimi, O. O., Fatoki, O. S., Odendaal, J. P. and Daso, A. P. (2012) 'Chemical monitoring and temporal variation in levels of endocrine disrupting chemicals (priority phenols and phthalate esters) from selected wastewater treatment plant and freshwater systems in Republic of South Africa', *Microchemical Journal.*, 101, 11–23. doi: 10.1016/j.microc.2011.09.011.

Olujimi, O., Fatoki, S., Odendaal, J., and Okonkwo, J. (2011) 'Solid-Phase Extraction Method for the Analysis of Eleven Phenolic Pollutants in Water Samples', *Asian Journal.*, 23(2), 657–662.

Olujimi, O. O., Fatoki, O. S., Odendaal, J. P. and Okonkwo, J. O. (2010) 'Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: A need for more monitoring', *Water SA*, 36(5), 671–682. doi: 10.4314/wsa.v36i5.62001.

Petrovic, M., de Alda, M. J. L., Diaz-Cruz, S., Postigo, C., Radjenovic, J., Gros, M. and Barcelo, D. (2009) 'Fate and removal of pharmaceuticals and illicit drugs in conventional and membrane bioreactor wastewater treatment plants and by riverbank filtration.', *Philosophical Transactions of the Royal Society A*, 367(1904), 3979–4003. doi: 10.1098/rsta.2009.0105.

Sánchez-Avila, J., Bonet, J., Velasco, G. and Lacorte, S. (2009) 'Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant', *Science of the Total Environment.*, 407(13), 4157–4167. doi: 10.1016/j.scitotenv.2009.03.016.

Santana, C., Ferrera, Z., Padron, M. and Rodriguez, J. (2009) 'Methodologies for the extraction of phenolic compounds from environmental samples: New approaches.', *Molecules*, 14, 298–320.

Schummer, C., Sadiki, M., Mirabel, P. and Millet, M. (2009) 'Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis', *Chromatographia*, 63, 189.

Silva, S. L., Alves, A., and Santos, L. (2009) 'Uncertainty measurement of chlorophenols and PCBs analyzed in aqueous media by SPME-GC-ECD.', *Journal of Chromatographic Science*, 47(2), 1 03-109.

Sim, W., Lee, S., Lee, I., Choi, S. and Oh, J. (2009) 'Distribution and formation of chlorophenols and bromophenols in marine and riverine environments.', *Chemosphere*, 77, 552–8.

Sukumaran, D., Saha, R., and Saxena, R. C. (2015) 'Performance Evaluation of Prevailing Biological Wastewater Treatment Systems in West Bengal, India'.

Tebbut, T. H. Y. (1998) *Principles of water quality . Oxford: Pergamon.* illustrate. ButterWorth-Heinemann,.

USEPA (2009) *National primary drinking water regulations. Safe Drinking Water Act EPA 816-F-09-004; http://water.epa.gov/drink/contaminants/upload/mcl-2.pdf. Retrieved: 26/01/2017.*

USEPA, (United States Environmental Protection Agency). (1982) *Appendix A to 40 CFR,* *Part 423--126 Priority Pollutants. Available from: https://www.epa.gov/eg/toxic-and-priority-pollutants-under-clean-water-act. Accessed 19.01.2017.*

Wang, Y., Hu, W., Cao, Z., Fu, X. and Zhu, T. (2005) ‘Occurrence of endocrine-disrupting com- pounds in reclaimed water from Tianjin, China’, *Analytical and Bioanalytical Chemistry,* 383, 857–863.

WHO (2004) *Rolling revision of the WHO guidelines for drinking-water quality, draft for review and comments. Nitrates and nitrites in drinking-water. World Health Organization (WHO/SDE/WSH/04.08/56).*

WHO. (1989) *Environmental health criteria 93. Chlorophenols other than pentachlorophenol. Geneva: World Health Organization;*

WHO, (1996) *Guidelines for Drinking Water Quality (2nd Edn) (Vol. 1–3). World Health Organization, Geneva, Switzerland.*

Williums, P. (2008) ‘Quantification and relative comparison of different type of uncertainties in sewer water quality modeling.’, *Water Research.,* 42, 3539–3551.

Zhou, F., Li, X., and Zeng, Z. (2005) ‘Determination of phenolic compounds in wastewater samples using a novel fiber by solid-phase microextraction coupled to gas chromatography’, *Analitica Chimica Acta*, 538, 63–70.

CHAPTER FIVE

Fate and impact of phthalates in activated sludge treated Municipal wastewater on the water bodies in the Eastern Cape, South Africa

Abstract

Phthalate esters (PAEs) are widely known plasticizer that have been implicated in endocrine disruption and are commonly found in wastewater treatment plants (WWTPs) effluents. The occurrence and fate of six priority PAEs; dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP), di(2-ethyl hexyl) phthalate (DEHP), and Di-n-octyl phthalate (DOP) were investigated as wastewaters passed through three WWTPs which adopted the activated sludge technology in the Amathole District Municipality, Eastern Cape, South Africa. Samples were extracted using solid-phase extraction (SPE) and analysed with gas chromatography-mass spectrometer (GC-MS). The six PAEs were detected in all the influents, treated sewage samples and sludge as well as in the receiving water bodies. DBP was most abundant in the inlet wastewater samples of Adelaide, Alice and Seymour WWTPs with concentration of 195 ± 62 µgL^{-1}, 1146 ± 385 µgL^{-1} and 78.3 ± 4.7 µgL^{-1}, respectively followed by DEHP and BBP in that order. The concentrations of DBP, BBP, DEHP and DOP detected in the sludge ranged between 130-1094 µg/g d.w. which is above the 100 µg/g d.w legislated by the European Union for agricultural use. DEHP and DBP were more pervasive in the sludge samples. The continuous flow activated sludge system used at Adelaide and Alice, with removal capacity; 67.99-99.47% outperformed a single tank system at Seymour (27.3-93.74%). The removal mechanism suggests more adsorption on settling particles and sludge than biodegradation as high significant correlation ($p<0.001$) was found between PAEs removal and total suspended solid (TSS) as well as turbidity. The final effluent impacted PAEs on the receiving water bodies and their concentrations were above US.EPA and European Environmental quality standard (EQS) of 3 µgL^{-1} and 1.3µgL^{-1}, respectively for DEHP in surface water for safe aquatic life. The high concentrations of PAEs detected in the treated and receiving waters will no doubt constitute health hazards to the dwellers and to aquatic life.

Key words: Phthalate esters, Wastewater, Treatment plants, sludge, final effluent, receiving water.

5.1. Introduction

Phthalates esters (PAEs) are esters of phthalic acid that are among the most important industrial chemicals because of their increasing production rate and eco-toxicological potential (ESIS, 2009; Clara *et al.*, 2010). They are synthetic compounds widely known in the environment for their wide applicability as plasticizers in polymers and additives in various industrial products such as insecticides, paints, coverings, insulators in electric disposals, personal care products, and cosmetics, amongst others. The global production of PAEs is approximately 6 million tons per year in spite of restrictions in some areas (Mackintosh *et al.*, 2006; Peijnenburg and Struijs, 2006; Abdel daiem *et al.*, 2012; Guo *et al.*, 2012). Instead, their production rate has increased globally. Bis(2-ethylhexyl) phthalate (DEHP) is considered the most widely used PAEs, being the main plasticizer used in polyvinyl chloride (PVC) production (ATSDR., 2000; Kotowska *et al.*, 2006; EU-RAR., 2008; Clara *et al.*, 2010). Benzylbutylphthalate (BBP) is another important PAEs representative commonly used in the manufacture of foamed PVC. Others, with relatively short alkyl chains include, dimethyl phthalate (DMP), diethyl phthalate (DEP), and di-n-butyl phthalate (DBP) which are commonly used as solvents in pesticides and perfumes (Oliver *et al.*, 2005; Gao *et al.*, 2014).

Unfortunately, PAEs are not bound chemically with their matrices and may therefore be dispersed or leached easily into the environment either during their production, uses or after disposal (Kotowska *et al.*, 2006; Yang *et al.*, 2006; Abdel daiem *et al.*, 2012). Their ubiquitous nature in the environment, including surface water, rainwater/storm-water, sediments, soils and sewage sludge has been reported in previous studies (Peijnenburg and Struijs, 2006; Roslev et al., 2007; Adeniyi et al., 2008; Gasperi et al., 2008; Fatoki et al., 2010; Gao et al., 2014). The presence of PAEs in treated and untreated wastewaters, and sewage sludge from full scale WWTPs has been widely reported in many countries (Gasperi et al., 2008; Dargnat et al., 2009; Clara et al., 2010; Olujimi et al., 2012; Gao et al., 2014). However, most conventional WWTPs are not designed to remove these persistent micro-pollutants from domestic and industrial influents. This has reduced the removal efficiency of WWTPs for these micro-pollutants, thereby increasing their presence in aquatic environments through the discharged effluents (Xue et al., 2010; Gao et al., 2014). Interestingly, previous study has shown that the removal of PAEs by WWTPs can vary from 60 - 100% (Oliver et al., 2005).

Many of these compounds have been implicated in endocrine disrupting effects due to their bioaccumulation potential in aquatic organisms. Human and aquatic exposures to some of these chemicals are also known to have carcinogenic and teratogenic effects (Becker *et al.*, 2004; Park *et al.*, 2012; Gao and Wen, 2016). The potential environmental hazard of these compounds has forced increasing global attention on their fate in the environment. This has also placed restriction or a complete phase out on their use in the European Union member countries (Becker *et al.*, 2004; Gao *et al.*, 2014). Similarly, ban on children's articles or toy containing more than 0.1% of six PAEs became a public law in USA under the Consumer Product Safety Improvement Act (CPSIA) in 2007 (Hileman, 2007). In 2000, the European community (DCE 2000/60/CE) listed DEHP among 33 hazardous substances to be placed under control in surface water and defined a concentration of lower than 1.3 μgL^{-1} for DEHP in surface water as an environmental quality standard (EQS) (Fromme *et al.*, 2002; EU, 2008).

Human and aquatic animals are unavoidably exposed to PAEs through drinking contaminated water. Though, health risk assessment of exposure to PEAs is a global concern but rural dwellers who depend more on stream water and river water for their daily domestic and agricultural use, are more prone to this health problem (Fatoki *et al.*, 2010). In the last decade in South Africa, it has been reported that 51-61% of its water is being consumed by farmers through irrigation of about 1.3 million hectares of land that are being managed by approximately 40,000 small-scale farmers, 15,000 medium to large scale farmers, 120,000 permanent workers, and an unknown number of seasonal workers (Blignaut and Heerden, 2009; Fatoki *et al.*, 2010). In South Africa, agriculture still remains crucial in food security especially for the poor, who constitute about 40% of the population and are predominantly concentrated in the rural areas and peri-urban townships. Irrigation faming contributes 25-30% of the country's agricultural output (Blignaut and Heerden, 2009).

Water scarcity is becoming a major problem in South Africa just as in many other countries in the world as dams in recent years have been less than 30% full (Marcucci and Tognotti, 2002; Malley *et al.*, 2009; Qiao *et al.*, 2009). As a result, river water, ground water, effluents from wastewater treatment plants have been considered suitable alternative sources of water for community daily household uses and portable drinking water (Blignaut and Heerden, 2009). Water quality in South Africa has been a major challenge as there are no interim guidelines for permissible levels of PAEs in fresh water system. This has given rise to indiscriminate pollution of fresh water systems through industrial discharge which in turn, makes surface water unfit for consumption purposes in the country (Fatoki *et al.*, 2012). According to a Green Drop report (2012), 12 out of the 15 treatment plants in the Amathole District Municipality (where we selected the three treatment plants under study; Adelaide, Alice, and Seymour), lack influent monitoring and poor effluent compliance (DWAF, 2012).

Therefore, this present study focuses on the removal efficiency of three selected WWTPs in the Amathole District Municipality in the Eastern Cape Province, South Africa for PAEs. Furthermore, the concentration levels of the six selected priority PAEs in the influent, final effluent and sludge of the respective WWTPs in the rural areas were investigated to determine the possible impact on the receiving water bodies and the quality of sludge in agriculture.

5.2. Materials and Methods

5.2.1. Study area

Influents, secondary effluent (water sample from the secondary clarifier) and final effluents from three wastewater treatment plants serving Adelaide, Alice, and Seymour all located in the Amathole district Municipality in the Eastern Cape, South Africa were investigated for the presence of PAEs. To assess the impact of the final effluent on the organic compound loads of the aquatic environment, water samples were also collected from about 500 m upstream and downstream of the discharge point. Table 5.1 illustrates the geographical location, population equivalent and technology in use in all the selected WWTPs. During the sampling period, the global position system was used to locate the position of the treatment plants. Geographical location of the sampling sites and the respective receiving water bodies are also shown in Figure 5.1.

Table 5.1 Brief description of selected wastewater treatment plants.

Plants	**Adelaide**	**Alice**	**Seymour**
Technology	Activated Sludge	Activated Sludge	Activated Sludge
Design capacity (ML/d)	0.5	0.5 - 2	0.25
Geographical location	S32º42.343ˈS and 26º18.790ˈE	32^{0}47.566ˈS and 26^0 50.958ˈE	32^{0}47.566ˈS and 26^0 50.958ˈE
Average flow rate in M^3/d	1,111,773	2,480,580	430,767
Treatment processes	Scr→ Gr→ Sed→ AS→ Sc→ Chl	Scr→ Gr→ Sed→ AS→ Sc→ Chl	Scr→ Gr→ Sed→ AS→ Chl
Population (2011)	12,191	15,143	2,467
Receiving river	Cobra	Tyume	Kat

*Abbreviations; Scr- Screening; Gr= Grit removal; Sed= Sedimentation; AS= Activated Sludge;

Sc= Secondary Clarifier; Chl= Chlorination

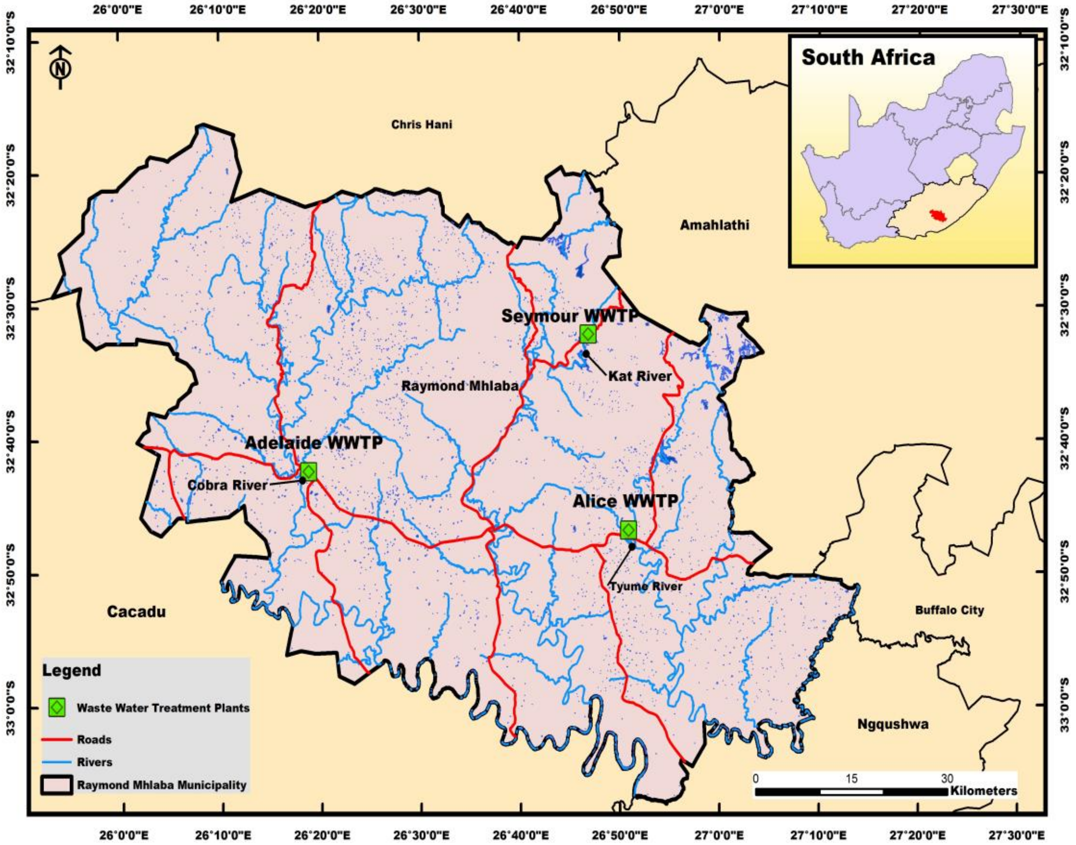


Figure 5.1 Location of sampling sites and receiving watersheds in Amathole District Municipality.

5.2.2. Chemicals and reagents

Standards of dimethyl phthalate (DMP) 98.0%, diethyl phthalate (DEP) 99.9%, di-n-butyl phthalate (DBP) 96.8%, benzyl butyl phthalate (BBP) 99.0%, di(2-ethyl hexyl) phthalate (DEHP) 99.6% and di-n-octyl phthalate (DOP) 99.1%, were all sourced from Accu Standard, Inc USA. A separate stock solution of each analyte was prepared in methanol at 2000 µgmL^{-1} and a working mixture of all the analytes was prepared at100 µgm/mL and stored at 4^{o}C in an amber bottle. The selected surrogate standard used, 2-Fluorobiphenyl (2FB) was purchased from Restek, USA. Anhydrous sodium sulphate and sodium thiosulphate pentahydrate were bought from Merck, Germany. Anhydrous sodium sulphate was purified by heating in a muffle furnace at 450^{o}C for 2 h. The physical-chemical properties (molecular weights, octanol-partition coefficient and water solubility) of the six selected PAEs, are summarised in Table 5.2.

High performance liquid chromatography (HPLC) grade methanol, n-hexane, dichloromethane and acetone, were all purchased from Merck, Germany. Double-distilled water was produced in our laboratory and the dry nitrogen gas used for drying with 99.99% purity was bottled by Afrox gas, South Africa. Solid phase extraction cartridges (SPE) C18-U (strata) 1000 mg/6mL, from Phenomenex were purchased from Separation, South Africa.

Table 5.2: Characterization of the six investigated phthalates

Compound	**Formula**	**Alkyl chain length**	**MW**	**S_w(mgL^{-1})**	**logK_{ow}**
DMP	$C_{10}H_{10}O_4$	1	194.19	400	1.61
DEP	$C_{12}H_{14}O_4$	2	222.24	1080	2.38
DBP	$C_{16}H_{22}O_4$	4	278.34	10	4.45
BBP	$C_{19}H_{20}O_4$	4	312.37	2.8	4.84
DEHP	$C_{24}H_{38}O_4$	8	390.57	0.003	7.5
DOP	$C_{24}H_{38}O_4$	8	390.57	0.022	8.1

*MW=Molecular weight; Sw=Solubility in water; Kow= Octanol-water partition coefficient

Data taken from (EU-RAR., 2007; EU-RAR, 2008; Clara *et al*., 2010)

5.2.3.1. Analytical methods

Taking cognisance of the ubiquitous nature of PAEs in the environment, the use of plastic materials throughout the procedure was completely avoided. All the materials used through extraction, transfer or storage of extracts were all glass made equipped with polytetrafluoroethylene (PTFE) lined screw or glass caps. All glass wares was soaked in 10% nitric acid overnight after thorough washing with tap water and finally rinsed with distilled water. This was followed by soaking in acetone (300mL) for 1 h and dried in the oven at 200°C for 4 h. All the glass wares were regularly rinsed with HPLC grade acetone and dichloromethane before use to eliminate any form of phthalate contaminants. The extraction method was developed from the reference methods of US EPA method 528, UCT, solid-phase extraction of EPA 8270 compounds in water and Juan Sanchez-Avila *et al.,* (2009) (Munch, 2000; Sánchez-Avila *et al.*, 2009; UCT, 2013).

5.2.3.2. Sampling protocol

A litre amber glass bottle fitted with PTFE lined polypropylene screw cap (to avoid phthalate contamination) was used for the collection of wastewater and river water samples throughout the sampling period. Composite samples of each of influent, secondary effluent and final effluents from all the selected WWTP were taken on a monthly basis for six months from February to July, 2016 including some from the receiving water body. One litre amber bottles previously cleaned in the laboratory were rinsed twice with the sample water before collection. De-chlorination and preservation of the samples were conducted at the time of collection with 40-50 mg of sodium thiosulphate and 50% $HCl_{(aq)},$ respectively. The pH value was adjusted to ≤ 2 which was the required pH for extraction after complete dissolution of the sodium thiosulphate. Routine physicochemical properties: turbidity, total dissolved solids (TDS) and total suspended solids (TDS) were measured *on-site* in triplicate for all samples collected using Hach instruments. All samples were kept in ice and transported to the Department of chemistry laboratory, University of Fort Hare, South Africa, stored at below 4 °C and extracted within three days of sample collection.

Dewatered sludge was collected in a glass jar with a polytetrafluoroethylene (PTFE) lined cap (to avoid phthalate contamination), previously rinsed with acetone and hexane and subsequently stored in an ice chest and later stored in the same laboratory at a temperature below 4 ^{o}C.

5.2.3.3. Effluent extraction method

Water samples collected were allowed to settle for a day under the specified storing temperature and later passed carefully through glass wool to remove all suspended solid particles that may clog the cartridges. Surrogate standard (2-Fluorobiphenyl) was added at 5 μgL^{-1} prior to the extraction to correct for possible losses during the experimental procedure. This also provides efficiency for the extraction procedure and subsequent corrections. C18-U cartridge (strata) 1000 mg/6mL was used for the solid phase extraction (SPE) method. The cartridges were fitted to a vacuum manifold (Supelco) connected to a vacuum pump prior to extraction and were preconditioned in succession with 7 mL each of n-hexane, dichloromethane, methanol and 10 mL double-distilled water, respectively at a flow rate of about 3 mL min^{-1}. Hydrochloric acid HCl (50% v/v) was used previously to adjust the pH of the water samples to ≤ 2 during collection and preservation. The respective water samples were then passed through the conditioned cartridges at a flow rate of 10 mL min^{-1}, rinsed with 5 mL of double-distilled water after complete run and left under vacuum for 1 h to dry. Cartridge elution was carried out with 7 mL mixture of dichloromethane:hexane (1:1) followed by 7 mL dichloromethane:acetone (1:1) and collected in a 60 mL amber glass vial. Each eluent was reduced to about 2 mL in a rotary evaporator and finally blown to 1 mL at 30 ^{o}C under a stream of dry nitrogen gas, ready for gas chromatography - mass spectrometry (GC-MS) analysis.

5.2.3.4 Removal efficiency for wastewater treatment plants

The removal efficiency of the WWTPs was calculated in percentage as the ratio of the difference between average influent and final effluent concentration to the average influent concentrations multiplied by 100 for each of the PAEs compounds.

$$100\times\{(\text{Inf}-\text{Ef})/\text{Inf}\}.$$

Where Inf = Average influent concentration and Ef = Average effluent concentration

5.2.3.5. Sludge extraction method

Sludge samples were air-dried in a room and ground with a mortar and pestle that had been previously cleaned and rinsed with HPLC grade acetone. For each of the WWTPs samples, 2 g of the homogenous sludge sample was extracted with 3×10 mL portion of dichloromethane in an ultrasonic bath for 15 min. The extracts were pulled together in a 60 mL amber glass vial and centrifuged at 5000 rpm for 30 min. The supernatant was reduced to about 5 mL in a rotary evaporator followed by clean-up.

5.2.3.6. Column chromatographic clean-up

A pre-cleaned column glass was filled with 5 g silica gel and topped with 2 g of anhydrous sodium sulphate previously treated as mentioned earlier. The column was preconditioned with 2×10 mL dichloromethane to remove trapped air and contaminants before the run of the extract. The respective extract was quantitatively transferred into the column and eluted with a 10 mL mixture of dichloromethane and n-hexane (1:1 v/v). The cleaned extract was reduced to about 2 mL in a rotary evaporator and blown to 1 mL at 30 ^{o}C under a stream of dry nitrogen gas (Sablayrolles *et al.*, 2005).

5.2.4. Method validation

A calibration solution containing all analyte of interest and standard surrogate was prepared in dichloromethane at concentration ranging from 0.1 - 15 µg/mL. An external standard calibration method was adopted with 6 points for each analyte and quantification was carried out within the linear range of the calibration curve. The regression coefficients for each of the analytes of interest; between the peak area and injected concentration were in the range of 0.99 (BBP) and 1.00 (DBP). Replicate analysis of laboratory fortified blanks (LFBs) spiked at 5 and 10 µgL^{-1} and carried out using the extraction procedure described above; gave a relative standard deviation of less than 15% indicating a good precision. The average percentage spike recovery of the replicate analysis for each of the analytes was between 75-116%**.** The limit of detection (LOD) and limit of quantification (LOQ) of the method ranges as 0.55-1.25 µgL^{-1} and 1.75-3.99 µgL^{-1}, respectively.

For each extraction batch of about 10-15 samples, LFB and laboratory reagent blank (LRB) (to determine the background system contamination) were included. The values obtained for LRB were below the detection limit of the respective analytes. A laboratory fortified sample matrix was also analysed to ensure that the sample matrix does not contain materials that adversely affect method performance. A duplicate analysis was performed for each of the series of batches.

5.2.4.2. Instrumental analysis

The analytical quantification of the targeted analytes was carried out by GC-MS (Agilent Technology 7890B coupled with 5977A MSD) with HP-5 MS capillary column (30 m × 250 µm i.d × 0.25 µm) with helium used as carrier gas at 90 kPa pressure. The injection port was maintained at 300 ^{o}C and sample injected in pulsed splitless mode. The injection pulse pressure was 90 kPa until 1 min and purge flow to split vent 50 mL/min at 2 min. The oven temperature was set at 50 ^{o}C for the first minute and heated to 310 ^{o}C at a rate of 10 ^{o}C/min and maintained for 5 min.

5.2.5. Statistical analysis

The box-whisker plots and mean values of the data obtained from the different parameters investigated were compared using SPSS (IBM SPSS Statistics 20), one-way analysis of variance and Tukey’s multiple range tests. The coefficient of correlation among PAEs and some physicochemical parameters was calculated by the Pearson correlations test. Significance for statistical analysis was set at p values < 0.05).

5.3. Results and discussion

5.3.1. Occurrence of phthalate esters in the wastewater of activated sludge treatment plants

The presence of six PAEs at the influents and final effluents of three selected wastewater treatment plants which adopted activated sludge technology in the Amathole District Municipality in the Eastern Cape Province, South Africa, and the frequencies of detection are as shown in Tables 5.3, 5.4, and 5.5. The mean values and standard error for the six months sampling were reported. A selected chromatogram of PAEs detected in one of the wastewater sample collected at Adelaide WWTP is also presented in Figure 5.2. These WWTPs were selected randomly as there was no previous work on phthalates in WWTPs in the Eastern Cape.

Table 5.3 Concentration ($\mu g L^{-1}$) of PAEs in Adelaide WWTP.

PAEs	**Influent**					**Final Effluent**				
	Mean ± SE	**Max**	**Min**	**Median**	**FD (%)**	**Mean ± SE**	**Max**	**Min**	**Median**	**FD (%)**
DMP	5.74 ± 1.83	12.07	1.35	5.36	100.00	1.48 ± 0.29	2.37	0.62	1.23	100.00
DEP	12.82 ± 3.85	24.42	2.53	10.92	100.00	3.39 ± 1.25	8.89	0.17	2.49	100.00
DBP	195.14 ± 61.53	451.48	ND	159.03	83.33	8.88 ± 1.12	26.47	ND	5.92	83.33
BBP	33.71 ± 13.76	80.70	2.38	17.90	100.00	5.10 ± 0.71	13.73	ND	3.95	83.33
DEHP	28.83 ± 7.33	48.16	3.44	36.06	100.00	9.23 ± 2.34	18.25	2.05	7.40	100.00
DOP	12.71 ± 3.99	21.75	ND	14.03	50.00	3.17 ± 0.37	4.15	ND	2.94	50.00
Total PAEs	288.96					31.24				

* ND= Not detected (Below limit of detection 0.102 – 0.586 µg/L); FD= Frequency of detection; SE = standard error; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate

Table 5.4 Concentration ($\mu g L^{-1}$) of PAEs in Alice WWTP.

PAEs	**Influent**					**Final Effluent**				
	Mean ± SE	**Max**	**Min**	**Median**	**FD (%)**	**Mean ± SE**	**Max**	**Min**	**Median**	**FD (%)**
DMP	8.37 ± 2.47	23.14	1.14	4.54	100.00	1.87 ± 0.57	4.57	0.64	1.54	100
DEP	12.60 ± 4.34	25.72	2.20	11.45	100.00	2.44 ± 0.80	5.97	0.12	2.08	100
DBP	1146 ± 385	2488.31	3.05	1185.13	100.00	6.08 ± 0.80	22.34	1.17	2.51	100
BBP	27.57 ± 9.22	52.12	ND	21.75	83.33	3.52 ± 1.33	8.75	0.75	2.04	100
DEHP	33.69 ± 8.41	94.87	6.13	21.52	100.00	5.41 ± 1.02	14.82	1.73	3.14	100
DOP	25.80 ±9.60	63.59	3.06	12.64	100.00	2.63 ± 1.21	6.02	ND	1.32	50
Total PAEs	1254.40					21.96				

* ND= Not detected (Below limit of detection 0.102 – 0.586 µg/L); FD= Frequency of detection; SE = standard error; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate

Table 5.5 Concentration ($\mu g L^{-1}$) of PAEs in Seymour WWTP.

PAEs						**Final Effluent**				
	Mean ± SE	**Max**	**Min**	**Median**	**FD (%)**	**Mean ± SE**	**Max**	**Min**	**Median**	**FD (%)**
DMP	3.05 ± 1.16	6.34	ND	1.47	75	2.21 ± 0.73	4.10	ND	1.99	75
DEP	6.49 ± 2.29	13.83	1.12	5.51	100	4.35 ± 0.76	7.32	ND	5.08	75
DBP	78.29 ± 4.71	277.89	2.70	16.29	100	4.90 ± 1.23	7.68	1.05	5.44	100
BBP	7.21 ± 2.14	14.32	1.73	6.40	100	4.15 ± 0.35	13.84	0.76	1.98	75
DEHP	20.72 ± 2.27	62.60	2.68	8.81	100	13.27 ± 4.84	24.91	2.42	12.87	100
DOP	6.37 ± 2.29	12.26	ND	5.75	75	4.19 ± 1.31	7.57	ND	3.79	75
Total PAEs	122.14					35.44				

* ND= Not detected (Below limit of detection 0.102 – 0.586 µg/L); FD= Frequency of detection; SE = standard error; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate

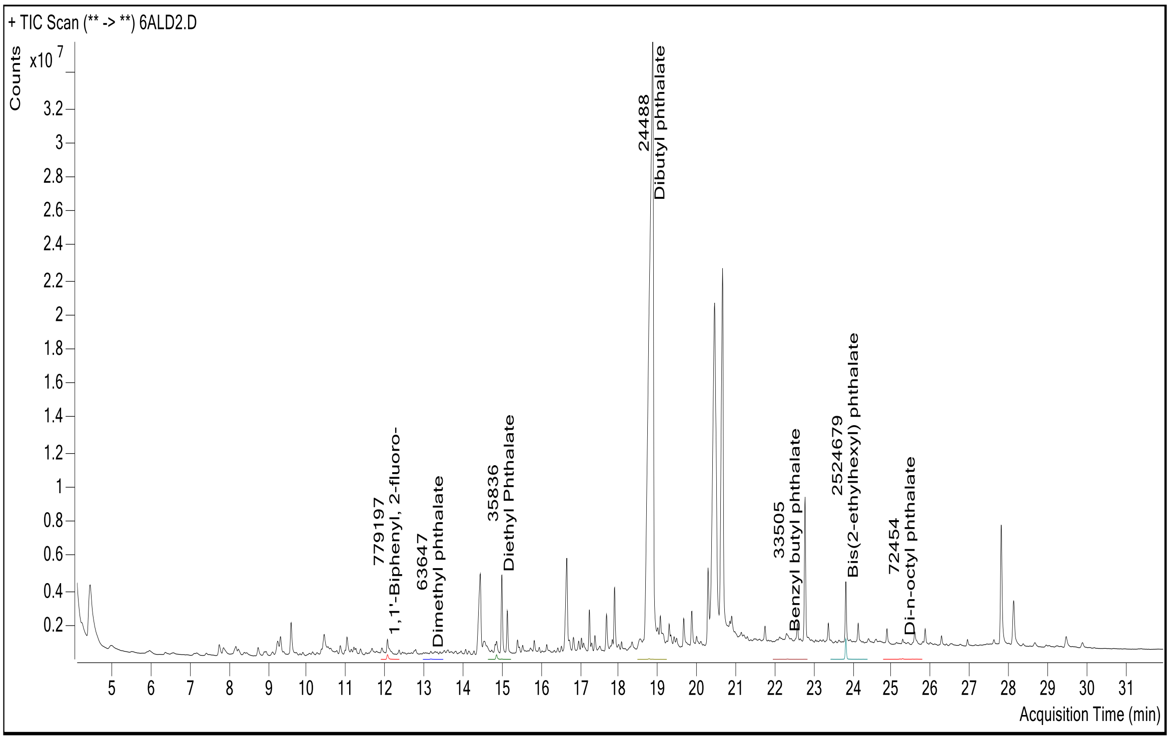


Figure 5.2 Sample of a PAEs chromatogram in Adelaide wastewater sample.

The concentration ranges for the most abundant PAEs (DBP) at the inlet of Adelaide WWTP, varied between <LOD and 451.5 µgL^{-1} with a concentration of 195.1 ± 61.5 µgL^{-1}. Alice had the highest concentration of 1146.4 ± 384.8 µgL^{-1} with a concentration range of 3.0-2488 µgL^{-1} and Seymour with the least concentration ranging between 2.7 µgL^{-1} and 277.8 µgL^{-1} and with a concentration of 78.3 ± 4.7 µgL^{-1}. It is important to note also, that the wastewater generated in all the sampling areas was predominately from domestic and agricultural runoff, as there was no reported industrial waste in these areas. This suggests that the compounds were probably associated with the leachate from the use of their products.

At Adelaide WWTP, DOP had the least detection frequency of 50% in the influent, and the final effluent. The concentration range in the influent was between ND (Not detected) and 20.5 µgL^{-1} with an average of 12.3 ± 5.4 µgL^{-1}. Apart from DOP, DBP and BBP also occurred less frequently with 83.33% at the influent and other sampling points. The second most abundant PAE in the influent was BBP with a concentration of 33.7 ± 13.8 µgL^{-1}, followed by DEHP with an average mean of 28.8 ± 7.3µgL^{-1}. DMP had the least concentration of 5.7 ± 1.8 µgL^{-1}.

The total average PAEs concentration detected in Alice WWTP happens to be the highest among the WWTPs under study (1254 µgL^{-1}). This may be attributed to the higher population recorded in this area which in turn increases anthropogenic activities and population from the high use of PAE based products. Alice also, had the highest concentration of DBP in the influent samples (1146 µgL^{-1}). This also depicts the highest concentration recorded in any of the WWTPs, followed by DEHP, BBP and DOP with a concentration of 33.7 ± 8.4, 27.6 ± 9.2, and 25.8 ± 7.3 µgL^{-1}, respectively. The concentration of DMP is of minor importance across the sampling points; with influent concentration occurring at a concentration lower than 10 µgL^{-1}.

At Seymour WWTP, the abundant distribution of PAEs in the influent is similar to that at the Alice WWTP but at a lower concentration. This is a plant serving a smaller population in a more remote area compared with others. It has the least total PAEs concentration in the influent (122 µgL^{-1}). DMP was as well the least concentrated PAE across the sampling points with concentrations ranging from <LOD to 6.3 µgL^{-1}with an average mean of 3.1 ± 1.2 µgL^{-1} in the influent.

The Box-whisker plots were used to present the concentrations of six PAEs in the influents and final effluents of the three WWTPs. The maximum, 75%, mean, 50%, 25% and the minimum concentrations of all target PAEs were shown in Figures 5.3, 5.4 and 5.5. All the targeted PAEs were detected in the influent samples collected throughout the sampling periods in all the selected

WWTPs serving Adelaide, Alice and Seymour. DEP and DEHP had 100% detection frequency (FD) across the three WWTPs whereas other PAEs, DMP, DBP, BBP and DOP occurred less frequently. The concentrations of all the PAEs in the final effluent were notably lower than what was detected in the influent, indicating a high removal capacity of the WWTPs for PAEs Figures 5.3, 5.4 and 5.5. Among the six PAEs investigated, DBP was the most abundant in the influents of the three WWTPs followed by DEHP, and BBP except at Adelaide WWTP where the concentration of BBP was slightly more than DEHP. Nevertheless, this may be in slight contrast to some reports identifying DEHP as the most abundant PAE in the influent of the WWTPs (Dargnat *et al.*, 2009; Clara *et al.*, 2010). However, there are other different reports rating DPB as the most abundant (Olujimi *et al.*, 2012; Gao *et al.*, 2014). This may not be unconnected to their important role as industrial additives in many products including flexible PVC materials and household products which suggests the main source of PAEs (Liu *et al.*, 2013). More so, the use of DBP in other products such as nail polish, aftershave, perfumes, pharmaceutical herbal and herbal coating that are commonly used in these areas may also have contributed to the abundance of DBP over DEHP. However, among the prominent compounds (DEHP, DBP, BBP and DOP) in the final effluents, DEHP was the most abundant which probably indicate its resistance to biodegradation.

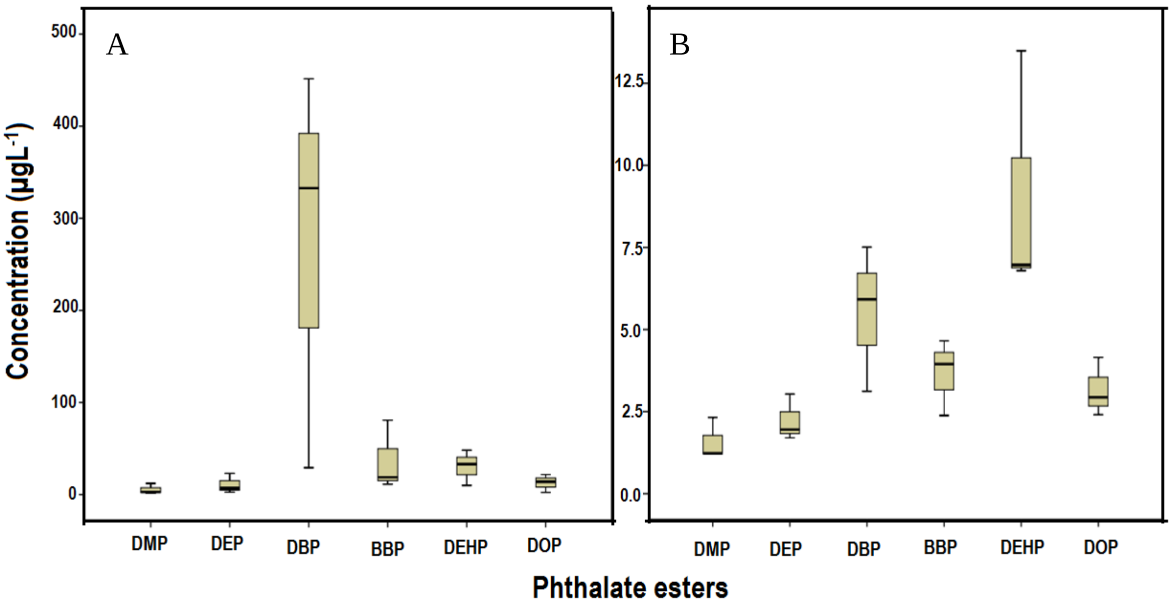


Figure 5.3 Box and whisker presentation of concentration of six PAEs in Adelaide WWTP (Plot A is the influent while Plot B is the effluent).

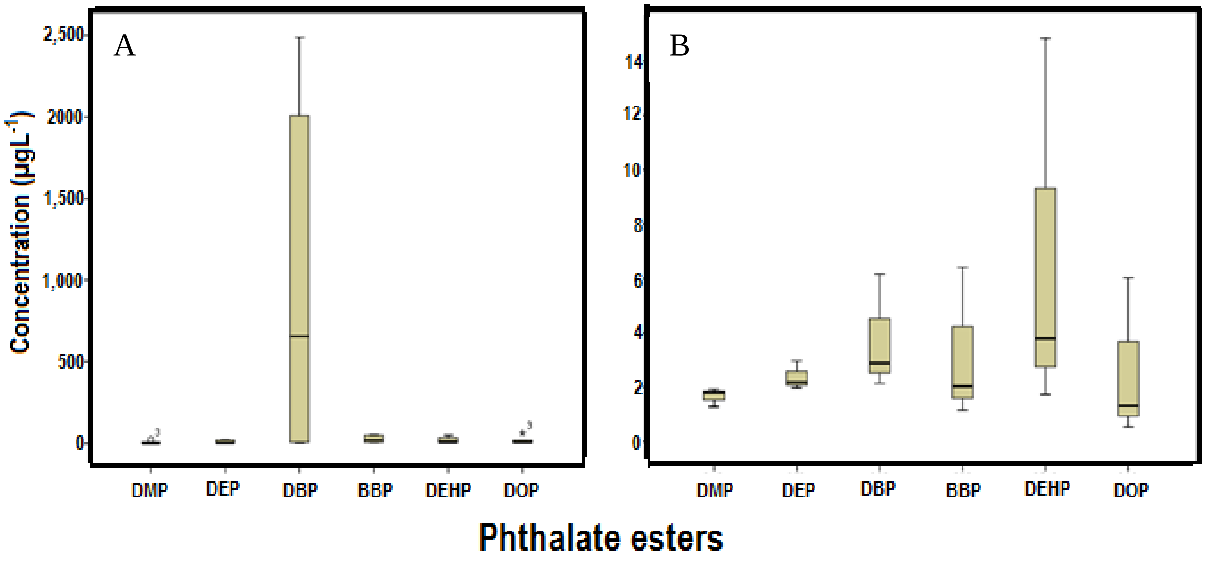


Figure 5.4 Box and whisker presentation of concentration of six PAEs in Alice WWTP (Plot A is the influent while Plot B is the effluent).

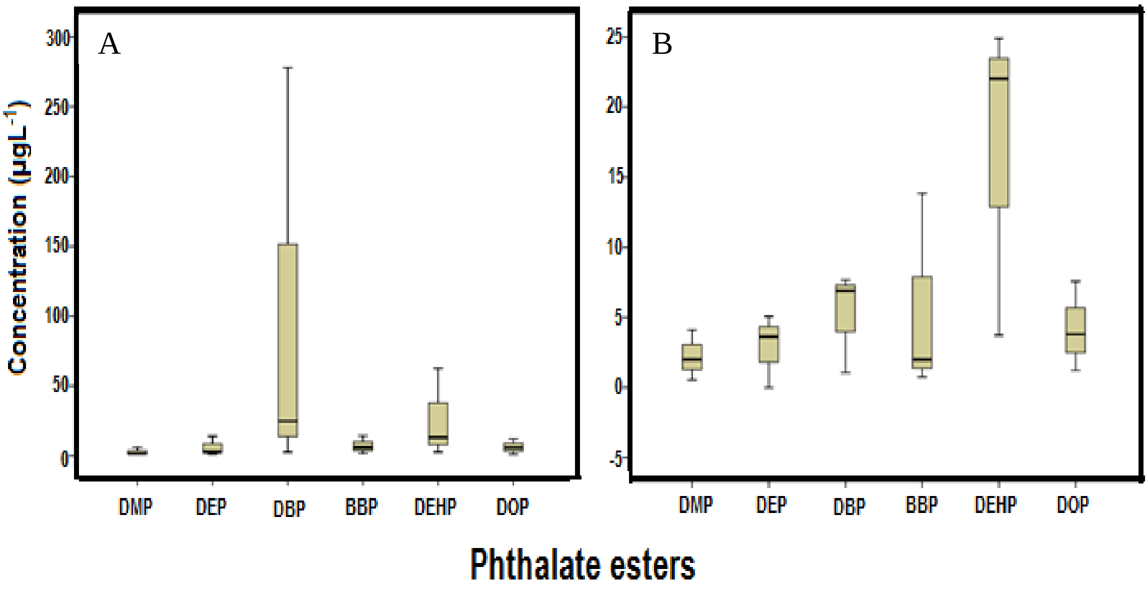


Figure 5.5 Box and whisker presentation of concentration of six PAEs in Seymour WWTP (Plot A is the influent while Plot B is the effluent).

5.3.2. The profile of PAE in wastewater and treated water

The measurement of the composition of each PAE helps tremendously in tracking the source of phthalate contaminant and reveals the transport and fate of these compounds in wastewater and treated water (Liu *et al.*, 2013). The relative contributions of each phthalate to the total PAEs across the treatment plants are presented in Figure 5.6. The preponderance of these three PAEs: DBP, DEHP and BBP at the influent, secondary and final effluent of all the WWTPs is significant to note.

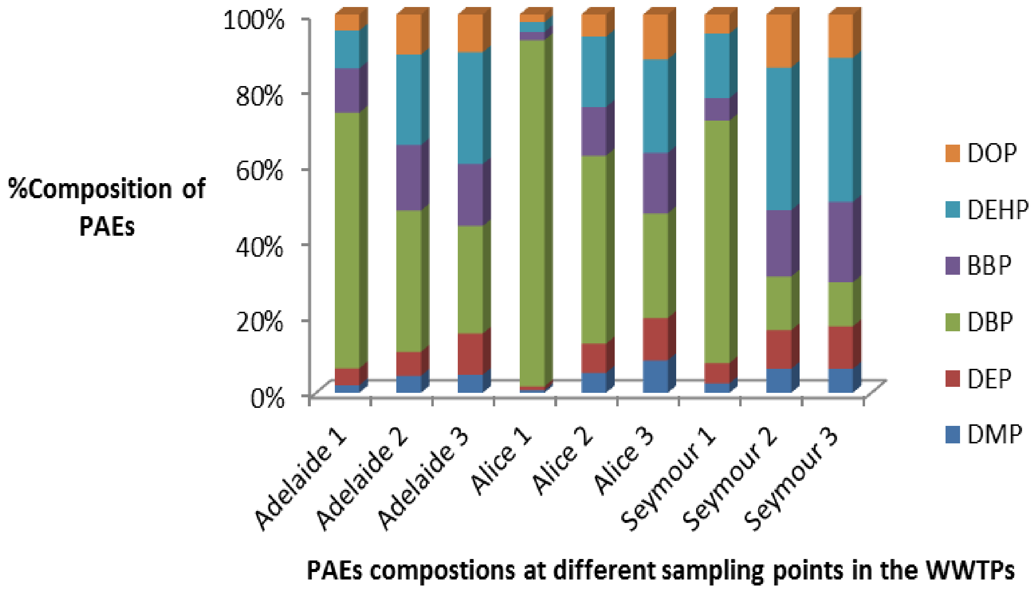


Figure 5.6 Relative abundance compositions of PAEs at three different treatment stages of the selected WWTPs; 1-Influent, 2-Secondary effluent and 3- Final effluent

It is obvious from the results that DBP is the most abundant compound in the influent samples which were over taken by DEHP at the secondary and final effluent. This was a true reflection of the contribution of plastic-based contaminants from domestic use during the sampling periods and the possible leaching of plasticizer from plastic materials in runoff water. DEHP is the only compound among others with a gradual increase in percentage composition across the sampling points. The relative abundance of DEHP in the treated effluent gave an indication of its possible escape or resistance to removal during treatment coupled with its persistent nature in the environment. On the other hand, the percentage composition of DBP in comparison with other PAEs decreases progressively across all the WWTPs. This probably suggests a significant removal tendency of DBP by the treatment plants.

5.3.3 Discharged effluent quality

The distributions of six PAEs in the secondary and final effluent of the three WWTPs are presented in Box-whisker plots as shown in Figures 5.7, 5.8 and 5.9. There was no significant difference in the concentrations of PAEs present in the discharged effluent both at the secondary and final effluent. However, there were seemingly reduction in PAEs concentration at Adelaide and Alice as effluents move from the secondary clarifier to the final treatment but not obvious at Seymour WWTP. The reduction in PAEs concentration was more pronounced at Alice WWTP. This probably suggests additional removal of these compounds through adsorption on settling particles at the secondary clarifier Figures 5.7 and 5.8. This also gave an indication that a more effective secondary clarifier exists at Alice WWTP compared to others. Unlike other PAEs, DEHP showed no compliance to the particle settling mechanism at the secondary clarifier.

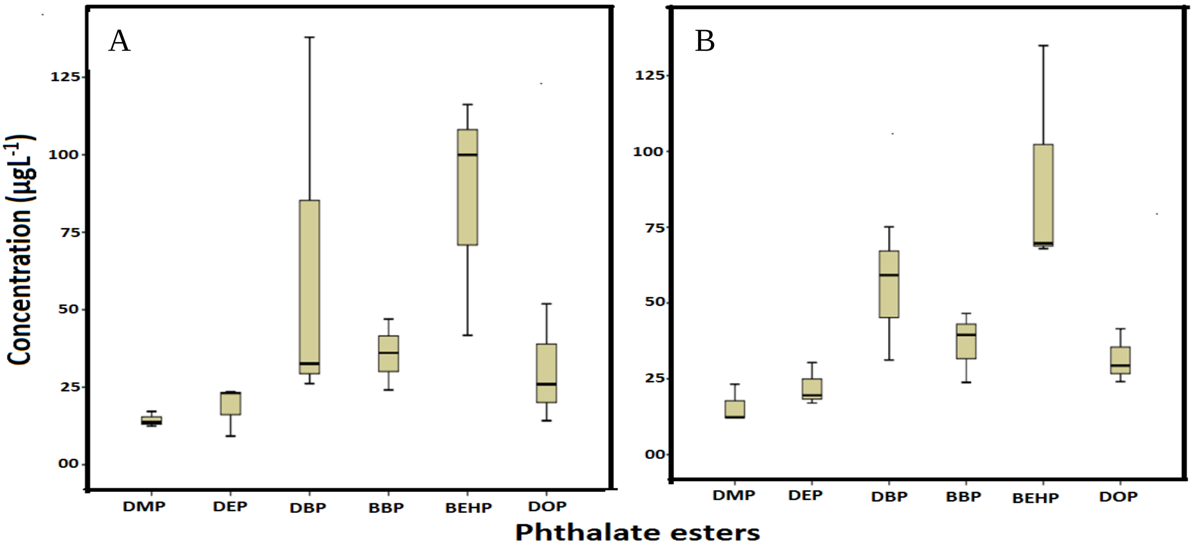


Figure 5.7 Box and whisker presentation of concentration of six PAEs in Adelaide WWTP (Plot A is the secondary effluent while Plot B is the final effluent)

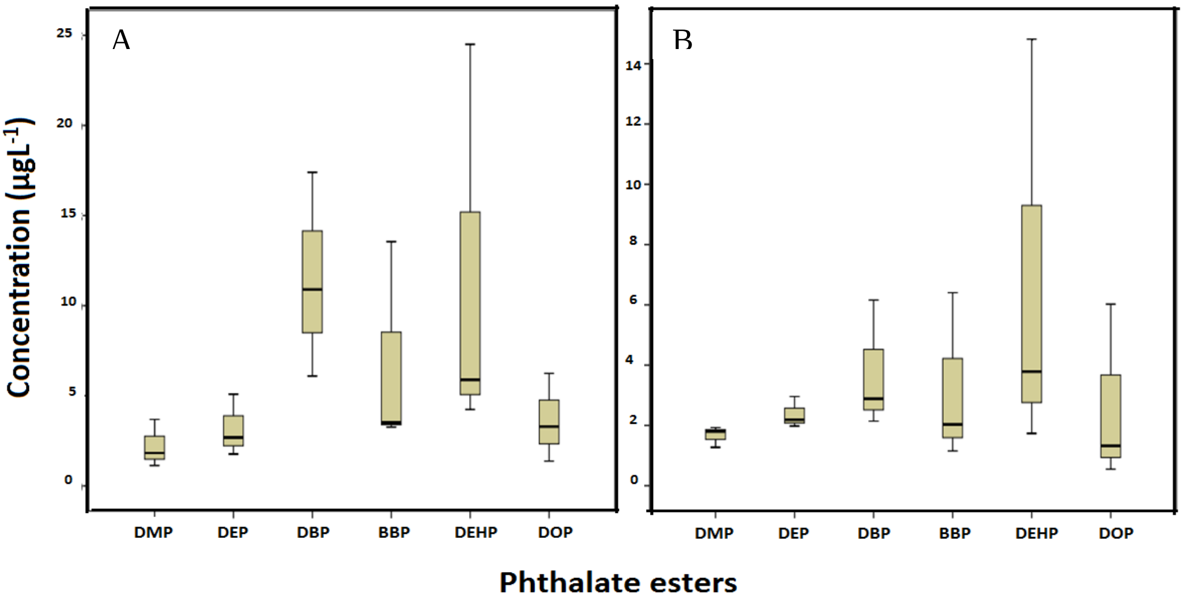


Figure 5.8 Box and whisker presentation of concentration of six PAEs in Alice WWTP (Plot A is the secondary effluent while Plot B is the final effluent)

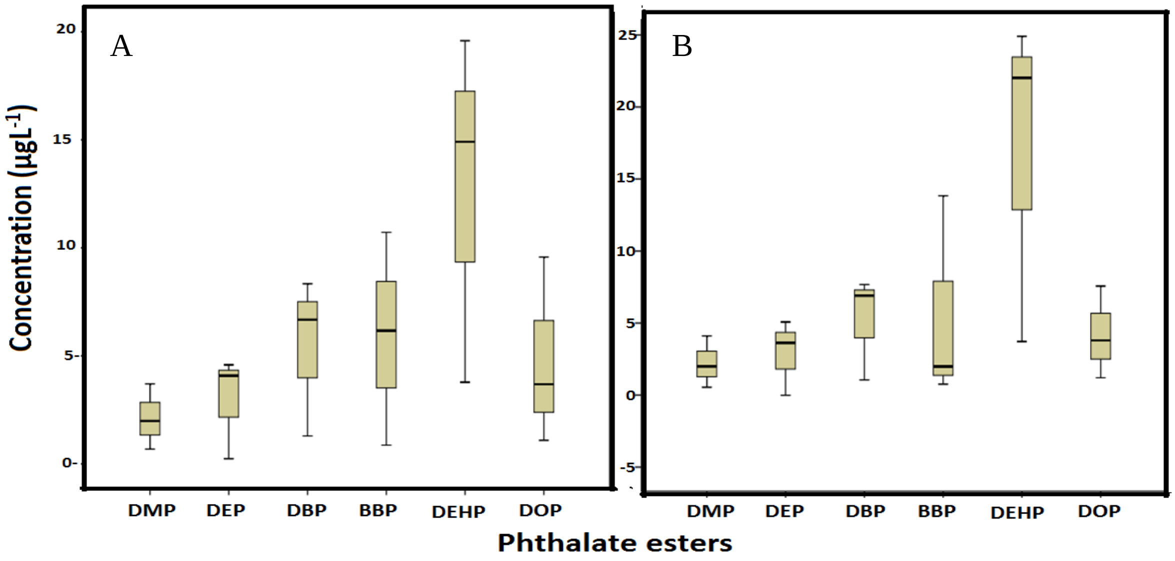


Figure 5.9 Box and whisker presentation of concentration of six PAEs in Seymour WWTP (Plot A is the secondary effluent while Plot B is the final effluent)

In Adelaide and Alice WWTPs, DBP constitutes the highest PAE contaminants in the secondary effluent with average concentrations of 10.7 ± 2.2 µgL^{-1} and 30.1 ± 2.7 µgL^{-1}, respectively, closely followed by DEHP with concentration levels of 6.8 ± 1.7 µgL^{-1}and 11.2 ± 3.9 µgL^{-1}, in that order. The reverse was the case in the final effluent where the average concentration of DEHP (5.41 µgL^{-1} at Alice to 13.3 µgL^{-1} at Seymour) was slightly above

DBP (4.9 µgL^{-1} at Seymour to 8.9 µgL^{-1} at Adelaide) in all the WWTPs.

Conversely, at Seymour WWTP the concentration of DEHP (12.8 µgL^{-1}) was much higher than that of DBP (4.8 µgL^{-1}) in the secondary effluent. DEHP was the highest contaminant both at the secondary and final effluents (12.8 ± 3.3 and 13.3 ± 4.8 µgL^{-1}). Other PAEs, i.e. DMP, DEP, BBP, and DOP are less concentrated at the two points of collection. This result is similar to the results obtained from the final effluent of WWTPs in most European countries, although, much higher concentrations were recorded in this study. For example, in France, Finland and, Sweden, the concentrations of DEHP in the final effluents were lower than 10 µgL^{-1} (Marttinen *et al.*, 2003; Dargnat *et al.*, 2009) whereas, a much lower concentration was reported in Denmark and Australia (Fauser *et al.*, 2003). Moreover, Olujimi *et al.*, (2012) reported much higher concentrations from WWTPs in Cape Town, South Africa. This was largely attributed to higher industrial effluent discharge from these areas. This result also agrees with previous findings establishing DEHP as the most abundant PAE contaminants in the final effluent of WWTPs.

5.3.4. Phthalate removal and fate in the selected wastewater treatment plants

A detailed investigation on three activated sludge WWTP technologies was conducted in order to assess the fate of six priority phthalates during wastewater treatment. The selected treatment plants operated primary clarification before activated sludge treatment followed by secondary clarification except in Seymour WWTP which has a single tank technology with no separate secondary clarification. In addition to the influent and final effluent samples collected, a sludge sample was also analysed for the six PAEs. Measured phthalate concentrations in the influent, and final effluent for the three WWTPs are presented in Tables

5.3, 5.4, and 5.5 above while Table 5.6 shows the removal efficiency of different WWTPs for phthalates.

On the average, there was a significant removal of all the compounds in Adelaide and Alice WWTPs with percentage removal ranging from 67.99 - 95.45% and 77.61 - 99.47% respectively, (Table 5.6). However, a relatively lower percentage removal was observed for the Seymour WWTP ranging from 27.29 to 93.74%. All the PAEs were effectively removed from the aqueous phase of the Adelaide and Alice wastewater treatment plants. The removal efficiency of DBP in all the WWTPs was the highest ranging from 93.84 to 97.37% and 93.74 to 99.47% at the secondary and final effluent, respectively for all the plants. Similarly, relatively high removal performance was also achieved with BBP in Adelaide (84.88%) and, Alice (87.23%) but below average in Seymour plant (42.53%).

Table 5.6 Removal efficiency of different WWTPs for phthalates (%)

	Adelaide		**Alice**		**Seymour**	
	S.E.	**F.E.**	**S.E.**	**F.E.**	**S.E.**	**F.E.**
DMP	77.76	74.26	61.89	77.61	30.27	27.29
DEP	85.88	73.58	63.28	80.60	46.90	32.95
DBP	94.53	95.45	97.37	99.47	93.84	93.74
BBP	85.30	84.88	71.90	87.23	38.47	42.53
DEHP	76.38	67.99	66.67	83.94	38.44	35.98
DOP	75.86	75.09	85.91	89.80	24.90	34.22

*S.E. = Secondary Effluent; F.E.= Final Effluent; DMP = dimethyl phthalate; DEP = diethyl phthalate;
DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate.

DEHP is the only phthalate compound with relatively low removal efficiency in all the treatment plants. It varied between 35.98% in Seymour, 67.99% in Adelaide but relatively higher in the Alice plant (83.94%) Table 5.6. This is consistent with results from many countries across the globe (Dargnat *et al.*, 2009; Clara *et al.*, 2010). PAEs with a shorter-chain such as DMP, DEP, and DBP are more easily degraded than longer-chain PAEs like

DOP and DEHP. Similarly, branch chain PAEs such as BBP exhibit lower degradation efficiency than straight-chain PAEs, such as DOP and DBP (Gao *et al.*, 2014).

There was no special advance treatment technology attached to these WWTPs compared to other WWTPs in advanced countries such as in Finland, Canada and Denmark (Fauser *et al.*, 2003; Marttinen *et al.*, 2003; Fernandez *et al.*, 2007; Dargnat *et al.*, 2009). The result is consistent with the conclusion made by Vogelsang *et al.*, (2006) when they compared different treatment devices; that DEHP was not efficiently removed by biological activity, unlike DEP, which is always eliminated. In a similar vein, the study carried out by Klöpffer, (1996) in Germany also acknowledged DEHP as a compound that is persistent with a high resistance to biodegradation.

Adelaide and Alice WWTPs performed relatively better than Seymour with respect to PAE removal efficiency. There was no notable difference in removal performance among the WWTPs for PAE at the secondary and final effluent. Post Hoc Tests revealed that the difference in concentration in the secondary and final effluents is not significant at $p > 0.05$. In terms of treatment plant performance, there was a significant difference between the Alice and Seymour plants at $p < 0.05$ but no substantial variation was found between Alice and Adelaide. This implies that removal performance was in the order Alice > Adelaide > Seymour.

The lower removal performance for PAEs recorded for Seymour WWTPs may be attributed to the number of clarification tank(s) which distinguished the two main existing activated sludge systems. The single tank technology which is used at the Seymour WWTP has no separate clarification tank unlike the continuous flow systems with a separate clarification tank referred to as secondary clarification (Wett and Buchauer, 2003).

In the study, an attempt was also made to determine the relationships between total PAE concentrations across the sampling points and the total organic load as total suspended solids in mg/L (TSS) and turbidity (Tables 5.7 and 5.8). There was a significant decrease in TSS and turbidity as the wastewater was passed through the treatment processes and as it got to the secondary and the final effluent (Table 5.7). The percentage reduction for turbidity at Adelaide, Alice and Seymour are 97.4, 96.75 and 72.8%, respectively. The TSS values are 97.74, 96.26, and 80.54% for Adelaide, Alice and Seymour respectively. It was obvious that the least percentage reduction was observed at the Seymour treatment plant. A high significant correlation $p < 0.001$ was found between TSS and all the PAEs except DBP, which was significant at $p < 0.05$. Similarly, a significant correlation at $p < 0.05$ was observed in the same manner with turbidity and all the PAEs except DBP. Likewise, there was a strong correlation between the removal of TSS and PAE across the sampling points as depicted in Table 5.8. This perhaps, suggests that the removal of PAEs from WWTPs is largely associated with the magnitude of settled particles to sludge which is achieved by the number of respective clarifiers existing in the treatment plants (Dargnat *et al.*, 2009; Clara *et al.*, 2010). This also indicates the role of adsorption on settling particles and centrifuged sludge. Thus, the relatively low performance removal efficiency observed from the results obtained at Seymour WWTP could be connected to its limited settling points in comparison with others. It is a small WWTP serving a smaller community with a lower population in a more remote area than the others. The removal efficiency of WWTPs from a global point of view is largely linked to the different treatment process characteristics. However, volatilization losses mostly considered for lighter compounds occur in the aeration basin and during sludge dehydration (Byrns, 2001; Dargnat *et al.*, 2009).

Table 5.7 Variations in physicochemical properties of wastewater samples collected from the influent and effluent of the WWTP.

	Adelaide			**Alice**			**Seymour**		
	Influent	**F. effluent**	**Red.(%)**	**Influent**	**F. effluent**	**%Red.**	**Influent**	**F .effluent**	**Red.(%)**
TDS mg/L	308.8 ± 20.3	265.2 ± 9.9	14.11	196.6 ± 12.3	147.2 ± 5.1	25.15	227.8 ± 30.0	100.3 ± 35.9	55.97
Turbidity (NTU)	560.3 ± 135	14.6 ± 5.5	97.4	547.67± 136	17.8 ± 6.9	96.75	266.9 ± 62.4	72.5 ± 45.2	72.8
TSS mg/L	195.1 ± 27.3	4.4 ± 1.0	97.74	179.9 ± 36.5	6.7 ± 2.6	96.26	79.7 ± 13.0	15.5 ± 6.4	80.54

*TDS = Total dissolved solids; TSS = Total suspended solids, F. effluent = Final effluent, Red = reduction

Table 5.8 Pearson correlations of physicochemical properties (TDS, TUR, TSS) with phthalate esters.

Correlations									
	DMP	DEP	DBP	BBP	DEHP	DOP	TDS	TUR	TSS
DMP	1								
DEP	0.938^{**}	1							
DBP	0.901^{**}	0.741^{*}	1						
BBP	0.897^{**}	0.969^{**}	0.695^{*}	1					
DEHP	0.921^{**}	0.961^{**}	0.778^{*}	0.897^{**}	1				
DOP	0.959^{**}	0.867^{**}	0.971^{**}	0.832^{**}	0.895^{**}	1*-			
TDS	0.254	0.505	0.120	0.540	0.422	0.246	1		
TUR	**0.726^{*}**	**0.784^{*}**	0.575	**0.750^{*}**	**0.838^{**}**	**0.704^{*}**	0.193	1	
TSS	**0.897^{**}**	**0.968^{**}**	**0.708^{*}**	**0.954^{**}**	**0.965^{**}**	**0.854^{**}**	**0.456**	**0.854^{**}**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

*TDS- Total dissolved solids; TUR- Turbidity; TSS- Total suspended solids

5.3.5. Phthalate in sludge sample

PAEs in dewatered sludge of all the WWTPs are presented in Table 5.9. The six priority PAEs were detected in Adelaide, Alice and Seymour with a total concentration of 334.30, 1948 and 1319 µg/g d.w. (dry weight) respectively. The PAEs distribution profile displayed DEHP as the most concentrated phthalate in Adelaide with a concentration of 120.5 ± 46.5 µg/g d.w. and DBP as the highest in Alice and Seymour with concentration of 1094 ± 89.32 µg/g d.w. and 429.67 ± 43.66 µg/g d.w. respectively. DBP, DEHP, BBP and DOP are the most abundant compounds in the sludge samples in that order for all the treatment plants.

Table 5.9 Phthalates concentration in sludge samples.

	Adelaide			**Alice**			**Seymour**		
PAEs	Sludge in μg/g dw			Sludge in μg/g dw			Sludge in μg/g dw		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
DMP	1.08 ± 0.45	0.31	1.86	6.10 ± 0.01	6.10	6.10	4.40 ± 0.76	2.27	5.71
DEP	4.84 ± 0.33	4.27	5.41	3.44 ± 0.03	3.44	3.45	5.82 ± 1.93	2.09	11.15
DBP	27.99 ± 13.70	4.27	51.72	1094 ± 89.32	939.16	1249	429.7 ± 43.66	311.38	519.47
BBP	76.36 ± 29.10	25.96	126.76	449.36 ± 99.56	276.91	621.81	383.0 ± 21.42	338.21	440.74
DEHP	120.48 ± 46.49	39.96	200.99	311.68 ± 23.68	270.66	352.70	353.8 ± 48.52	246.04	481.27
DOP	103.55 ± 34.59	43.64	163.46	83.45 ± 6.62	71.98	94.92	142.78 ± 27.15	82.82	214.30
Total PAEs	334.30			1947.90			1319.43		

A slight deviation from this trend was observed at the Adelaide WWTP where DBP came as fourth most abundant PAE with a concentration of 27.99 ± 13.70 µg/g d.w. A higher concentration for DBP in sludge from this plant (Adelaide) was expected considering the high level of removal efficiency exhibited by the plant for DBP. However, it is possible that the PAEs got degraded during activated sludge treatment in the plant. This was also evident in the low (total) concentration of PAEs recorded for this plant in comparison with others. Structural changes of suspended organic matter through degradation and hydrolysis could take place during the biological process thereby forcing the adsorbed organic PAEs to be released with the effluent (Gao *et al.*, 2014).

Another significant concern here, in terms of the abundance of PAEs is DOP. It is a compound that was scarcely detected in wastewater and is a minor phthalate in industrial use and production. Ellington (1999) explained that it has the tendency to get adsorbed on particulate matter because of its high octanol water partition coefficient ($\log K_{ow}$) as shown in Table 5.2. Byrns (2001) also showed that compounds with high $\log K_{ow} \geq 4$, have the tendency to be adsorbed onto particulate matter (Dargnat *et al.*, 2009). DMP and DEP are of minor concern in any of the treatment plants as the concentrations were lower than 10 µg/g d,w.

This is in accordance with the octanol water partition coefficient ($\log K_{ow}$) of these PAEs which is lower than 4 (Tan, *et al.*, 2007; Dargnat *et al.*, 2009).

The total concentrations of sludge recorded in this study are relatively higher than that in some results in European countries (Marttinen *et al.*, 2003; Dargnat *et al.*, 2009). The concentration for the dominant PAEs in this study, which varied between 103 and 1094 µg/g d.w (DBP, BBP, DEHP and DOP) in the activated sludge were considerably above the 100 µg/g d.w recommended by the European Union for agriculture use (EU legislation 86/278/EEC). Among fertilizers used for the purpose of agriculture, sewage sludge is considered as carrying the highest load of organic contaminants. This calls for concern, especially in the event of the sludge being used in enriching soil for farming. Since agriculture still remains crucial in food security for the predominantly rural poor who constitute up to 40% of the population in South Africa, serious attention has to be paid to the quality of sludge generated from WWTPs for farming. However, it is also important to note that these results are much lower than the report of Olujimi *et al.* (2014) documented for the upstream and downstream sediment of an oxidation pond in Nigeria. As at the time of this report, we could not find other information on phthalate in WWTP sludge in South Africa for comparison.

5.3.6. Impact of final effluent on receiving water quality

The effluent discharge from WWTPs has been identified among the main sources of phthalate and other micro-pollutants in the aquatic environment. Therefore, it is imperative to examine and monitor the amount of these pollutants that find its way into receiving water for the purpose of preserving a safe aquatic environment. The impact of the final effluent discharged from the three WWTPs which adopted activated sludge technology on the quality of the receiving watershed was also assessed in this present study. This was conducted by comparing the concentration of PAEs in the final discharged effluent with the concentration in the main stream. The variation of the total PAEs and separate concentration of the six PAEs under investigation upstream and downstream of discharge point of the WWTPs are shown in Tables 5.10, 5.11, and 5.12.

Table 5.10 Concentrations of PAEs at Adelaide upstream and downstream.

PAEs	**Upstream**				**Downstream**				**Final Effluent**			
	Mean	**Max**	**Min**	**FD (%)**	**Mean**	**Max**	**Min**	**FD (%)**	**Mean**	**Max**	**Min**	**FD (%)**
DMP	1.00 ± 0.20	1.73	0.39	100	1.12 ± 0.18	1.73	0.59	100	1.48 ± 0.29	2.37	0.62	100
DEP	1.58 ± 0.45	2.83	0.03	100	1.79 ± 0.46	3.29	0.02	100	3.39 ± 1.25	8.89	0.17	100
DBP	3.87 ± 0.91	7.52	ND	83	6.29 ± 2.16	16.04	1.07	100	8.88 ± 1.12	26.47	ND	83
BBP	2.23 ± 0.54	3.64	ND	83	2.34 ± 0.68	4.96	ND	83	5.10 ± 0.71	13.73	ND	83
DEHP	6.73 ± 2.27	16.58	1.11	100	6.87 ± 2.02	13.40	0.98	100	9.23 ± 2.34	18.25	2.05	100
DOP	2.96 ± 0.67	4.24	ND	50	2.22 ± 0.79	4.77	ND	67	3.17 ± 0.37	4.15	ND	50
Total PAEs	**18.36**				**20.63**				**31.24**			

* ND= Not detected (Below limit of detection 0.102 – 0.586 µg/L); FD= Frequency of detection; SE = standard error; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate

Table 5.11 Concentrations of PAEs at Alice upstream and downstream.

PAEs	Upstream				Downstream				Final Effluent			
	Mean ± SE	**Max**	**Min**	**FD (%)**	**Mean** ± SE	**Max**	**Min**	**FD (%)**	**Mean** ± SE	**Max**	**Min**	**FD (%)**
DMP	1.26 ± 0.20	2.13	0.65	100	1.51 ± 0.37	3.14	0.56	100	1.87 ± 0.57	4.57	0.64	100
DEP	1.56 ± 0.58	3.76	0.04	100	1.80 ± 0.51	3.08	0.10	100	2.44 ± 0.80	5.97	0.12	100
DBP	6.86 ± 1.83	16.80	1.06	100	8.74 ± 1.01	32.16	1.17	100	6.08 ± 0.80	22.34	1.17	100
BBP	2.32 ± 0.62	4.23	0.82	100	2.98 ± 1.19	7.23	0.66	100	3.52 ± 1.33	8.75	0.75	100
DEHP	4.79 ± 1.39	11.18	2.27	100	6.32 ± 1.80	11.80	1.01	100	5.41 ± 1.02	14.82	1.73	100
DOP	1.75 ± 0.52	3.07	ND	67	2.36 ± 0.96	5.32	ND	67	2.63 ± 1.21	6.02	ND	50
Total PAEs	**18.54**				**23.72**				**21.96**			

* ND= Not detected (Below limit of detection 0.102 – 0.586 **µg/L**), FD= Frequency of detection;DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate

Table 5.12 Concentrations of PAEs at Seymour upstream and downstream.

PAEs	**Upstream**				**Downstream**				**Final Effluent**			
	Mean ± SE	Max	Min	FD (%)	Mean ± SE	Max	Min	FD (%)	Mean ± SE	Max	Min	FD (%)
DMP	1.08 ± 0.20	1.90	ND	83	1.19 ± 0.20	1.78	ND	83	2.22 ± 0.73	4.10	ND	75
DEP	2.61± 0.87	6.09	ND	83	3.86 ± 1.04	7.72	ND	83	4.35 ± 0.42	5.08	ND	75
DBP	4.64 ± 1.20	8.55	1.24	100	22.70 ± 18.55	115.3	1.13	100	4.90 ± 1.23	7.68	1.05	100
BBP	3.38 ± 1.74	9.69	ND	66	3.30 ± 1.15	5.81	ND	67	4.14 ± 0.41	13.84	0.00	75
DEHP	5.12 ± 2.57	17.53	0.69	100	6.64 ± 2.20	12.48	1.14	100	13.27 ± 4.84	24.91	2.42	100
DOP	2.53 ± 0.96	5.91	ND	67	3.34 ± 1.33	6.70	ND	67	4.19 ± 1.31	7.57	ND	75
Total PAEs	**19.36**				**41.03**				**33.07**			

* ND= Not detected (Below limit of detection 0.102 – 0.586 **μg/L**), FD= Frequency of detection;DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate

Throughout the sampling periods, all the six PAEs (DMP, DEP, DBP, BBP, DEHP and DOP) were detected both in the upstream and downstream of all the treatment plants under investigation, but with varying detection frequencies. This further confirms the ubiquitous nature of phthalates in the environment. In all the treatment plants, DEHP was the most frequently detected both in the upstream and the downstream of the discharge point while DOP had the least frequency of detection. This finding was similar to other studies carried out by some researchers (Dargnat *et al.*, 2009; Clara *et al.*, 2010; Gao *et al.*, 2014). The total phthalate concentrations in the upstream for the three plants were notably lower than what were detected in the downstream, indicating the influence of the WWTPs effluent on the receiving water. The total PAE concentrations at the upstream of the Adelaide, Alice, and Seymour WWTPs were 18.4, 18.5, and 19.4 µgL^{-1}, respectively, which are lower than the total concentration in the downstream. The increase in concentration of the total PAEs in the downstream of Adelaide (20.6 µgL^{-1}) and Alice (23.7 µgL^{-1}) was not as pronounced as what was detected at Seymour (41.0 µgL^{-1}). This may further justify the relatively lower removal efficiency initially recorded for this treatment plant. During the sampling periods, there were periods when the treatment plant suffered a pump breakdown which also reduced the level of treatment received by the upcoming influent before it was discharged into the receiving water. It could be said that the final effluent discharged from the WWTPs relatively increased the total concentration of PAEs at the receiving watershed. However, these concentrations were not as high as the report of Olujimi *et al.* (2012) on the concentration of PAEs from five WWTPs in Cape Town, South Africa.

At the Adelaide treatment plant, DEHP and DBP had the dominant concentration both in the upstream and downstream of the discharging point with concentration ranging from 0.98 - 16.58 µgL^{-1} and from below limit of detection (LOD) to 16.04 µgL^{-1}, respectively. The same trend was also observed for both Alice and Seymour treatment plants but with higher concentrations. This observation is similar to many other results recorded in some parts of Europe and other parts of Africa (Fatoki and Noma, 2002; Dargnat *et al.*, 2009; Olujimi *et al.*, 2012; Gao *et al.*, 2014). It is worth mentioning, a dramatic increase in DBP concentration (115.3 µg/L) in the downstream of the Seymour treatment plant which is a cause of major concern (Figure 5.10c). This could be attributed to the frequent pump breakdowns which led to redirection of the influent to a temporal arrangement of pit sedimentation/filtration process. In Figure 5.10a, there was a clear indication that the final effluent discharged from the Adelaide treatment plant contributed to the concentration of the six PAEs in the downstream. At the Alice WWTP (Figure 5.10b), DBP and DEHP concentration were higher downstream than what was generated in the treatment plant as final effluent. This may be the result an accumulation of the compounds from the effluent over time. However, an increase in the concentration of PAE pollution in the downstream could also be influenced by other point sources having PAE concentrations higher than what was released from the treatment plant.

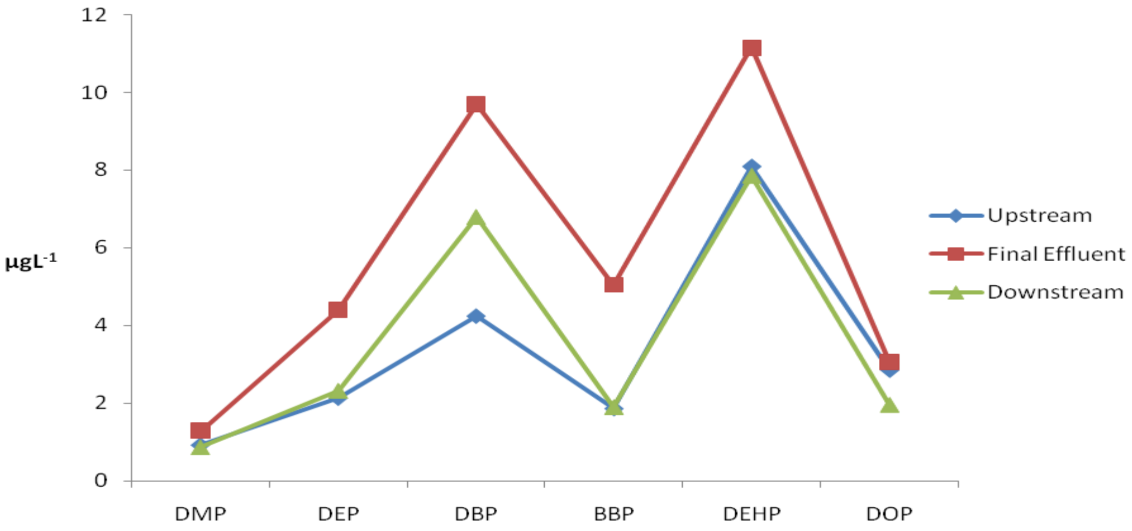


Figure 5.10a Impact of final effluent at Adelaide WWTP on receiving water bodies (Cobra river).

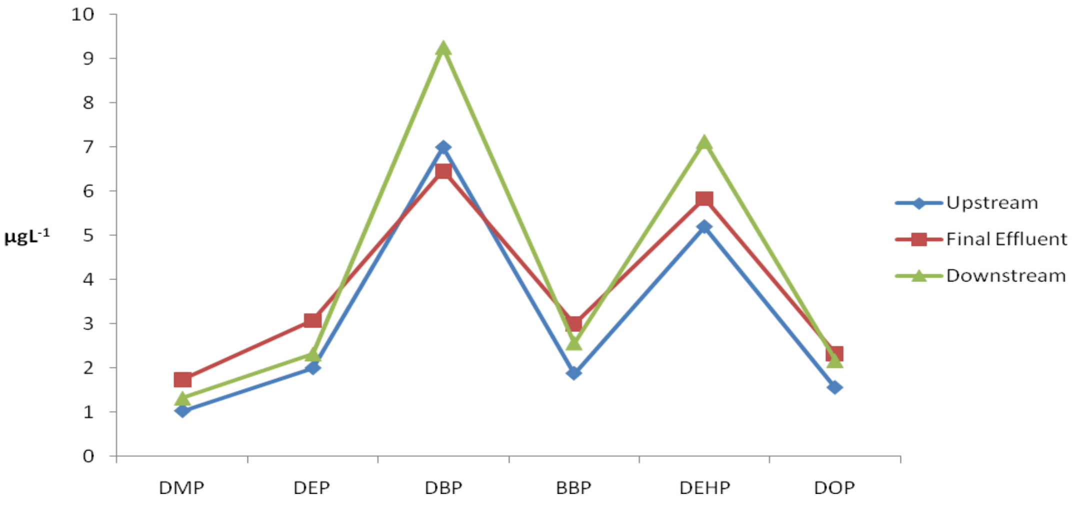


Figure 5.10b Impact of final effluent at Alice WWTP on receiving water bodies (Tyhume river).

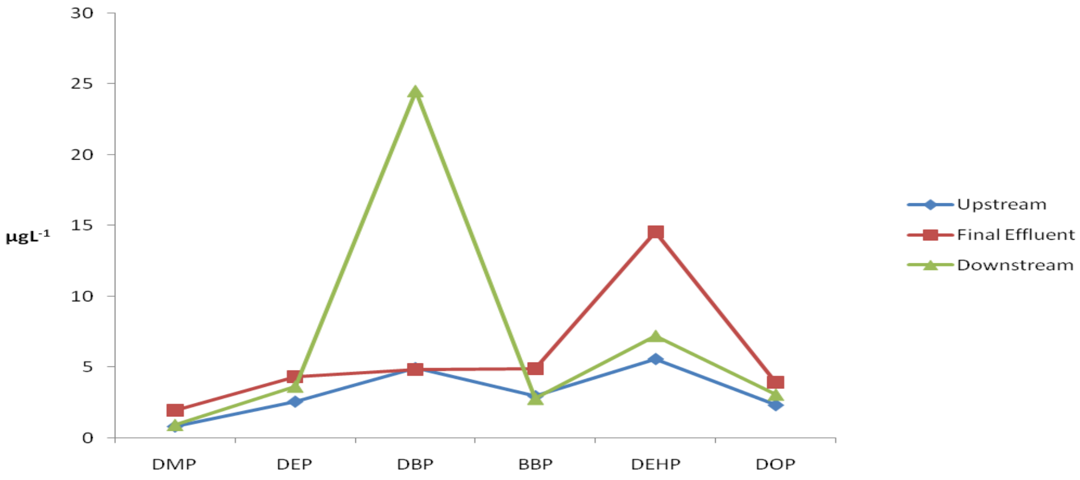


Figure 5.10c Impact of final effluent at Seymour WWTP on receiving water bodies (Gesi river).

At Seymour WWTP Figure 5.10c, the concentration of PAEs discharged in the final effluent of the WWTP was higher than what was detected in the upstream and downstream of the discharging point. Generally, the concentrations of PAEs detected in the entire receiving watershed connected to the three investigated treatment plants were above the EQU of 1.3 μgL^{-1} set by the European Union for DEHP in surface water and the above 3 criteria standard set by USEPA for the protection of aquatic lives. Apart from DEHP and DBP, other PAEs have concentration lower than 10 μgL^{-1}. Nevertheless, PAEs in this study are considerably lower compared to similar studies carried out in Cape Town South Africa, and were not as high as PAEs detected in some selected Rivers and dams in the Eastern Cape (Fatoki and Noma, 2002; Fatoki *et al.,* 2010). The high concentrations of PAEs in these other areas in South Africa are attributed largely to their discharge from industries using them. In this present study, the wastes generated into the sewer in the selected study areas were largely domestic and agricultural runoffs. PAEs are not persistent in the outdoor environment as they are subjected to biodegradation, photo-degradation, and anaerobic degradation but the outdoor concentrations are much higher in urban and suburban areas than in rural and remote areas (Rudel and Perovich, 2009; Olujimi *et al.*, 2012).

5.4. Conclusions

The provision of adequate information on micro-pollutants (EDCs) that are being continuously discharged from our wastewater treatment plants and their fate in the environment will no doubt provide a convenient platform on which an ensured protection plan for our water resources and ecosystem can be based. The six selected phthalate esters assessed in this study were detected in all the raw and treated wastewater, sludge and receiving water bodies for all the wastewater treatment plants investigated in Amathole

Municipality with DBP, DEHP and BBP being the prominent compounds. The concentrations detected in the final effluent and sludge samples exceeded acceptable levels allowed for a safe aquatic environment by the European Union and the US EPA legislation as there was no stipulated legislation in South Africa and other Africa countries. The relative concentrations found in these environments are largely influenced by the level of anthropogenic activities around the areas, which in turn, is dependent on population. This was consistent with similar findings in other parts of the world and Africa. This study also found it possible that other point sources for these micro-pollutants to the receiving water bodies may exist apart from WWTPs discharged effluents. It is therefore imperative for future research to identify other point source(s) of contaminants to these receiving water bodies with a view to controlling the environmental pollution and reducing the health hazard posed to those dependent on these waters. With the increasing diversity in the chemicals that are being released into our environment, WWTPs still remain a panacea and hope for the aquatic life and our human water resources. As a result, it is worth of considering, the adequacy and proper management of these WWTPs especially in Africa where industrial and domestic wastewaters are still being discharged without treatment.

References

Abdel daiem, M. M., Rivera-Utrilla, J., Ocampo-Pérez, R., Méndez-Díaz, J. D. and Sánchez-Polo, M. (2012) 'Environmental impact of phthalic acid esters and their removal from water and sediments by different technologies - A review', *Journal of Environmental Management,* 109, 164–178. doi: 10.1016/j.jenvman.2012.05.014.

Adeniyi, A., Dayomi, M., Siebe, P. and Okedeyi, O. (2008) 'An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo Province, South Africa.', *Chemistry Central Journal*, 2, 1-9. doi: 10.1186/1752-153X-2-9.

ATSDR. (2000) *Toxicological profile for di(2-ethylhexyl)phthalate (DEHP). Atlanta, GA: ATSDR,.*

Becker, K., Seiwert, M., Angerer, J., Heger, W., Koch, H. M., Nagorka, R., Roßkamp, E., Schlüter, C., Seifert, B. and Ullrich, D. (2004) 'DEHP metabolites in urine of children and DEHP in house dust.', *International Journal of Hygiene and Environmental Health*, 20, 409–417.

Blignaut, J. and Van Heerden, J. (2009) 'The impact of water scarcity on economic development initiatives', *Water SA*, 35(4), 415–420.

Byrns, G. (2001) 'The fate of xenobiotic organic compounds in wastewater treatment plants.', *Water Research*, 35(25), 23–33.

Caruso, A. and Santoro, M. (2014) 'Detection of Organochlorine Pesticides by GC-ECD Following U.S. EPA Method 8081', 4.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C. and Chovanec, A. (2010) Occurrence of phthalates in surface runoff , untreated and treated wastewater and fate during wastewater treatment', *Chemosphere*, 78(9), 1078–1084. doi: 10.1016/j.chemosphere.2009.12.052.

Dargnat, C., Teil, M. J., Chevreuil, M. and Blanchard, M. (2009) 'Phthalate removal throughout wastewater treatment plant. Case study of Marne Aval station (France)', *Science of the Total Environment*, 407, 1235–1244. doi: 10.1016/j.scitotenv.2008.10.027.

DWAF, D. of W. A. (2012) *Chapter 5 – Eastern Cape Province, Green Drop Report* 460

Ellington, J. (1999) 'Octanol/water partition coefficient and water solubilities of phthalate esters.', *Journal of Chemical & Engineering Data*, 44, 1414–8.

ESIS (2009) *European Chemical Substances Information System. <http:// Ecb.jrc.ec.europa.eu/esis/> (22.01.17).*

EU (2008) 'European Union Risk Assessment Report: Bis(2-ethylhexyl)phthalate (DEHP).', *European Commission - Joint research Centre. Luxembourg*, 80, 588.

EU-RAR. (2007) *European Union Risk Assessment Report on Benzyl Butyl Phthalate (BBP)., Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau, 3rd Priority List, vol. 76. <http://ecb.jrc.ec.europa.eu/DOCUMENTS/ Existing-Chemicals/RISK_ASSESSMENT/RE accessed 11/01/2017.*

EU-RAR. (2008) *European Union Risk Assessment Report on Bis(2-ethylhexyl) Phthalate (DEHP)., Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau, 2nd Priority List, vol. 80. <http://ecb.jrc. Ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/ Dehpreport042.pdf>. accessed 11/01/2017.*

Fatoki, O., Olujimi1, O., Odendaal, J. and Genthe., B. (2012) 'Health Risk Assessment of Plasticizer in Wastewater Effluents and Receiving Freshwater Systems, Recent Advances in

Plasticizers, Dr. Mohammad Luqman (Ed.), ISBN: 978-953-51-0363-9, InT ech, Available from: http://www.intechopen.com/books/recent-a', *InTechOpen*.

Fatoki, O. S., Bornman, M., Ravandhalala, L., Chimuka, L., Genthe, B. and Adeniyi, A. (2010) 'Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects', *Water SA*, 36(1), 117–126. doi: 10.4314/wsa.v36i1.50916.

Fatoki, O. S. and Noma, A. (2002) 'Solid phase extraction method for selective determination of phthalate esters in the aquatic environment.', *Water, Air and Soil Pollution*, 140, 85–98.

Fauser, P., Vikelsoe, J., Sorensen, P. and Carlsen, L. (2003) 'Phthalates Res, nonylphenols and LAS in an alternately operated wastewater treatment plant — fate modelling based on measured concentrations in wastewater and sludge.', *Water,* 37, 1288–1295.

Fernandez, M. P., Ikonomou, M. G., and Buchanan, I. (2007) 'An assessment of estrogenic organic contaminants in Canadian wastewaters.', *Science of the Total Environment.*, 373, 250–69.

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J. and Wenzel, A. (2002) 'Occurrence of phthalates and bisphenol A and F in the environment.', *Water Research.*, 36, 1429– 1438.

Gao, D., Li, Z., Wen, Z. and Ren, N. (2014) 'Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China', *Chemosphere,* 95, 24–32. doi: 10.1016/j.chemosphere.2013.08.009.

Gao, D. W. and Wen, Z. D. (2016) 'Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes', *Science of the Total Environment*. 541, 986–1001. doi: 10.1016/j.scitotenv.2015.09.148.

Gasperi, J., Garnaud, S., Rocher, V. and R., M. (2008) 'Priority pollutants in wastewater and combined sewer overflow', *Science of the Total Environment*, 407(1), 263–272.

Guo, Y., Zhang, Z., Liu, L., Li, Y., Ren, N. and Kannan, K. (2012) 'Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure.', *Journal of Agricultural and Food Chemistry*, 60, 6913–6919.

Hileman, B. (2007) 'California bans phthalates in toys for children', *Chemical Engineering News*, 85(43), 12. doi: 10.1021/cen-v085n043.p012a.

Kumar, B., Verma, V. K., Sharma, C. S. and Akolkar, A. B. (2014) 'Quick and easy method for determination of priority phenolic compounds in water and wastewater', *Journal of Xenobiotics*, 4(1), 46–52. doi: 10.4081/xeno.2014.4680.

Klöpffer, W. (1996) 'Environmental hazard assessment of chemicals and products. Part V. Anthropogenic chemicals in sewage sludge.', *Chemosphere*, 33, 1067–1081.

Kotowska, U., Garbowska, K., and Isidorov, V. (2006) 'Distribution coefficients of phthalates between absorption fiber and water and its using in quantitative analysis.', *Analytica Chimica Acta*, 560,110–117.

Liu, Y., Chen, Z., and Shen, J. (2013) 'Occurrence and removal characteristics of phthalate esters from typical water sources in northeast China', *Journal of Analytical Methods in Chemistry*, 2013. doi: 10.1155/2013/419349.

Mackintosh, C. E., Maldonado, J. A., Ikonomou, M. G. and Gobas, F. A. P. C. (2006) 'Sorption of 40, phthalate esters and PCBs in a marine ecosystem.', *Environmental Science & Technology*, 40(11), 3481–3488.

Malley, Z. J. U., Taeb, M., Matsumoto, T. and Takeya, H. (2009) 'Environmental sustainability and water availability: Analyses of the scarcity and improvement opportunities in the Usangu plain, Tanzania.’, *Physics and Chemistry of the Earth*, 34, 3–13.

Marcucci, M. and Tognotti, L. (2002) ‘Reuse of wastewater for industrial needs: the Pontedera case.’, *Resources, Conservation and Recycling*, 34, 249–259.

Marttinen, S., Kettunen, R., Sormunen, K. and Rintala, J. (2003) ‘Removal of bis Res, (2-ethylhexyl) phtalate at a sewage treatment plant.’, *Water*, 37, 1385–93.

Munch, J. W. (US. E. (2000) *Determination of Phenols in drinking water by solid phase extraction and capillary column gas chromatography/mass spectrometry (GC/MS)., U.S. Environmental Protection Agency, Washington D.C. EPA/815/R-00/014*.

Oliver, R., May, E., and Williams, J. (2005) ‘The occurrence and removal of phthalates in a trickle filter STW.’, *Water Research.*, 39, 4436–4444.

Olujimi, O. O., Fatoki, O. S., Odendaal, J. P. and Daso, A. P. (2012) ‘Chemical monitoring and temporal variation in levels of endocrine disrupting chemicals (priority phenols and phthalate esters) from selected wastewater treatment plant and freshwater systems in Republic of South Africa’, *Microchemical Journal.*, 101, 11–23. doi: 10.1016/j.microc.2011.09.011.

Olujimi, O. O., Aroyeun, O. A., Akinhanmi, T. F. and Arowolo, T. A. (2014) ‘Occurrence and Concentration of Phthalate Esters in River Sediment Around Covenant Oxidation Ponds, Covenant University, Ota, Ogun State’, *International Journal of Environment and Sustainability (IJES),* 3(2), 1–7. Available at: https://www.sciencetarget.com/Journal/index.php/IJES/article/view/517.

Park, M. A., Hwang, K. A., Lee, H. R., Yi, B. R., Jeung, E. B. and Choi, K. C. (2012) ‘Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. *Molecular Medicine Reports*, 5(3), 761–766. http://dx.doi.org/10.3892/mmr.2011.712.

Peijnenburg, W. and Struijs, J. (2006) ‘Occurrence of phthalate esters in the environment of the Netherlands.’, *Ecotoxicology and Environmental Safety*, 63(2), 204–215.

Qiao, G., Zhao, L., and Klein, K. K. (2009) ‘Water user associations in Inner Mongolia: Factors that influence farmers to join.’, *Agricultural Water Management*, 96, 822–830.

Roslev, P., Vorkamp, K., Aarup, J., Frederiksen, K. and Nielsen, P. H. (2007) ‘Degradation of phthalate esters in an activated sludge wastewater treatment plant’, *Water Research*, 41(5), 969–976. doi: 10.1016/j.watres.2006.11.049.

Rudel, R. and Perovich, L. (2009) ‘“Endocrine disrupting chemicals in indoor and outdoor air”’, *Atmospheric Environment*, 43(1), 170–181.

Sablayrolles, C., Montr, M., Benanou, D., Patria, L. and Treilhou, M. (2005) ‘Development and validation of methods for the trace determination of phthalates in sludge and vegetables’, *Journal of Chromatography A*, 1072, 233–242. doi: 10.1016/j.chroma.2005.02.074.

Sánchez-Avila, J., Bonet, J., Velasco, G. and Lacorte, S. (2009) ‘Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant’, *Science of the Total Environment*, 407(13), 4157–4167. doi: 10.1016/j.scitotenv.2009.03.016.

Tan, B., Hawker, D., Müller, J., Leush, F., Tremblay, L. and Chapman, H. (2007) ‘Modelling of the fate of selected endocrine disruptors in a municipal wastewater treatment plant in South East Queensland, Australia.’, *Chemosphere*, 69, 644–54.

U C T (2000) *EPA Method 8270 : Determination of Semi-volatile Organic Compounds in Water using Solid Phase Extraction and GC / MS SPE Procedure :*

Vogelsang, C., Grung, M., Jantsch, T., Tollefsen, K. and Liltved, H. (2006) 'Occurrence and removal of selected organic micropollutants at mechanical, chemical and advanced wastewater treatment plants in Norway.', *Water Research*, 40, 3559–3570.

Wett, B. and Buchauer, K. (2003) 'Comparison of aerobic and anaerobic technologies for domestic wastewater treatment based on case studies in Latin America', *Seminario Problemas y Soluciones Ambientales, Aguas Residuales y Residuos Solidos*, 1–14. Available at: http://www.araconsult.at/download/literature/bogota_wett_lg.pdf.

Xue, W. C., Wu, C. Y., Xiao, K., Huang, X., Zhou, H. D., Tsuno, H. and Tanaka, H. (2010) 'Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation', *Water Research.*, 44, 5999–6010.

Yang, M., Park, M. S., and Lee, H. (2006) 'Endocrine Disrupting Chemicals: Human Exposure and Health Risks', *Journal of Environmental Science and Health, Part C*, 24(2), 183–224. doi: 10.1080/10590500600936474.

CHAPTER SIX

Phthalates removal efficiency in different wastewater treatment technology adopted in the selected WWTPs in the Eastern Cape, South Africa

Abstract

The removal capacity of different wastewater treatment plants (WWTPs) technologies adopted in rural areas for phthalate was investigated in the Eastern Cape, South Africa. Wastewater samples collected from three WWTPs which use activated sludge (AS), trickling filter (TF) and oxidation pond (OP) technology were extracted using the solid-phase extraction (SPE) method followed by gas chromatography-mass spectrometry (GC-MS) analysis. The six selected phthalate esters (PAEs) dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP), di(2-ethyl hexyl) phthalate (DEHP), and di-n-octyl phthalate (DOP) were detected in all the influent and effluent samples of all the WWTPs while DBP was the most abundant compound in the influent with a maximum detection of 2497 μgL^{-1} followed by DEHP and BBP. There was a notable reduction in the amount of all the PAEs in the final effluent of AS in Alice, TF in Berlin and OP technology in Bedford with a removal efficiency that varied between 77-99%, 76-98%, and 61-98%, respectively. The high significant correlation of PAEs removal with total suspended solids (TSS) and turbidity suggests that the removal performance proceeded more on adsorption on settling particles and sludge than on biodegradation. Concentration of all the compounds in the final effluent except for DEP and DMP which varied on the average 3.00-9.84 μgL^{-1} were above the USEPA criteria standard of 3 μgL^{-1} for DEHP in surface water. Similarly, DBP, DEHP and BBP were the prominent compounds in the sludge with average values 123-1094 µg/gd.w exceeding the European Union standard limit (100 µg/gd.w) for agricultural use. AS exhibited a more stable and better performance across the different seasons (except spring). As water remained the major source of human and aquatic life exposure to PAEs, pollution source control deserves a special attention to prevent the risk posed by these micro pollutants.

Key words; Wastewater treatment plants, phthalate esters, activated sludge, trickling filters, oxidation pond, sludge.

6.1. Introduction

In recent years, some chemicals have been implicated in endocrine system interference, resulting in negative effects on hormonal control and on the development of aquatic organisms, wildlife, and even human (Matsui, 2008). They are known to be mutagenic and carcinogenic in a number of human tissues, causing a shortened menstrual cycle, female breast cancer, hypospadias, cryptorchidism, and endometriosis (Becker *et al.*, 2004; Horn *et al.*, 2004; Balabanič *et al.*, 2011; Park *et al.*, 2012). Phthalate esters (PAEs) are among chemicals considered to be endocrine disrupting chemicals (EDCs) (Horn *et al.*, 2004) and the potential hazardous nature of these chemicals has increased the global attention on their environmental fate. This has also made the European Union place restrictions on the use of some PAEs (Becker *et al.*, 2004; Gao *et al.*, 2014). Some PAEs are known to be toxic to reproduction, impair development in aquatic animals (amphibians and crustaceans) and induce genetic mutation (EU-RAR, 2004; EU-RAR., 2007, 2008; Clara *et al.*, 2010). They are easily leached into the environment during manufacturing processes, uses, or decomposition of plastic because they are not chemically bound to the polymeric matrix in soft polyvinyl chloride (PVC) when applied as plasticizer (Dargnat *et al.*, 2009; Clara *et al.*, 2010).

The primary route of these PAEs (plasticizer) in the aquatic environment has been linked to the effluent and sludge discharged from wastewater treatment plants (WWTPs) (Kolpin *et al.*, 2002; Loraine and Pettigrove, 2006; Gao *et al.*, 2014). These traces of dangerous organic contaminants often escape the conventional wastewater treatment processes which are not designed to specifically degrade them. This has made the performance of WWTPs usually unsatisfactory for PAE removal (Nakada *et al.*, 2006; Xue *et al.*, 2010). Aquatic organisms are exposed to the escaped PAEs and consequently, they pose a hazard to the whole food chain (Kraigher *et al.*, 2008; Balabanici *et al.*, 2012). Sources of PAEs in municipal wastewater may include; domestic and industrial waste, atmospheric deposit or urban run-off.

Phthalate esters (PAEs) have been detected in natural water bodies, urban, and municipal water in concentrations that raise a great concern for the protection of the aquatic environment and conservation of drinking water resources especially in the rural areas (Löwenberg *et al.*, 2014). More so, the US Environmental protection agency (EPA) has classified six PAEs: (dimethyl (DMP), diethyl (DEP), di-butyl (DBP), benzyl-butyl (BBP) di(2-ethyylhexyl) (DEHP) and di-n-octyl (DOP) as priority pollutants (Liu *et al.*, 2013).

The primary essence of WWTPs, among other things, is to enable the disposal of domestic and industrial effluents without posing danger to human health and the ecosystem. The design of a WWTP and the selection of an appropriate technology is predicated on the nature and sources of the discharged water (ESCWA, 2003). The ease, with which WWTPs provide these essential services, provides a basis for standard measurements. Therefore, it is important that the performance record of a wide range WWTP technologies is made available (Pescod, 1992; ESCWA, 2003; Coskuner and Ozdemir, 2006). Recently, several studies have focused investigation on PAEs in influents, effluents and sludge but little did we know about the fate of PAEs throughout the entire process of WWTPs. Investigating the removal of these organic pollutants as they are passed through the WWTP processes is crucial to ecological and environmental health (Gao and Wen, 2016).

In some rural areas in South Africa, scarcity of potable water is still a serious problem for millions of people as a result of climate change which increases flooding and drought in many parts of the country (Jonathan, 2011). Water availability is being severely impacted by population growth and urbanization and the deteriorating quality of the existing water has also increased the problem of finding suitable and sustainable treatment technology (Jonathan, 2011).

Optimizing the existing treatment operations, or incorporating new specific treatment operations for micro-pollutant removal are additional processes adopted especially in developed countries to improve WWTP performance (Batt *et al.*, 2007; Cai *et al.*, 2012). Among the major factors influencing the removal of micro-pollutants in treatment plant facilities is the type of wastewater treatment technology adopted. Many past studies on the occurrence and removal of EDCs from WWTPs have focused mainly on large-scale treatment plants using the activated sludge process (Leech *et al.*, 2009; Qiang *et al.*, 2013). It is also important that attention needs to be focused on the removal of EDCs in other smaller scale treatment plants (that usually serve smaller communities) with technology other than activated sludge to assess their performance.

WWTPs are designed with many treatment processes and operated in order to replicate the natural treatment processes which reduce contaminant loads to a level that nature can handle (Ravi *et al.*, 2010). As a result, special attention on the environmental impacts of existing WWTPs is of paramount importance. The type of wastewater treatment technology adopted also plays a vital role among factors influencing PAE removal (Qiang *et al.*, 2013). Most of the previous investigations on the occurrence and removal of PAEs have been on large-scale WWTPs using activated sludge technology (Andersen *et al.*, 2003; Joss *et al.*, 2004). It is important for us to know the relative performance of other treatment technologies in relation to PAE removal, especially in the rural areas where communities still rely heavily on surface water for their domestic and agricultural use.

This present study focuses on the influence of different types of treatment technologies in rural areas on the removal of six selected PAEs; DMP, DEP, DBP, BBP, DEHP and DOP in municipal WWTPs in the Amathole and Buffalo District Municipalities in the Eastern Cape, South Africa.

6.2. Materials and Methods

6.2.1. Study area

Three wastewater treatment plants located in Alice, Bedford and Berlin in the Eastern Cape of South Africa were investigated for their PAEs removal efficiency. WWTPs in Alice and Bedford are both in the Amathole District Municipality and they have adopted activated sludge (AS) and oxidation ponds (OP) technology, respectively. Alice is a well-known town because of the presence of University of Fort Hare and has a land area of 9.85. Bedford on the other hand, is a town in the centre of the Eastern Cape with a total land area of 14.6 while Berlin is the only town in Buffalo District Municipality selected for this study. It has a total land area of about 38.22 and uses trickling filter (TF) as wastewater treatment technology. These are all treatment facilities serving small populations in the rural areas. Table 6.1, illustrates the geographical location, population equivalent and receiving river in all the selected WWTPs. During sampling period, the global position system was used to locate the position of the treatment plants and reference was made to the 2011 census results. Figure 6.1 also show the geographical location of the sampling sites and receiving water bodies. It shows the distinct distance of Amathole from Buffalo District Municipality.

Table 6.1; Brief Description of the selected Wastewater Treatment Plants.

Plants	Alice	Bedford	Berlin
Technology	Activated sludge	Oxidation pond	Biofilters, anaerobic digestion and sludge drying bed.
Design capacity (ML/d)	0.5	0.5	1.0
Geographical location	32º47.566'S and 26º 50'E	32º41.157'S and 26º 05'E	32º50.700'S and 27º 37'E
Population (2011)	15,143	8,770	3,048
Receiving River	Tyhume	No discharge	Tributary of Nahoon

Figure 6.1 Location of sampling sites and receiving watersheds at Amathole and Buffalo District Municipalities.

6.2.2. Reagents

Dimethyl phthalate (DMP) 98.0%, diethyl phthalate (DEP) 99.9%, di-n-butyl phthalate (DBP) 96.8%, benzyl butyl phthalate (BBP) 99.0%, di(2-ethyl hexyl) phthalate (DEHP) 99.6% and di-n-octyl phthalate (DOP) 99.1 %, were all from Accu Standard, Inc USA and were supplied in 100 mg each. Surrogate standard 2-fluorobiphenyl (2FB) was sourced from Restek, USA. A working standard (100 µg/mL) was prepared from the stock solution (2000 µg/mL) and stored under 4 ^{o}C in amber bottles. A standard working mixture of all the analytes was later prepared at 100 µg/mL also in methanol. Sodium thiosulphatepentahydrate, anhydrous sodium sulphate as well as high performance liquid chromatography (HPLC) grade dichloromethane, n-hexane, acetone, and methanol including glass wool were all from Darmstadt, Germany. Afrox gas, South Africa supplied the nitrogen gas used for drying, with

99.99% purity while double-distilled water was produced in the Department of chemistry laboratory at the University of Fort Hare. Solid-phase extraction cartridges (SPE) C18-U (strata) 1000 mg/6mL, were purchased from Phenomenex supplied by Separations, South Africa. Physical properties of the selected phthalates are shown in Table 6.2.

Table 6.2; Characterization of the six investigated phthalates

Compound	Formula	Alkyl chain length	MW	S_w(mgL^{-1})	logK_{ow}
DMP	$C_{10}H_{10}O_4$	1	194.19	400	1.61
DEP	$C_{12}H_{14}O_4$	2	222.24	1080	2.38
DBP	$C_{16}H_{22}O_4$	4	278.34	10	4.45
BBP	$C_{19}H_{20}O_4$	4	312.37	2.8	4.84
DEHP	$C_{24}H_{38}O_4$	8	390.57	0.003	7.5
DOP	$C_{24}H_{38}O_4$	8	390.57	0.022	8.1

MW = Molecular weight; Sw = Solubility in water; Kow = Octanol-water partition coefficient; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate.

Data taken from (EU-RAR, 2004; EU-RAR., 2007, 2008; Clara *et al.*, 2010)

6.2.3. Sample collection

Wastewater samples of from the influent, final effluent and sludge were collected from each of the three selected WWTPs on a monthly basis for a period of six months from February-July, 2016. Two sampling campaigns were carried out in the winter (June-July), three in autumn (March-May) and one in the summer (February) to assess the seasonal variation of phthalate removal. One litre amber glass bottles fitted with PTFE lined polypropylene screw caps, previously cleaned and rinsed were used to collect composite samples of the influent and final effluent. Each water sample was first dechlorinated by adding 40-50 mg of sodium thiosulphate followed by acidification to a pH of ≤ 2 with 50% HCl. Some physicochemical parameters (pH, temperature, total dissolved solids, total suspended solids, turbidity) were measured *on-site* in triplicate on a routine basis using Hach instruments. Centrifuged sludge was collected in Berlin WWTP (TF) in a glass jar with a PTFE lined cap while exposed sludge samples from the drying floor were collected in Alice and Bedford WWTPs. All samples were stored in an ice chest during transportation and kept under a temperature of 4 ^{o}C in the Department of Chemistry laboratory of the University of Fort Hare and analysed within 3 days of sample collection.

6.2.4. Sample preparation

6.2.4.1. Effluent extraction

The six target phthalates in wastewater were analysed in duplicate following a modified method of Sánchez-Avila *et al.*, (2009). Prior to extraction, samples were allowed to settle for 24 h. and carefully filtered through glass wool to remove all suspended particles that may clog the solid-phase extraction (SPE) cartridges. The separate filtrates (500 mL each) at a pH ≤ 2 were spiked with 25 µL of the surrogate standard (5 µg/L) to measure method efficiency and error correction. The C18-U cartridges (strata) 1000 mg/6mL used for the SPE were fitted to a vacuum manifold (Supelco) connected to a vacuum pump and were conditioned successively with 7 mL each of n-hexane, dichloromethane, and methanol followed by 10 mL double-distilled water at a flow rate of 3 mL min^{-1}. The cartridges were rinsed with 5 mL of double-distilled water after a complete run of the sample water at a flow rate of 10 mL min^{-1} and left to dry under vacuum for about 1 h. A 7 mL mixture of dichloromethane:n-hexane (1:1) and dichloromethane: acetone (1:1) was used for elution of the analytes. The eluents were reduced to about 2 mL in a rotary evaporator and blown to 1 mL under a dry stream of nitrogen at 30 ^{o}C.

6.2.4.2 Sludge preparation

Sludge samples were air-dried at room temperature for about 7 days and ground into fine powder with a mortar and pestle. Two grams of the homogenous dried sludge was extracted three times each with 10 mL of dichloromethane for 15 min. in an ultrasonic bath at 60 ^{o}C. The combined extracts separately collected in 60 mL amber bottles were centrifuged at 5000 RPM for 30 min to push the particulate matter down. The supernatant was filtered and reduced to about 5 mL in a rotary evaporator and subjected to a clean-up.

6.2.4.3. Column chromatographic clean-up

A pre-cleaned glass column was filled with 5 g of silica gel and topped with 2g anhydrous sodium sulphate which was previously heated in a muffle furnace at 450 ^{o}C for 2 h. The respective columns were conditioned twice with 10 mL dichloromethane to remove contaminants and trapped air. The extract was quantitatively transferred into to the column and eluted with 10 mL mixture of dichloromethane and n-hexane (1:1). The eluents were reduced to about 2 mL in a rotary evaporator and finally blown to 1 mL in a 5 mL amber glass vial under a stream of dry nitrogen at 30 ^{o}C (Sablayrolles *et al.*, 2005).

6.2.5. Wastewater treatment plants removal efficiency

The removal efficiency of the WWTPs was calculated in percentage. The difference between the average influent and final effluent concentration is divided by the average influent concentrations multiplied by 100 for each of the PAEs compounds.

$$100\times\{(\text{Inf}-\text{Ef})/\text{Inf}\}.$$

Where Inf = average influent concentration and Ef = Average effluent concentration

6.2.6. Quality assurance and quality control

Throughout the sampling procedure and analysis, no plastic material was introduced, considering the ubiquitous nature of PAEs. All bottles and glassware were thoroughly washed with detergent, rinsed with tap water and finally with distilled water before they were soaked in 10% nitric acid (500 mL) for 24 h. This was followed with soaking in HPLC grade acetone for 2 h before oven drying at 200 ^{o}C for about 4 h. Regular rinse of the glassware with HPLC acetone or dichloromethane before use was carried out.

For each batch of 15-20 samples analysed, one laboratory reagent blank (LRB) was analysed together in order to determine background contamination of phthalate and subsequent background subtraction was performed. All samples were fortified with 2-fluorobiphenyl surrogate (2FB) prior analysis to correct for any error that might occur due to method performance. Four replicate analysis of laboratory fortified blanks (LFBs) spiked differently at 5 and 10 μgL^{-1} levels gave a relative standard deviation of less than 15% indicating good precision.

Five points external standard calibration was carried out for each analyte and concentration was determined within the linear range of the curve. Analyses of calibration standards ranging from 0.1 - 15 μgmL^{-1} for each analyte including surrogate standard were performed to generate the calibration curve. The regression coefficient between the peak area and injected concentration for each of the six PAEs and surrogate varied from 9.93E-01 (BBP) to 1.00E+00 (DBP). Recoveries for each of the PAEs spiked in water at 5 and 10 μgL^{-1} including surrogate standard ranged between 75 and 123%. A midpoint continuous calibration check standard was injected into the instrument before and after every 10 samples analysed to verify sensitivity.

6.2.7. Instrumental analysis

All extracts were quantified by gas chromatography coupled with mass spectrometer (Agilent 7890B coupled with 5977A MSD). The HP-5 MS capillary column which is 30 m long × 250 µm internal diameter × 0.25 µm thick uses helium as carrier gas at 90 kPa. The oven temperature was initially set at 50 ^{o}C for 1min, ramped at 10 ^{o}C/min to 310 ^{o}C and held constant for 5 min. The transfer line and injection port were maintained at 280 ^{o}C and 300 ^{o}C respectively. A 2 µL of the sample was injected in paused splitless mode with injection pulse pressure of 90 kPa for 1 min and purge flow to split vent of 50 mL/min at 2 min.

6.2.8. Statistical analysis

The mean values of data obtained from the different parameters investigated were compared using SPSS (IBM SPSS Statistics 20), one-way analysis of variance and Tukey's multiple range tests. The coefficient of correlation among PAEs and some physicochemical parameters was calculated by the Pearson correlations test. Significance for statistical analysis was set at p values < 0.05).

6.3. Results and discussion

6.3.1. Occurrence and concentration of six PAEs compounds in wastewater of three selected WWTPs

The concentration of the six targeted phthalates in the influent and final effluent from each of the selected WWTPs in Alice, Bedford, and Berlin as well as the detection frequencies are presented in Tables 6.3, 6.4, and 6.5. DBP was the most abundant phthalate compound in the influent of all the WWTPs followed by DEHP, BBP, and DOP. The concentration range of DBP in order of abundance in Alice, Bedford, and Berlin WWTP were 3.12-2497 µgL^{-1} (mean 1100 ± 429 µgL^{-1}), 6.92 - 1494 µgL^{-1}, (mean 595.0 ± 282.3 µgL^{-1}) and 1.59 - 791.2 µgL^{-1} (mean 180.69 ± 129 µgL^{-1}), respectively. This was followed by DEHP with a similar concentration distribution of 35.6 ± 14.7 µgL^{-1} in Alice, 46.43 ± 8.6 µgL^{-1} in Bedford and 16.44 ± 2.9 µgL^{-1} in Berlin. The least concentration of compound among the six selected phthalates was DMP. On the average, the concentrations of DMP for the three WWTPs were lower than 10 µgL^{-1}. From this present study, the prevalence of DBP over DEHP in the influent of a WWTP is similar to the findings of Olujimi *et al.*, (2012). Unlike in many other countries in Europe and in China where DEHP was more prevalent than DBP (Dargnat *et al.*, 2009; Clara *et al.*, 2010; Gao *et al.*, 2014).

The abundance of DBP and DEHP compounds in this present study and other similar ones explains their importance and popularity as additives in many industrial products including flexible PVC materials and household products suggesting a major phthalate contaminant source in water (Liu *et al.*, 2013).

In the influent and effluent samples of the treatment plants, DMP, DEP, DBP and DEHP were detected in all the samples analysed for all the sampling periods whereas, BBP and DOP, varied in detection frequencies both in the treated and untreated wastewater. Except for Bedford which had all the six compounds detected at the influent samples, detection frequencies for both BBP in Alice and DOP in Berlin amounted to 83%. This suggests that the ubiquitous nature of phthalates is also predominant in domestic and agricultural runoff water just as expected in industrial discharge. The values obtained in this study were true representations of PAEs in domestic and agriculture runoffs because; there was no evidence of industrial effluent discharge in any of the areas which these WWTPs served. At the final effluent, detection frequencies for DOP were 50%, 83%, and 50% in Alice, Bedford and Berlin, respectively; whereas, only Bedford and Berlin had varying detection frequencies of 83% and 75% for BBP, respectively. The PAEs concentration measured in the influent samples were notably higher than the concentration at the final effluent signifying good removal capacity of the municipal WWTPs.

Table 6.3 Concentration (μgL^{-1}) of PAEs in the wastewater samples of WWTP in Alice.

PAEs	Influent				Final Effluent				
	Mean ± SE	Min	Max	FD (%)	Mean ± SE	Min	Max	FD (%)	Removal (%)
DMP	8.40 ± 3.88	0.89	24.51	100	1.73 ± 0.67	0.34	4.87	100	79.38
DEP	13.42 ± 4.53	2.29	27.19	100	3.08 ± 0.93	0.40	7.32	100	76.99
DBP	1100 ± 429	3.12	2497	100	6.47 ± 3.60	1.23	24.19	100	99.41
BBP	26.82 ± 10.04	ND	52.25	83	3.00 ± 1.45	0.28	8.95	100	86.54
DEHP	35.66 ± 14.71	6.16	96.18	100	5.84 ± 2.16	1.84	15.13	100	83.62
DOP	26.11 ± 11.66	3.08	67.37	100	2.32 ± 1.75	ND	5.78	50	95.55
Total PAEs	1210.83				22.47				

*ND = Not detected (Below limit of detection 0.102 – 0.586 **μg/L**), FD = Frequency of detection, Min = Minimum, Max = Maximum; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate.

Table 6.4 Concentration (μgL^{-1}) of PAEs in the wastewater samples of WWTP in Bedford.

PAEs	Influent				Final Effluent				
	Mean ± SE	Min	Max	FD (%)	Mean ± SE	Min	Max	FD (%)	Removal (%)
DMP	5.96 ± 1.47	2.27	11.74	100	2.31 ± 0.68	0.53	5.15	100	61.24
DEP	16.55 ± 5.05	4.02	35.29	100	2.97 ± 0.80	1.02	6.74	100	82.05
DBP	594.99 ± 282.3	6.92	1494	100	9.84 ± 2.89	3.36	22.08	100	98.34
BBP	44.60 ± 10.42	5.09	71.62	100	6.00 ± 2.56	ND	15.84	83	88.79
DEHP	46.43 ± 8.64	21.36	85.04	100	10.79 ± 4.23	1.47	30.99	100	76.75
DOP	23.09 ± 6.32	3.11	42.48	100	5.81 ± 2.16	ND	12.88	83	79.02
Total PAEs	731.65				35.77				

*ND = Not detected (Below limit of detection 0.102 – 0.586 **μg/L**), FD = Frequency of detection, Min = Minimum, Max = Maximum; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate.

Table 6.5 Concentration (μgL^{-1}) of PAEs in the wastewater samples of WWTP in Berlin.

PAEs	Influent				Final Effluent				
	Mean ± SE	Min	Max	FD (%)	Mean ± SE	Min	Max	FD (%)	Removal (%)
DMP	3.20 ± 0.63	1.12	5.52	100	1.12 ± 0.36	0.53	2.02	100	76.59
DEP	6.02 ± 1.93	2.15	15.32	100	2.20 ± 0.61	0.91	3.78	100	75.62
DBP	180.69 ± 128.96	1.59	791.19	100	4.18 ± 0.85	2.77	6.25	100	98.46
BBP	15.48 ± 4.68	6.32	36.42	100	3.70 ± 0.55	ND	4.39	75	88.02
DEHP	16.44 ± 2.89	8.80	27.89	100	4.40 ± 0.96	1.54	5.64	100	82.13
DOP	9.73 ± 1.51	ND	11.55	83	2.77 ± 1.92	ND	4.69	50	88.63
Total PAEs	231.59				17.47				

*ND = Not detected (Below limit of detection 0.102 – 0.586 **μg/L**), FD = Frequency of detection, Min = Minimum, Max = Maximum; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate.

At the final effluent, DEHP and DBP were also the most abundant compounds with similar concentrations across the three WWTPs. Apart from Alice treatment plant, DEHP was the compound with the highest concentration of 10.79 ± 4.23μgL^{-1} and 4.40 ± 0.96 μgL^{-1} in Bedford, and Berlin, respectively. This was closely followed by DBP with concentration of 9.84 ± 2.9μgL^{-1} and 4.18 ± 0.85 μgL^{-1} in Bedford and Berlin, respectively. In Alice WWTP, DBP had the highest concentration of 6.08 ± 0.8 μgL^{-1}in the effluent closely followed by DEHP 5.84 ± 2.16 μgL^{-1}. In Bedford, BBP and DOP were the other compounds with a concentration above 6.00 μgL^{-1}; whereas, other phthalate compounds; DEP and DMP were the least concentrated compounds in the final effluent, and are of minor significance in terms of concentrations. Comparable distributions in the effluent samples were reported in the previous studies (Roslev *et al.*, 2007; Dargnat *et al.*, 2009; Clara *et al.*, 2010). Also, in another studies carried out by Fatoki and Noma (2000) and Olujimi *et al.*, (2012) similar distribution of phthalates was observed in Rivers and WWTPs effluent, respectively but with much higher concentrations. The concentrations of the prominent PAEs; DEHP, DBP, BBP and DOP in the final effluent are relatively higher at Bedford than Alice and Berlin WWTPs. Likewise; the final effluent concentrations of these same compounds from all the treatment processes were above the 3 μgL^{-1} criteria standard set by USEPA for the protection of aquatic life.

6.3.2. Comparative removal efficiency of different wastewater treatment technology

The removals of PAEs from three selected wastewater treatment plants are illustrated in Figure 6.2. On a general note, phthalates esters were significantly removed in all the treatment plants. The removal capacity varied between 76.99 - 99.41% in Alice, 61.24 - 98.34% in Bedford and 75.59 - 98.46% in Berlin. DBP was most efficiently removed in all the WWTPs with removal efficiency of over 98%, followed by BBP and DOP with removal efficiency of over 80% except in the oxidation pond where DOP was 79.02%. However, there was no notable difference in the removal capacity for DBP in any of the treatment plants (Figure 6.2). The same trend was observed for BBP with a removal efficiency of 87%. A distinct difference in removal capacity for any of the WWTPs was only observed with respect to DMP, DEP, DEHP, and DOP. Activated sludge (AS) treatment performed better than trickling filter (TF) and the oxidation pond (OP) in that order for the removal of DMP, DEHP, and DOP. The only difference in this trend was observed for the removal of DEP where OP outperformed AS and TF, with removal efficiencies of 82.05%, 76.99%, and 75.62%, respectively. DMP, DEP and DEHP were the phthalate compounds that were least removed among the different treatment technologies: although, the removal capacity for these compounds were above 70% except in OP where DMP was removed at a lower efficiency of 61.24%.

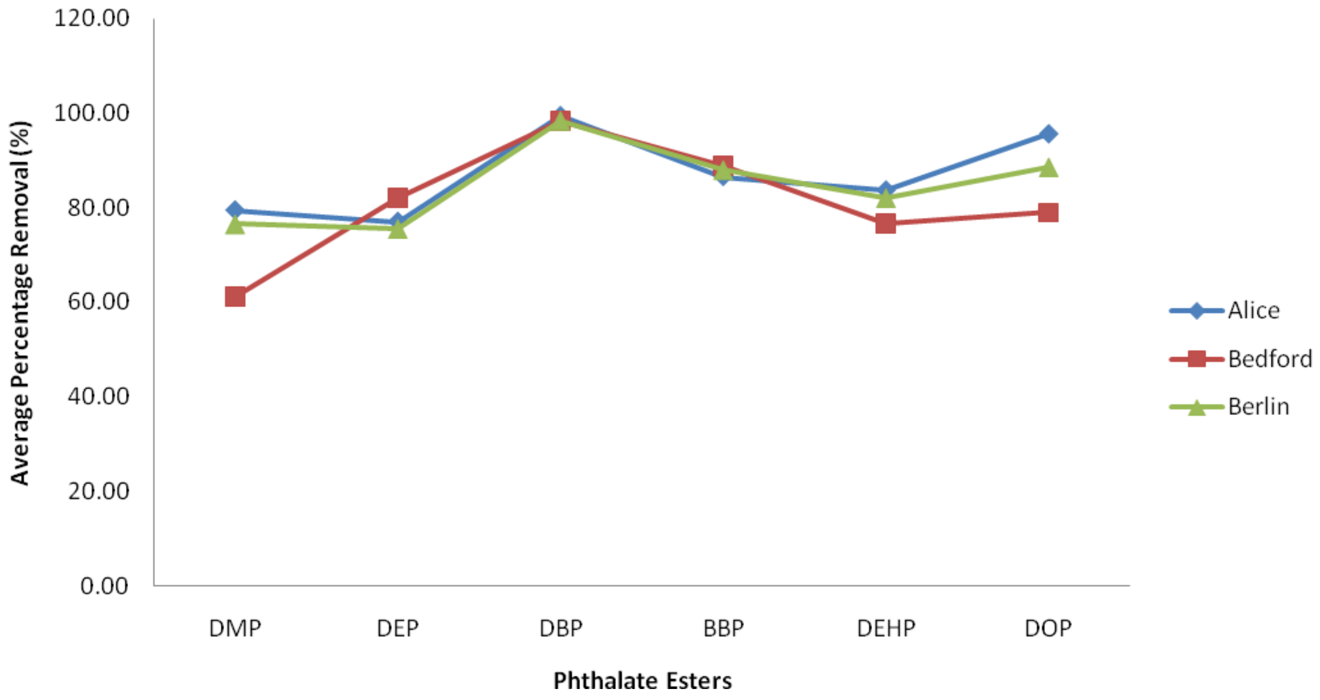


Figure 6.2 Removal efficiency of different wastewater treatment processes

The efficient performance of these treatment plants was attributed to their strong ability to remove suspended solids since it was not designed to remove micro-pollutants such as PAEs. The high percentage reduction in turbidity and total suspended solids (TSS) was directly proportional to phthalate removal as shown in Tables 6.6 and 6.7. There was a high significant correlation between TSS, turbidity and all the phthalates ($p < 0.001$) except DBP that was significant at $p < 0.05$. This shows a direct proportionality between phthalate compounds and both the TSS and turbidity (Dargnat *et al.*, 2009; Clara *et al.*, 2010). This also suggests the possible adsorption of the compounds on settling particles and centrifuged sludge. Elimination of particulate matters could either be explained by the TSS retention in bio-filter, or biodegradation of the compounds, or adsorption on the biomass or volatilization of the compounds. Berge *et al.* (2012) concluded that removal of particulate matter is certainly linked to removal of TSS.

Table 6.6 Physicochemical properties of wastewater samples in the selected WWTPs.

PLANTS	Alice			Bedford			Berlin		
	Influent	Effluent	Red. (%)	Influent	Effluent	%Red.	Influent	Effluent	Red(%)
TDS mg/L	196.6 ±12.3	147 ±5.1	25.15	342.67±70.2	188.59±4.1	44.96	380.61 ±42.5	389.83±30.8	-2.42
Turb. NTU	547.67 ±136.2	17.82±6.9	96.75	637.67±133.9	119.12±18.9	81.32	129.43 ±36.2	6.49±4.6	94.99
TSS mg/L	179.9±36.5	6.73 ±2.6	96.26	184.87±18.8	57.4±10.8	68.95	49.07 ±17.5	1.53±0.6	96.88

*TDS – Total dissolved solids, Turb- Turbidity, TSS – Total suspended solids, Red – Reduction, (below limit of detection LOD), FD = Frequency of detection, Min = Minimum, Max = Maximum; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate.

Table 6.7 Pearson correlation of the six phthalates with some physicochemical properties.

Correlations

	DMP	DEP	DBP	BBP	DEHP	DOP	TDS	TUR	TSS
DMP	1								
DEP	0.900*	1							
DBP	0.987**	0.861*	1						
BBP	0.804	0.978**	0.747	1					
DEHP	0.888*	0.994**	0.837*	0.984**	1				
DOP	0.979**	0.961**	0.955**	0.902*	0.957**	1			
TDS	0.125	0.102	0.110	0.241	0.107	0.020	1		
TUR	0.923**	0.986**	0.882*	0.953**	0.990**	0.974**	0.021	1	
TSS	0.945**	0.963**	0.901*	0.920**	0.974**	0.981**	0.038	0.992**	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

The duration of settling at both the primary and secondary clarifiers will no doubt have a strong influence on the removal capacity of phthalates and other related micro-pollutants. However, the level of reduction recorded for TSS and turbidity in the OP (81.32% and

68.95%) was slightly lower than respective average reduction observed in AS (96.75% and 96.26%) and TF (94.99% and 96.88%). The removal efficiency of WWTPs from a global point of view is largely linked to the different treatment process characteristics. However, volatilization losses mostly considered for lighter compounds occur in the aeration basin and during sludge dehydration (Byrns, 2001; Dargnat *et al.*, 2009).

There was a relatively low percentage reduction of total dissolved solids (TDS) in all the treatment plants of which TF was significant (Table 6.6). The average TDS at the final effluent of TF in Berlin was considerably higher than what was recorded in the influent. This may suggest possible dissolution of soluble matter along the treatment path way or the dissolution of part of the suspended solids in the cause of agitation during treatment. In addition, a different dose of chlorine disinfectant is another factor that may increase the amount of dissolved ions in the treated effluent. The percentage reduction of TDS recorded for TF in Berlin was -2.42% while AS in Alice and OP in Bedford were 25.15% and 44.96%, respectively. Minor elimination of dissolved compounds as compared with major elimination of particulate matter was also observed by Berge *et al.*, (2012) in his work on physicochemical lamellar clarification and bio-filtration.

6.3.3. Occurrence of phthalates in sludge samples

All the six phthalate compounds were detected in sludge samples of all three treatment technologies shown in Table 6.8. The total concentration of all phthalates in AS (Alice), OP (Bedford) and TF (Berlin) WWTPs were 1948, 1346, and 1228 µg g^{-1}d.w. (dry weight), respectively. It varies from below the limit of detection (LOD) to 1249 µg g^{-1}d.w. in AS technology (Alice), 2.1 - 877.2 µg g^{-1} d.w.in OP (Bedford) and from 2.5 - 799.2 µg g^{-1}d.w. in TF (Berlin). All the phthalate compounds were consistently detected throughout the sampling period in all the WWTPs other than DMP in Alice's treatment plant. The phthalate profile showed that DBP, BBP, DEHP and DOP as the most abundant compounds, respectively for Alice and Bedford with concentration of 1094 ± 89.3, 449.36 ± 99.6, 311.68 ± 23.7 and 83.45 ± 6.6µg g^{-1}d.w. and 592.91 ± 100.7, 322.45 ± 32.4, 288.63 ± 36.0 and 123.93 ± 27.8 µg g^{-1}d.w, respectively. However, BBP was the most abundant in Berlin followed by DBP, DEHP and DOP concentration of 448.38 ± 87.9, 399.45 ± 151.6, 234.93 ± 12.4, and 129.44 ± 18.8µg g^{-1}d.w. respectively. The relative contributions of DMP and DEP are of minor importance to all the WWTPs with concentration of DMP which was lower than 10 µg g^{-1}d.w. This distribution pattern conforms with the octanol/water partition coefficient logK_{ow} of all the PAEs (Table 6.1) as observed by (Tan, 2007; Dargnat *et al.*, 2009). Byrns (2001) also showed that compounds that are poorly degraded have log$K_{ow} \geq 4$. DMP and DEP are also considered as volatile PAEs that could be lost during treatment or preparation of sludge materials (Berge *et al.*, 2012). Kong *et al.*, (2008) and Dargnat *et al.* (2009), also observed over 50- 90% degradation of DMP and DEP compound in aerobic treatments.

Table 6.8 Concentration of sludge in µg g^{-1}d.w.

PAEs	Alice				Bedford				Berlin			
	Mean	Min	Max	FD (%)	Mean	Min	Max	FD (%)	Mean	Min	Max	FD (%)
DMP	6.10 ± 0.1	6.0	6.1	75	5.06 ± 1.3	2.1	8.2	100	4.54 ± 0.9	2.5	6.8	100
DEP	3.44 ± 0.1	3.4	3.5	100	13.16 ± 1.7	8.5	16.5	100	11.67 ± 2.1	6.9	17.3	100
DBP	1094 ± 89.3	939.2	1248.6	100	592.91 ± 100.7	436.5	877.2	100	399.45 ± 151.6	64.9	799.2	100
BBP	449.36 ± 99.6	276.9	621.8	100	322.45 ± 32.4	233.6	386.5	100	448.38 ± 87.9	220.9	649.4	100
DEHP	311.68 ± 23.7	270.7	352.7	100	288.63 ± 36.0	217.5	387.4	100	234.93 ± 12.4	200.3	257.6	100
DOP	83.45 ± 6.6	71.9	94.9	100	123.93 ± 27.8	72.9	201.4	100	129.44± 18.8	96.3	181.9	100
Total	1947.90				1346.14				1228.41			

*FD= Frequency of detection; Min = Minimum; Max = Maximum; DMP = dimethyl phthalate; DEP = diethyl phthalate; DBP = di-n-butyl phthalate; BBP = benzyl butyl phthalate; DEHP = di (2-ethylhexyl phthalate; DOP = di-n-octyl phthalate.

Generally, the concentrations of DBP, BBP, DEHP and DOP in these studies were above the European Union legislation of 100 µg/g d.w on the use of sludge for agriculture (EU legislation 86/278/EEC) (Inglezakis *et al.*, 2014). This calls for considerable treatment of this sludge if it is to be considered for agricultural use especially in these areas where livelihood depends largely on agriculture. Contamination of groundwater through the soil or surface water by runoffs is possible consequence of the use of this untreated sludge for agricultural purposes. This will in turn pose a serious health challenge to the ecosystem as human and aquatic animals are exposed to EDCs. These results are also higher in comparison with similar studies carried out in Europe and China (Dargnat *et al.*, 2009; Gao *et al.*, 2014) but not as high as what Olujimi *et al.*, (2014) detected in the upstream and downstream sediment of an oxidation pond in Nigeria. To the best of my knowledge, there was no other similar work on PAEs in WWTPs sludge for comparison in South Africa.

6.3.4. Seasonal variations of phthalate removal in different wastewater treatment technology

The removal efficiency of the different treatment technologies; AS, OP and TF for the six targeted phthalates across three seasons; summer (Feb), autumn (Mar- May) and winter (Jun - Jul) are presented in Figures 6.3, 6.4 and 6.5. The temperature for the three treatment plants AS, OP and TF were 30.67, 35.72 and 26.10 ^{o}C in summer, 24.07, 24.63 and 25.29 ^{o}C in autumn and 14.65, 14.41 and 16.32 ^{o}C in winter, respectively. The results indicated a notable difference in removal efficiency for most PAEs across the seasons in OP processes. Figure 6.4 shows lower removal efficiency for all the phthalates in summer in OP except for DEP and DEHP which shows no significant influence of temperature change. There was a gradual increase in the removal efficiency as the temperature dropped. There was also a negative removal observed for DOP and DMP in the summer. This may be as a result of increased perturbation of the pond which may in turn influenced level of adsorption on settling particles. This may lead to possible re-suspension of the sediment and the analytes into the water system.

OP process was the least effective in the removal of PAEs in the summer. The removal for DOP and DMP were This may have been influenced by the limited aeration and poor biomass in the system (Camacho-Munoz *et al.*, 2012). A longer hydraulic retention time (HRT) could be an advantage for an improved removal of EDCs in decentralized treatment processes, although, the high microbial activity, thorough mixing of microbial diversity and artificial aeration that increased EDCs bioavailability compensated for short HRT in AS processes (Koh *et al.*, 2008).

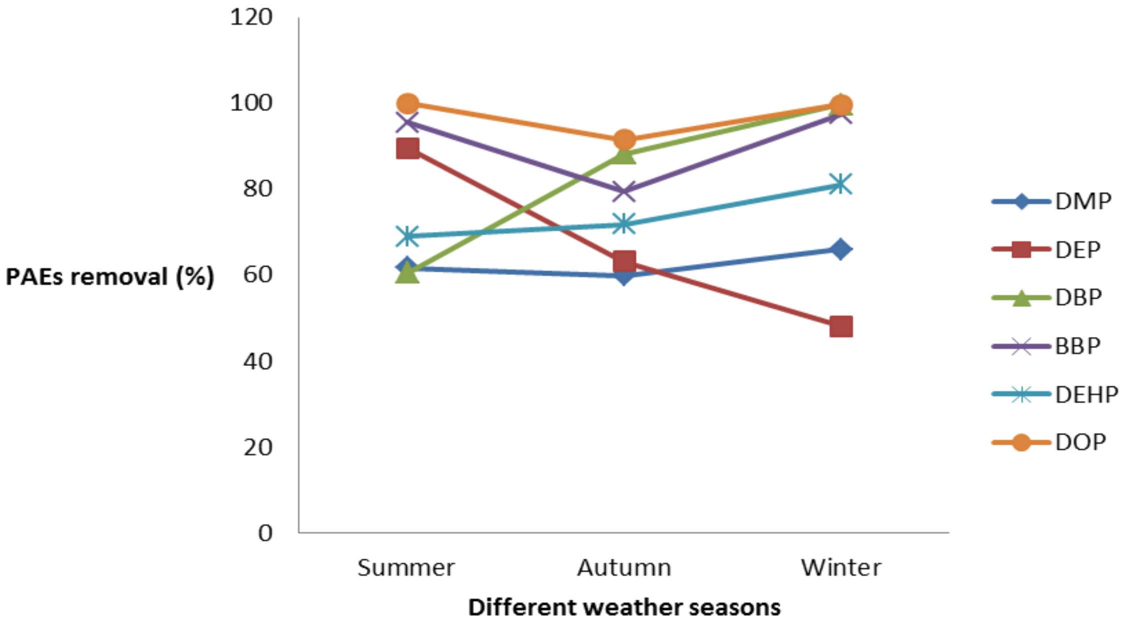


Figure 6.3, Seasonal variation in PAEs removal in AS WWTPs in Alice.

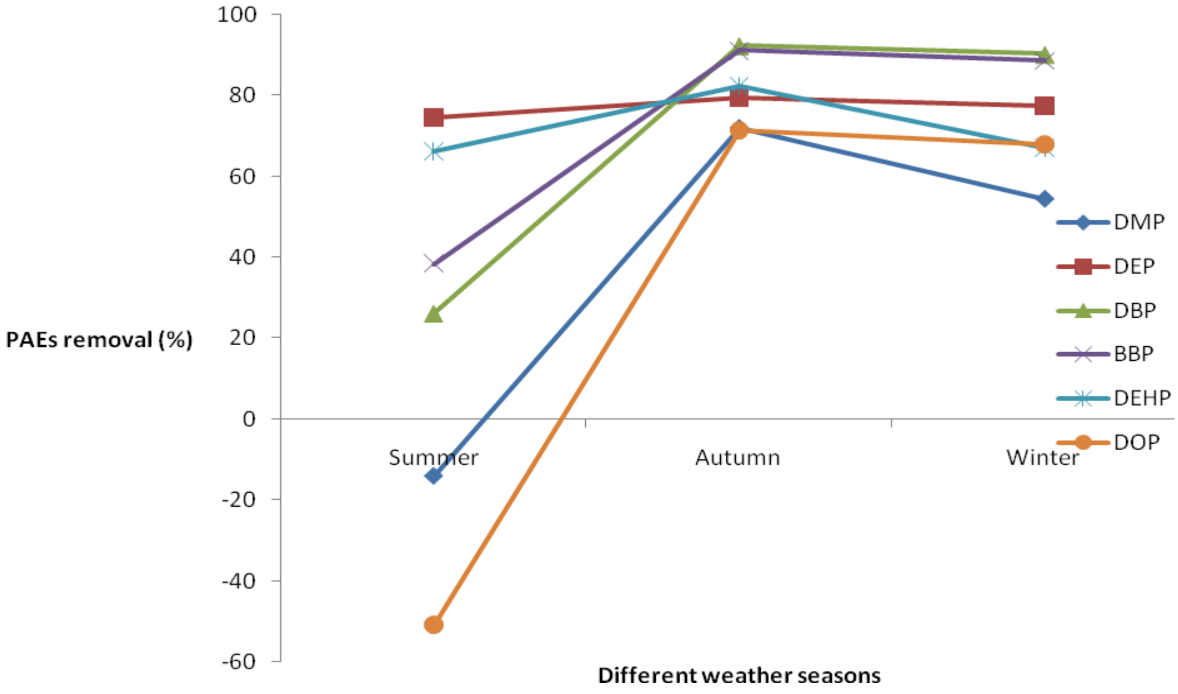


Figure 6.4, Seasonal variation in PAEs removal in OP WWTPs in Bedford.

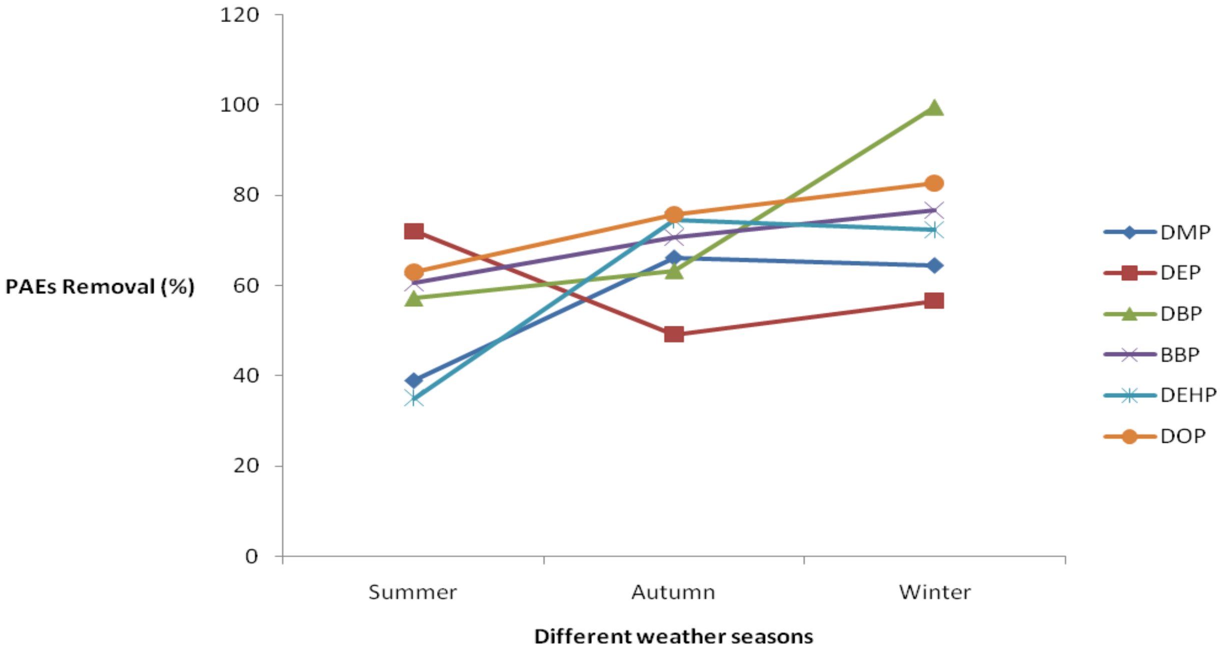


Figure 6.5, Seasonal variation in PAEs removal in TF WWTPs in Berlin

In a similar order but at a lower magnitude, TF also shows a gradual improvement in percentage removal as temperature decreases (Figure 6.5). The removal efficiency for most of the PAEs in TF is in the order summer < autumn < winter. DEP was the only compound that showed a sharp contrast with this trend. The order of removal efficiency for DEP was the reverse of the others.

However, in the AS plant treatment technology; there was no sharp distinction in percentage removal for most phthalates across the different weather conditions. The percentage removal efficiency for most of the phthalates ranged between over 60 - 100% across the different weather conditions. This suggests that the performance of the AS treatment plant is probably independent of temperature. This results were also similar to the high removal performance reported by Qiang *et al.* (2013) on the removal of some endocrine disrupting chemicals (EDCs) in AS technology. It is to be expected that microbial activity will decrease in summer because of the increase in temperature, but another fact that the removal of the bulk of phthalates and other EDCs is largely attributed to adsorption on particulate matter in the primary clarifier cannot be ignored either, although, Hashimoto and Murakami (2009) also argued that the degradation of EDCs is more favoured in AS process than other treatment processes by the diverse microbial colonies. In addition, the increase in the bioavailability of EDCs or other micro-pollutants as a result of their adsorption onto activated sludge which is less important in other treatment technologies could probably give AS an advantage over others.

It was presumed that seasonal temperature would influence the removal capacity of the AS treatment processes for EDCs since their operation was largely dependent on microbial activities and growth rate. However, previous studies have shown inconsistency in results with respect to this assumption. For example, Koh *et al.*, (2009) could not detect any notable effect in the removal capacity of AS for EDCs at 6 ^{o}C decrease in temperature. On the contrary, Nie *et al.*, (2012) observed an enhanced removal of micro-pollutants (pharmaceuticals and EDCs) with an increase in temperature. It would be wise to go by the general statement that suggests that EDC removal in WWTPs technology is generally influenced by the combined performance of adsorption and biodegradation (Qiang *et al.*, 2013).

AS exhibited a more stable and better performance for PAE removal across the different weather conditions than OP and TF. However, the only compound that showed exclusion to this performance trend was DEP.

DEP seems to be the only compound that was greatly impacted by increases in temperature. In all the three treatment technologies, the removal efficiency for DEP increased with increased temperature. This probably supports the earlier conclusions that the compound is volatile and has the tendency to be lost during treatment technology (Berge *et al.*, 2012).

DBP on the other hand, was the only compound that showed a sharp increase in removal as the temperature decreased across all the treatment processes. In AS, the removal efficiency for DBP increased from 69%, 71% and 81% in summer, autumn and winter, respectively. The same order of magnitude was observed with OP and TF treatment processes, with a relative increase from 26%, 92%, 90% and 57%, 63%, 99% for summer, autumn and winter, respectively. It is the only compound that gave a statistical significance at $p<0.05$ between summer and winter across all the treatment plants.

6.4. Conclusions

The presence of all the priority PAEs in the influent, treated water and sludge of WWTPs in the rural areas further confirms the ubiquitous nature of these hazardous micro-pollutants in the environment especially in water. The relative concentrations found in these environments are largely influenced by the level of anthropogenic activities around the areas which, in turn, are dependent on population. The three different WWTPs technologies (AS, TF, OP) successfully removed over 60% of these compounds from wastewater through their capacity to remove suspended particles as the combined treatment processes could not degrade them. The corresponding deposit of these PAEs in the WWTPs sludge, and their escape through the final effluent shifted the consequent health hazard of these micro-pollutants to human and aquatic life. The reliance of rural dwellers on surface water and the possible transfer of these pollutants to the food chain through the use of phthalate-laden sludge in agriculture calls for a holistic remedial approach especially in the regulation of the use of the prominent compounds DBP and DEHP in industries. In addition to regulation, it is recommended that degradation processes like advanced oxidation processes, bioremediation among others may be studied and WWTPs redesigned for removal by degradation of the compounds to none toxic compounds.

References

Andersen, H., Siegrist, H., Halling-Sorensen, B. and Ternes, T. A. (2003) ‘Fate of estrogens in a municipal sewage treatment plant.’, *Environmental Science and Technology.*, 37(18), 4021– 4026.

Balabanič, D., Rupnik, M., and Krivograd Klemenčič, A. (2011) ‘Negative impact of endocrine-disrupting compounds on human reproductive health.’, *Reproduction Fertility and Development*, 23, 403–411.

Balabanici, D., Hermosilla, D., Merayo, N., Klemencic, A. K. and Blanco, Á. (2012) ‘Comparison of different wastewater treatments for removal of selected endocrine disruptors from paper mill wastewaters’, *Journal of environmental science and health. Part A, Toxic/hazardous substance & environmental engineering*, 47(10), 1350–63. doi: 10.1111/cobi.12645.This.

Batt, A. L., Kim, S., and Aga, D. S. (2007) ‘Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations.’, *Chemosphere*, 68(3), 428–435.

Becker, K., Seiwert, M., Angerer, J., Heger, W., Koch, H. M., Nagorka, R., Roßkamp, E., Schlüter, C., Seifert, B. and Ullrich, D. (2004) ‘DEHP metabolites in urine of children and DEHP in house dust.’, *International Journal of Hygiene and Environmental Health*, 20, 409–417.

Berge, A., Gasperi, J., Rocher, V., Coursimault, A. and Moilleron, R. (2012) ‘Phthalate and alkylphenol removal within wastewater treatment plants using physicochemical lamellar clarification and biofiltration’, *WIT Transactions on Ecology and the Environment*, 164, 357–368. doi: 10.2495/WP120311.

Byrns, G. (2001) ‘The fate of xenobiotic organic compounds in wastewater treatment plants.’, *Water Research*, 35(25), 23–33.

Cai, K., Elliott, C. T., Phillips, D. H., Scippo, M. L., Muller, M. and Connolly, L. (2012) ‘Treatment of estrogens and androgens in dairy wastewater by a constructed wetland system.’, *Water Research.*, 46(7), 2333–2343.

Camacho-Munoz, D., Martin, J., Santos, J. L., Aparicio, I. and Alonso, E. (2012) ‘Effectiveness of conventional and low-cost wastewater treatments in the removal of pharmaceutically active compounds.’, *Water, Air, & Soil Pollution,* 223(5), 2611–2621.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C. and Chovanec, A. (2010) ‘Chemosphere Occurrence of phthalates in surface runoff , untreated and treated wastewater and fate during wastewater treatment’, *Chemosphere*. 78(9), 1078–1084. doi: 10.1016/j.chemosphere.2009.12.052.

Coskuner, G. and Ozdemir, N. . (2006) ‘Performance assessment of a wastewater treatment plant treating weak campus wastewater’, *International Journal of Environment and Pollution*, 28(1/2), 185–197.

Dargnat, C., Teil, M. J., Chevreuil, M. and Blanchard, M. (2009) ‘Phthalate removal throughout wastewater treatment plant. Case study of Marne Aval station (France)’, *Science of the Total Environment*. 1235–1244. doi: 10.1016/j.scitotenv.2008.10.027.

ESCWA, (Economic and Social Commission For Western Asia) (2003) *Waste-Water Treatment Technologies : a General Review*. United Nation, 1320.

EU-RAR (2004) *European Union Risk Assessment Report on Dibutyl Phthalate. Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau, 1st Priority List, vol. 29. <http://ecb.jrc.ec.europa.eu/DOCUMENTS/ Existing-*

Chemicals/RISK_ASSESSMENT/REPORT/dibuty (accessed 17/02/2017).

EU-RAR. (2007) *European Union Risk Assessment Report on Benzyl Butyl Phthalate (BBP)., Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau, 3rd Priority List, vol. 76. <http://ecb.jrc.ec.europa.eu/DOCUMENTS/ Existing-Chemicals/RISK_ASSESSMENT/RE* (accessed 11/01/2017).

EU-RAR. (2008) *European Union Risk Assessment Report on Bis(2-ethylhexyl) Phthalate (DEHP)., Institute of Health and Consumer Protection (IHCP), European Chemicals Bureau, 2nd Priority List, vol. 80. <http://ecb.jrc. Ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/ Dehpreport042.pdf>.(accessed 11/01/2017).*

Fatoki, O. S., and Noma, A. (2002). Solid phase extraction method for selective determination of phthalate esters in the aquatic environment. *Water, Air and Soil Pollution*, *140*, 85–98.

Gao, D., Li, Z., Wen, Z. and Ren, N. (2014) 'Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China', *Chemosphere*. 95, 24–32. doi: 10.1016/j.chemosphere.2013.08.009.

Gao, D. W. and Wen, Z. D. (2016) 'Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes', *Science of the Total Environment*. 541, 986–1001. doi: 10.1016/j.scitotenv.2015.09.148.

Hashimoto, T. and Murakami, T. (2009) 'Removal and degradation characteristics of natural and synthetic estrogens by activated sludge in batch experiments.', *Water Research.*, 43(3), 573–582.

Horn, O., Nalli, S., Cooper, D. and Nicell, J. (2004) 'Plasticizer metabolites in the environment.', *Water Research.*, 38, 3693–3698.

Inglezakis, V., Zorpas, A. A., Karagiannidis, A., Samaras, P., Voukkali, I. and Sklari, S. (2014) 'European Union legislation on sewage sludge management', *Fresenius Environmental Bulletin*, 23(2A), 635–639.

Jonathan, L. (SBHSA) (2011) *South Africa Water Treatment technologies in SA*. Pretoria. 1-9.

Joss, A., Andersen, H., Ternes, T., Richle, P. R. and Siegrist, H. (2004) 'Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization.', *Environmental Science & Technology. Technol.*, 38(11), 3047–3055.

Koh, Y. K. K., Chiu, T. Y., Boobis, A. R., Cartmell, E., Scrimshaw, M. D. and Lester, J. N. (2008) 'Treatment and removal strategies for estrogen from wastewater.', *Environ. Technol.*, 29(3), 245–267.

Koh, Y. K. K., Chiu, T. Y., Boobis, A. R., Scrimshaw, M. D., Bagnall, J. P., Soares, A., Pollard, S., Cartmell, E. and Lester, J. N. (2009) 'Influence of operating parameters on the biodegradation of steroid estrogens and nonylphenolic compounds during biological wastewater treatment processes.', *Environmental Technology*, 43(17), 6646–6654.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. T. (2002) 'Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance.', *Environmental Science & Technology*, 36, 1202–1211.

Kong, X.-J., Li, D., Cao, L.-Q., Zhang, X.-M., Zhao, Y., Lv, Y. and Zhang, J. (2008) 'Evaluation of municipal sewage treatment systems for pollutant removal efficiency by measuring levels of micropollutants', *Chemosphere*, 72, 59–66.

Kraigher, B. ., Kosjek, T. ., Heath, E. ., Kompare, B. . and Mandić-Mulec, I. (2008) 'Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors', *Water Research.*, 42(4578–4588).

Leech, D. M., Snyder, M. T., and Wetzel, R. G. (2009) 'Natural organic matter and sunlight accelerate the degradation of 17ß-estradiol in water', *Science of the Total Environment*, 407(6), 2087–2092.

Liu, Y., Chen, Z., and Shen, J. (2013) 'Occurrence and removal characteristics of phthalate esters from typical water sources in northeast China', *Journal of Analytical Methods in Chemistry*, 2013. doi: 10.1155/2013/419349.

Loraine, G. A. and Pettigrove, M. E. (2006) 'Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California.', *Environmental Science & Technology*, 40, 687–695.

Löwenberg, J., Zenker, A., Baggenstos, M., Koch, G., Kazner, C. and Wintgens, T. (2014) 'Comparison of two PAC/UF processes for the removal of micropollutants from wastewater treatment plant effluent: Process performance and removal efficiency', *Water Research*, 56, 26–36. doi: 10.1016/j.watres.2014.02.038.

Matsui, S. (2008) 'Endocrine Disruptors', *Encyclopedia of Toxicology*, (May), 1259–1260. doi: http://dx.doi.org/10.1016/B978-008045405-4.00402-X.

Nakada, N., Tanishima, T., Shinohara, H. and Kiri, K. (2006) 'Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment', *Water Research*, 40(17), 3297–3303. doi: 10.1016/j.watres.2006.06.039.

Nie, Y. F., Qiang, Z. M., Zhang, H. Q. and Ben, W. 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(S1), 9–15.

Olujimi, O., Fatoki, S., Odendaal, J., and Daso, A. P. (2012) ‘Chemical monitoring and temporal variation in levels of endocrine disrupting chemicals (priority phenols and phthalate esters) from selected wastewater treatment plant and freshwater systems in Republic of South Africa’, *Microchemical Journal.*, 101, 11–23. doi: 10.1016/j.microc.2011.09.011.

Olujimi, O. O., Aroyeun, O. A., Akinhanmi, T. F. and Arowolo, T. A. (2014) ‘Occurrence and Concentration of Phthalate Esters in River Sediment Around Covenant Oxidation Ponds, Covenant University, Ota, Ogun State’, *International Journal of Environment and Sustainability (IJES)*, 3(2), 1–7. Available at: https://www.sciencetarget.com/Journal/index.php/IJES/article/view/517.

Park, M. A., Hwang, K. A., Lee, H. R., Yi, B. R., Jeung, E. B. and Choi, K. C. (2012) ‘Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. *Molecular Medicine Reports,* 5(3), 761–766. http://dx.doi.org/10.3892/mmr.2011.712.

Pescod, M. . (FAO) (1992) *Wastewater treatment and use in agriculture [WWW Document]. URL http://www.fao.org/docrep/t0551e/t0551e05.htm accessed 17/02/2017*.

Qiang, Z., Dong, H., Zhu, B., Qu, J. and Nie, Y. (2013) ‘A comparison of various rural wastewater treatment processes for the removal of endocrine-disrupting chemicals (EDCs)’, *Chemosphere*, 92(8), 986–992. doi: 10.1016/j.chemosphere.2013.03.019.

Ravi, K. P., Liza, B. P., and Somashekar, R. K. (2010) ‘Assessment of the efficiency of sewage treatment plants: a comparative study between Nagasandra and Mailasandra sewage treatment plants', *Kathmandu University Journal of Science, Engineering and Technology*, 6(2), pp. 115–125. doi: 10.3126/kuset.v6i2.4020.

Roslev, P., Vorkamp, K., Aarup, J., Frederiksen, K. and Nielsen, P. H. (2007) 'Degradation of phthalate esters in an activated sludge wastewater treatment plant', *Water Research*, 41(5), 969–976. doi: 10.1016/j.watres.2006.11.049.

Sablayrolles, C., Montr, M., Benanou, D., Patria, L. and Treilhou, M. (2005) 'Development and validation of methods for the trace determination of phthalates in sludge and vegetables', *Journal of Chromatography A*, 1072, 233–242. doi: 10.1016/j.chroma.2005.02.074.

Sánchez-Avila, J., Bonet, J., Velasco, G. and Lacorte, S. (2009) 'Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant', *Science of the Total Environment*. 407(13), 4157–4167. doi: 10.1016/j.scitotenv.2009.03.016.

Tan, B., Hawker, D., Müller, J., Leush, F. and Tremblay, L. (2007) 'Chapman HF. Modelling of the fate of selected endocrine disruptors in a municipal wastewater treatment plant in South East Queensland, Australia.', *Chemosphere*, 69(6), 44–54.

Xue, W. C., Wu, C. Y., Xiao, K., Huang, X., Zhou, H. D., Tsuno, H. and Tanaka, H. (2010) 'Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation', *Water Research.*, 44, 5999–6010.

CHAPTER SEVEN

GENERAL DISCUSSION AND RECOMMENDATION

7.0 General Introduction

Some exogenous synthetic or natural chemicals that cause changes in endocrine functions and consequently cause adverse health effects in organisms and their progeny are now found widely in the environment as a result of their widespread use (Damstra *et al.*, 2002; Kookana *et al.*, 2007; Schiliro *et al.*, 2009; WHO-UNEP, 2013).

These chemicals generally referred to as endocrine disrupting chemicals (EDCs) find wide applicability in various essential commodities used in residential, industrial and agricultural applications which also make them ubiquitous in the environment especially in surface water, rain or storm-water, sediments, soils and sewage sludge (Roslev *et al.*, 2007; Adeniyi *et al.*, 2008; Gasperi *et al.*, 2008; Schiliro *et al.*, 2009; Fatoki *et al.*, 2010; Gao *et al.*, 2014). Various research outcomes have revealed their carcinogenic, mutagenic and teratogenic potentials. They have also been linked to other diseases in humans and aquatic organisms after exposure to them, necessitating increasing global attention to their environmental fate (Horn *et al.*, 2004; Abdel daiem *et al.*, 2012; Park *et al.*, 2012; Gao and Wen, 2016). This has also made some European countries and the USA, under the Consumer Product Safety Improvement Act (CPSIA) to place restrictions on or ban their use altogether (Hileman, 2007; Gao *et al.*, 2014). Some of the EDCs that are widely reported in wastewater facilities include; alkyl-phenol, phthalates, bisphenol A, pharmaceutical products, pesticides, alky-phenol ethoxylates, steroid sex hormones and poly-brominate compounds (Roslev *et al.*, 2007; Dargnat *et al.*, 2009; Olujimi *et al.*, 2012).

The primary route that these EDCs particularly phthalates and phenolic compounds take into the environment has been through effluent and sludge that are continually discharged from conventional wastewater treatment plants (WWTPs), which are not designed to remove these organic pollutants (Loraine and Pettigrove, 2006; Nakada *et al.*, 2006; Xue *et al.*, 2010; Gao *et al.*, 2014). This has made the performance of WWTPs unsatisfactory for their removal and consequently, aquatic organisms are unavoidably exposed to the escaped EDCs which in turn, introduced hazards to the whole food chain (Kraigher *et al.*, 2008; Balabanici *et al.*, 2012). Sources of EDCs (phthalates and phenolic compounds) include; domestic and industrial waste, atmospheric deposition or urban and agricultural run-offs. Investigating the removal of these organic pollutants as they pass through the WWTP processes is crucial to ecological and environmental health (Gao and Wen, 2016).

In developed countries, efforts are geared towards optimizing the existing treatment operations, or incorporating new specific treatment operations for micro-pollutants removal as additional processes to improve WWTP performance apart from specific restriction placed on the use of these compounds (Batt *et al.*, 2007; Cai *et al.*, 2012). In developing countries such as South Africa however, there is limited or no information on the occurrence and level of these organic micro-pollutants in the environment that may serve as a basis for interim guidelines for permissible levels in fresh water systems (Fatoki *et al.*, 2012). This has given rise to indiscriminate pollution of fresh water systems through industrial discharge, which has made surface water quality in South Africa a major challenge, especially to rural dwellers, who still rely strongly on surface water for their daily domestic, recreational and agricultural use.

Now that river water, ground water, effluents from WWTPs have been considered as suitable alternative sources of water to alleviate the problem of water scarcity in South Africa, poor management and inadequate maintenance of wastewater and municipal sewage treatment infrastructure in some Municipalities in the Eastern Cape, South Africa have also been identified as one of the reasons for the pollution of their respective receiving watersheds upon which most rural community depend (Momba *et al.*, 2006).

In a bid to protect the communities from the risk of water borne diseases and several negative health effects, emphasis has been placed on the quality of effluents from WWTPs being released into the water bodies.

Therefore, this present study focused on quantifying endocrine disrupting chemicals (phthalates and phenolic compounds) in municipal wastewater in the Eastern Cape Province, South Africa and their removal in selected WWTPs with different treatment technology processes. Since there was no prior work on wastewater in the WWTPs in these rural areas, a random selection was made of five WWTPs in the Amathole and Buffalo District Municipalities both in the Eastern Cape with emphasis on different treatment technologies.

7.1. Method validation for quantitation of phthalates and phenolic compounds in wastewater samples

The growing and persistent presence of EDCs and the continuous and their frequent detection in the effluent of WWTPs in the environment in the past decades has been considered a serious health issue (Cesaro and Belgiorno, 2015). The complications that are also associated with the detection and analytical procedures of these diverse compounds of low concentration (a few ng/L to several µg/L) create a challenge for water and wastewater treatment facilities. In order to ensure that the intended method is specific, accurate, reproducible and robust over the specified range of analysis, continuous validation and modification of the existing methods are still a norm for a better and more suitable instrumental technique (Olujimi *et al.*, 2011). The analytical procedure for the determination of trace organics in a complex matrix such as in wastewater is usually accomplished by extraction which is sometimes followed by a clean-up and further separation by instrumented analysis usually chromatographic method (Cai *et al.*, 2003; Ling *et al.*, 2007; Damas *et al.*, 2009).

For this study, the solid-phase extraction (SPE) method, which is faster, easy to manipulate and requires the use of small amounts of solvent, was adopted for the simultaneous separation of groups of EDCs. The percentage recoveries for phthalates using three different sorbent sizes of SPE (C-18X (strata) 500 mg/6mL, C18-U (strata) 1000 mg/6mL, and C18 500 mg/6mL) falls within the standard permissible range (70 -130%) for semi-volatile compounds such as EDCs, which suggests their suitability. The extraction methods that gave good recoveries followed modified guidelines of Sánchez-Avila *et al.* (2009) and US Environmental Protection Agency (EPA) method 8270 (U C T, 2013) using different organic solvent combinations for analyte elution. The third extraction method investigated gave lower recoveries which can probably be attributed to an insufficient amount of solvent used for elution. However, C18-U (strata) 1000 mg/6mL and the modified method of Sánchez-Avila *et al.*, (2009) were adopted for better retention capacity and recovery for wastewater. Results obtained for all experimental recoveries and in the application of adopted method on real wastewater samples showed that the method was sensitive, accurate, reproducible, with good precision and reliable.

7.2. Assessment of physicochemical qualities of wastewater samples in the selected wastewater treatment plants' effluents

The performance evaluation of the five selected WWTPs in the two Municipalities in the Eastern Cape Province, South Africa, using selected physicochemical qualities; temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), turbidity and total suspended solids (TSS) of discharged effluents was also presented in this study. The treatment processes of the three different technologies (AS, TF, and OP) showed a significant influence on the turbidity, TSS, TDS, DO and EC of the wastewater whereas, temperature and pH were not significantly influenced by the processes. The non-significant difference in temperature and pH across the treatment processes suggests dependence of temperature on atmospheric weather conditions and pH on composition of the type of household, business, industry, level of treatment received by the wastewater and public facilities discharging into the water system (Olujimi *et al.*, 2010).

Apart from DO, the values of TSS, turbidity, TDS, and EC, reduced substantially as the wastewater was passed through the treatment processes. Except for turbidity, the quality of effluent released from these WWTPs into the river fall within the South African standard limits for domestic and recreational water (DWAF, 1996a, 1996b). However, this was not the case at all times for the WWTP at Seymour and Bedford. This may be attributed to the difference in volume of wastewater received by these treatment plants at a point in time. Although, the Seymour treatment plant was working below capacity, the incessant wastewater pump breakdown reduced desired treatment on the incoming wastewater thereby, impacting negatively on the receiving water bodies. It was noteworthy to find an unusual high EC in the effluent of TF in Berlin which suggested possible dissolution of substances along its flowing path to the receiving water. There was also a significant increase in turbidity in the effluent of the Bedford treatment plant probably due to algal growth in the effluents that are usually under storage for later use. The WWTPs effluent may not have added additional organic load to the receiving water but the additional nutrient load, in turn, increased the DO in the receiving water. The effluent may have impacted negatively on the receiving water. There were indications that the receiving water also had other point sources of pollution apart from the WWTP's effluents.

7.3. Occurrence and fate of phenolic compounds in wastewater treatment plants

The occurrence of the nine targeted phenolic compounds namely; phenol (PH), 2-chlorophenol (2-CP), 2,4-dimethylphenol (2,4-DMP), 2,4-dichlorophenol (2,4-DCP), 4-

chloro-3-methylphenol (4-C-3MP), 2-nitrophenol (2-NP), 4-nitrophenol, pentachlorophenol (PCP), and 2,4,6-trichlorophenol (2,4,6-TCP) in the influents, effluents from four WWTPs at Adelaide, Alice, Bedford and Berlin as well as their frequencies of detection were presented in this study. All the phenolic compounds were detected at deferent frequencies in the four WWTPs. The removal capacity for all the WWTPs varied between 33 - 96%. The prominent phenolic compounds detected in these areas include; 2-NP, 4-C-3MP, PCP, and 2,4-DMP. These were the same compounds detected in abundance in a similar work carried out in Cape Town South Africa by Olujimi *et al.* (2012) except for phenol. However, the influent concentrations in this study are no match for some of the effluent concentrations in the Cape Town WWTPs which received wastewater from industries that used these chemicals.

This suggests that these compounds are more likely introduced to surface water more from industries that use these chemicals than from domestic waste and agricultural runoff. It could therefore be inferred that these meagre amounts in the influent and receiving water bodies could probably be a result of the decomposition or leachate from the use of products containing these compounds such as plastics, dyes, drugs, pesticides, paper and petrochemical products (Zhou, *et al.*, 2005). The concentration values of phenolic compounds both in the final effluent and receiving waters for all the treatment plants were below the tolerable limits of 5 $\mu g L^{-1}$set by USEPA and the European Union for bathing water (domestic use) (Llompart *et al.*, 2002; Czaplicka, 2004; Fattahi *et al.*, 2007; Petrovic *et al.*, 2009; Silva *et al.*, 2009). It may be safe to say that the relative amounts of these compounds produced in these rural areas were not above the removal capacities of the existing WWTPs.

7.4. Phthalates in municipal wastewater in the Eastern Cape, South Africa and their removal in different wastewater treatment technologies.

The ubiquitous nature of PAEs and their presence in treated and untreated wastewaters and sewage sludge of full scale WWTPs have been widely reported in many countries (Gasperi *et al.*, 2008; Dargnat *et al.*, 2009; Fatoki *et al.*, 2010; Gao *et al.*, 2014). The occurrence of six targeted phthalates namely dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP), di(2-ethyl hexyl) phthalate (DEHP), and di-n-octyl phthalate (DOP)in the influents and final effluents from three WWTPs using activated sludge (AS) in Alice, oxidation pond (OP) in Bedford, and trickling filters (TF) in Berlin as well as their detection frequencies have been presented in this study. In addition, the results of the investigation of the six PAEs at different treatment points of three selected WWTPs which adopted the AS technology namely; Adelaide, Alice and Seymour all in the Amathole District Municipality in the Eastern Cape Province, South Africa and their frequencies of detection were also presented. DBP was the most abundant phthalate found in the influent of all the WWTPs.

However, unlike in similar previous studies around the globe (Dargnat *et al.*, 2009; Clara *et al.*, 2010; Gao *et al.*, 2014), DBP took precedence in order of abundance over DEHP. The reason may be that many industrial products such as PVC and other household materials being consumed contain DBP as an additive. Moreover, the use of DBP in other products commonly used in areas such as nail polish, aftershave, perfumes, herbal, pharmaceuticals and herbal coating may also have contributed to the abundance of DBP over DEHP. The dominance of these three compounds DBP, DEHP and BBP across the treatment processes is worthy of note. The results of the analysis showed that lower levels of the target phthalates were present in the final effluents when compared with the levels in the influents, showing good removal efficiency of the WWTPs for the phthalates under consideration. DBP has the highest removal rate ranging between (92 - 99% removal) whereas; DEHP showed some level of resistance with removal range (36 – 84%). This supports the statement that PAEs with shorter-chain such as DBP are more easily degraded than longer-chain PAEs like DEHP (Gao *et al.*, 2014).

Of all the phthalates, DMP was the compound that least occurred. WWTPs with activated sludge technology appeared to be the most effective based on their efficient removal of DMP, DEHP and DOP. Of all the PAEs, DEP and DEHP had 100% detection frequency across the three WWTPs in Adelaide, Alice and Seymour. The WWTPs showed efficient removal of the PAEs with Alice and Adelaide being the most efficient with removal performance ranging from 64 - 99%. However, a lower performance was recorded with Seymour WWTP (33 - 94%). This was attributed to the single tank technology in use in the area that allowed limited time for clarification. In a similar vein, AS technology, showed better performance in the removal of PAEs (77 - 99%), followed by TF technology (76 - 98%) and OP (61 - 98%) in that order. Influents of Alice WWTP appeared to have the highest concentration of PAE, probably due to the high population in this area including the presence of the University population.

Of the PAE compounds, DBP, DEHP and BBP occurred most frequently in the influents, secondary and final effluents of all the three AS-operated WWTPs and other treatment technologies. Seymour (AS) plant appeared to be the weakest of the plants with respect to the six targeted PAEs removal. The poor performance of this plant was found to impact negatively on the receiving watershed by increasing the total PAE concentrations. Generally, the concentrations of PAEs detected in the entire receiving watershed connected to all the investigated treatment plants were above the EQU of 1.3 μgL^{-1} set by the European Union for DEHP in surface water and above 3 μgL^{-1} criteria standard set by USEPA for the protection of aquatic life. DEHP was frequently detected both in the upstream and downstream signifying its importance in the production of polyvinyl chloride (PVC) based products, as PAE concentrations in these areas were largely attributed to domestic and agricultural runoffs.

Although, the plants were not designed to remove micro-pollutants, the efficient performances of these treatment plants are connected to their strong ability to remove suspended solids. This study has found highly significant positive correlations between total suspended solids (TSS), turbidity and all the phthalates. Berge *et al.* (2012) concluded that removal of particulate matter is certainly linked to removal of TSS.

All the six phthalate compounds were also detected in the sludge samples from all the treatment plants. DBP was the most highly detected in the sludge from Alice and Bedford while BBP was the most abundant in Berlin treatment plant. It is assumed that the disappearance of the compounds from the sludge has to do with their partition coefficients. Byrns, (2001) has shown that compounds that are poorly degraded have $\log K_{ow} \geq 4$. Generally, the concentrations of DBP, BBP, DEHP and DOP in these studies were above the European Union legislation of 100 µg/g d.w on the use of sludge for agriculture (EU legislation 86/278/EEC) (Inglezakis *et al.*, 2014).

In all the three treatment technologies, the removal efficiency for DEP increased with an increase in temperature. This probably supports the earlier conclusions that the compound is volatile and has the tendency to be lost during treatment technology (Berge *et al.*, 2012).

In conclusion, it will be wise to go with the general statement that suggests that EDCs removal in WWTPs technology is generally influenced more by adsorption than biodegradation (Qiang *et al.*, 2013).

7.5. Conclusions and future prospects

The existing WWTPs in Adelaide, Alice, Bedford, Berlin and Seymour in the Eastern Cape Province, South Africa, reduced substantially the levels of EDCs; phthalates and phenolic compounds detected in wastewaters in these rural areas. The combined effects of low solubility of phthalates in water and strong affinity for adsorption on particulate matter enhanced the removal performance of these WWTPs that are not designed specifically to remove them. The relative amount of EDCs generated as a result of leachate of these contaminants from domestic products containing them and agricultural runoffs were lower than the potential amounts that may be released by industries using these chemicals.

Although, activated sludge technology showed better removal performance than the trickling filter (TF) and the oxidation pond (OP), it is sufficient to say that these technologies have the potential capacity to remove at least over 50% of these compounds from wastewater and to concentrate them in wastewater sludge. The health risks that are associated with the use of this sludge in agriculture without treatment may be more than what is anticipated from the presence of these EDCs in water. Contamination of groundwater through percolation of agricultural runoffs through the soil is possible consequence from the use of such sludge for agricultural purposes. This will in turn pose serious health challenges to the ecosystem as human and aquatic animals are exposed to EDCs.

The concentration levels of EDCs especially phthalates found in WWTPs effluents, receiving water bodies and particularly wastewater sludge in these area of study call for the urgent attention of the constituted authorities if the consequent negative health effects on humans and aquatic life is to be avoided. Agriculture still remains crucial in food security for the poor in South Africa who constitutes up to 40% of the population. Since they are predominantly concentrated in the rural areas, serious attention has to be paid to the quality of sludge generated from WWTPs for farming.

In advanced countries, the incorporation of tertiary treatment process such as advanced oxidation process with the existing WWTPs specifically for micro-pollutants removal and legislation of policies that place restrictions on the use of these dangerous chemicals are the two remedial actions that have been adopted. However, in developing countries such as South Africa, if the hydraulic retention time (HRT) of the wastewater in the conventional treatment plants can be optimized, it may enhance the biodegradation and adsorption routes of removal of EDCs in the WWTPs. In addition, increased sludge retention time (SRT) and microbial diversity may further increase biodegradation and adsorption of micro-pollutants.

There is the urgent need for the constituted authorities in South Africa to consider upgrading the standard of the old wastewater treatment facilities in order to meet the strict effluent standards for a safe environment and aquatic life.

The restrictions on the use of these EDC compounds or the environmental quality standards set for their limits in surface water in some advanced countries in the world such as US, European countries (France, Italy, Finland) and China was based on information that was available on the levels of these compounds in their environment. In South Africa today, there is no information on the concentration levels of phthalates and phenolic compounds in WWTPs upon which any policy can be based. It is therefore expedient to commit to more research work in this area considering the fact that the adverse health effects of these micro-pollutants is a challenge not only to organisms but their progeny as well.

To achieve this noble task, a routine monitoring of the performance of these WWTPs is vital so that abnormal fluctuations can easily be detected and addressed by the relevant authorities. Moreover, less expensive but efficient extraction methods should be developed in future research in order to encourage the routine investigation of pollutants in the environment especially in Africa.

References

Abdel daiem, M. M., Rivera-Utrilla, J., Ocampo-Pérez, R., Méndez-Díaz, J. D. and Sánchez-Polo, M. (2012) ‘Environmental impact of phthalic acid esters and their removal from water and sediments by different technologies - A review’, *Journal of Environmental Management*, 109, 164–178. doi: 10.1016/j.jenvman.2012.05.014.

Adeniyi, A., Dayomi, M., Siebe, P. and Okedeyi, O. (2008) ‘An assessment of the levels of phthalate esters and metals in the Muledane open dump, Thohoyandou, Limpopo Province, South Africa.’, *Chemistry Central journal*, 2, 1–9. doi: 10.1186/1752-153X-2-9.

Balabanici, D., Hermosilla, D., Merayo, N., Klemencic, A. K. and Blanco, Á. (2012) ‘Comparison of different wastewater treatments for removal of selected endocrine disruptors from paper mill wastewaters’, *Journal of environmental science and health. Part A, Toxic/hazardous substance & environmental engineering*, 47(10), 1350–63. doi: 10.1111/cobi.12645.

Batt, A. L., Kim, S., and Aga, D. S. (2007) ‘Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations.’, *Chemosphere*, 68(3), 428–435.

Berge, A., Gasperi, J., Rocher, V., Coursimault, A. and Moilleron, R. (2012) ‘Phthalate and alkylphenol removal within wastewater treatment plants using physicochemical lamellar clarification and biofiltration’, *WIT Transactions on Ecology and the Environment*, 164, 357–368. doi: 10.2495/WP120311.

Byrns, G. (2001) ‘The fate of xenobiotic organic compounds in wastewater treatment plants.’, *Water Research*, 35(25), 23–33.

Cai, K., Elliott, C. T., Phillips, D. H., Scippo, M. L., Muller, M. and Connolly, L. (2012)

‘Treatment of estrogens and androgens in dairy wastewater by a constructed wetland system.’, *Water Research.*, 46(7), 2333–2343.

Cai, Y. ., Jang, G., and Liu, J. . (2003) ‘Solid-phase extraction of sev- eral phthalate esters from environmental water samples on col- umn packed with polytetrafluoroethylene turning’, *Analytical Sciences*, 19, 1491–1494.

Cesaro, A. and Belgiorno, V. (2015) ‘Removal of endocrine disruptors from urban wastewater by advanced oxidation processes (AOPs): a review’, *The Open Biotechnology Journal*, 10(1), 0–28. doi: 10.2174/1874070720151112E012.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C. and Chovanec, A. (2010) ‘Chemosphere Occurrence of phthalates in surface runoff , untreated and treated wastewater and fate during wastewater treatment’, *Chemosphere*. 78(9), 1078–1084. doi: 10.1016/j.chemosphere.2009.12.052.

Czaplicka, M. (2004) ‘Sources and transformations of chlorophenols in the natural environment’, *Science of the Total Environment.*, 322(1–3), 21–39.

Damas, E. Y. C., Medina, M. O. C., Clemente, A. C. N., Diaz, M. A. D., Bravo, L. G., Ramada, R. M. and Porto, R. M. D. (2009) ‘Validation of an analytical methodology for the quantitative analysis of petroleum hydrocarbons in marine sediment samples.’, *Quimica Nova*, 32(4), 855–860. Available at: wos:000267331400006.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R. J. and Van der Kraak, G. (2002) *Global assessment of the state-of-the-science of endocrine disruptors, WHO/PCS/EDC/02.2, International Programme on Chemical Safety, World Health Organisation.*

Dargnat, C., Teil, M. J., Chevreuil, M. and Blanchard, M. (2009) ‘Phthalate removal throughout wastewater treatment plant. Case study of Marne Aval station (France)’, *Science* *of the Total Environment*. 1235–1244. doi: 10.1016/j.scitotenv.2008.10.027.

DWAF (1996a) *South African Water Quality Guidelines, (Volume 2), Recreational Water Use (2nd edn.). Department of Water Affairs & Forestry, Pretoria.*

DWAF (1996b) *South African water quality guidelines (Vol.1), domesticuses(2nded.)Pretoria:Department of Water Affairs and Forestry.*

Fatoki, O., Olujimi1, O., Odendaal, J. and Genthe., B. (2012) *Health Risk Assessment of Plasticizer in Wastewater Effluents and Receiving Freshwater Systems, InTechOpen*.

Fatoki, O. S., Bornman, M., Ravandhalala, L., Chimuka, L., Genthe, B. and Adeniyi, A. (2010) 'Phthalate ester plasticizers in freshwater systems of Venda, South Africa and potential health effects', *Water SA*, 36(1), 117–126. doi: 10.4314/wsa.v36i1.50916.

Fattahi, N., Assadi, Y., Hosseini, M. R. M., and Jahromi, E. Z. (2007) 'Determination of chlorophenols in water samples using simultaneous dispersive liquid-liquid microextraction and derivatization followed by gas chromatography-electron-capture detection.', *Journal of Chromatography A*, 1157(1–2), 23–9.

Gao, D., Li, Z., Wen, Z. and Ren, N. (2014) 'Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China', *Chemosphere*. 95, 24–32. doi: 10.1016/j.chemosphere.2013.08.009.

Gao, D. W. and Wen, Z. D. (2016) 'Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes', *Science of the Total Environment*. 541, 986–1001. doi: 10.1016/j.scitotenv.2015.09.148.

Gasperi, J., Garnaud, S., Rocher, V. and R., M. (2008) 'Priority pollutants in wastewater and combined sewer overflow', *Science of the Total Environment*, 407(1), 263–272.

Hileman, B. (2007) ‘California bans phthalates in toys for children’, *Chem. Eng. News*, 85(43), 12. doi: 10.1021/cen-v085n043.p012a.

Hileman, B. (2007) *California bans phthalates in toys for children*, *Chem. Eng. News*.

Horn, O., Nalli, S., Cooper, D. and Nicell, J. (2004) ‘Plasticizer metabolites in the environment.’, *Water Research.*, 38, 3693–3698.

Inglezakis, V., Zorpas, A. A., Karagiannidis, A., Samaras, P., Voukkali, I. and Sklari, S. (2014) ‘European Union legislation on sewage sludge management’, *Fresenius Environmental Bulletin*, 23(2A), 635–639.

Kookana, R., Williams, M., and Waller, N. (2007) *Endocrine- disrupting chemicals in the Australian environment*, *Land & Water Australia* 1-14.

Kraigher, B., Kosjek, T., Heath, E., Kompare, B. and Mandić-Mulec, I. (2008) ‘Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors’, *Water Research.*, 42(4578–4588).

Ling, W., Gui-biin, J., Ya-qi, C., Bin, H., Ya-wei, W. and Da-zhong, S. (2007) ‘Cloud point extraction coupled with HPLC-UV for the determination of phthalate esters in environmental water samples.’, *Journal of Environmental Sciences*, 19, 874–878.

Llompart, M., Lourido, M., Landin, P., Garcia-Jares, C., and R. Cela, J. (2002) ‘Optimization of a derivatization-solid-phase microextraction method for the analysis of thirty phenolic pollutants in water samples.’, *Journal of Chromatography A*, 963(1–2), 137–148.

Loraine, G. A. and Pettigrove, M. E. (2006) ‘Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California.’, *Environmental Science & Technology*, 40, 687–695.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S. and Wang, X. C. (2014) ‘A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment’, *Science of the Total Environment*, 619–641. doi: 10.1016/j.scitotenv.2013.12.065.

Momba, M. N. B., Osode, A. N., and Sibewu, M. (2006) ‘The impact of inadequate wastewater treatment on the receiving watebodies - Case study: Buffalo City and Nkonkobe Municipalities of the Eastern Cape Province.’, in *Durban, South Africa : Water SA, Water Institute of South Africa (WISA) Bennial Conference.*, 0378–4738.

Nakada, N., Tanishima, T., Shinohara, H. and Kiri, K. (2006) ‘Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment’, *Water Research*, 40(17), 3297–3303. doi: 10.1016/j.watres.2006.06.039.

Olujimi, O., Fatoki, S., Odendaal, J., and Daso, A. P. (2012) ‘Chemical monitoring and temporal variation in levels of endocrine disrupting chemicals (priority phenols and phthalate esters) from selected wastewater treatment plant and freshwater systems in Republic of South Africa’, *Microchemical Journal*. 101, 11–23. doi: 10.1016/j.microc.2011.09.011.

Olujimi, O., Fatoki, S., Odendaal, J., and Okonkwo, J. (2011) ‘Solid-Phase Extraction Method for the Analysis of Eleven Phenolic Pollutants in Water Samples’, *Asian Journal.*, 23(2), 657–662.

Olujimi, O. O., Fatoki, O. S., Odendaal, J. P. and Okonkwo, J. O. (2010) ‘Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: A need for more monitoring’, *Water SA*, 36(5), 671–682. doi: 10.4314/wsa.v36i5.62001.

Park, M. A., Hwang, K. A., Lee, H. R., Yi, B. R., Jeung, E. B. and Choi, K. C. (2012) ‘Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. *Molecular Medicine Reports*, 5(3), 761–766. http://dx.doi.org/10.3892/mmr.2011.712.

Petrovic, M., de Alda, M. J. L., Diaz-Cruz, S., Postigo, C., Radjenovic, J., Gros, M. and Barcelo, D. (2009) ‘Fate and removal of pharmaceuticals and illicit drugs in conventional and membrane bioreactor wastewater treatment plants and by riverbank filtration.’, *Philosophical Transactions of the Royal Society A*, 367(1904), 3979–4003. doi: 10.1098/rsta.2009.0105.

Qiang, Z., Dong, H., Zhu, B., Qu, J. and Nie, Y. (2013) ‘A comparison of various rural wastewater treatment processes for the removal of endocrine-disrupting chemicals (EDCs)’, *Chemosphere*, 92(8), 986–992. doi: 10.1016/j.chemosphere.2013.03.019.

Roslev, P., Vorkamp, K., Aarup, J., Frederiksen, K. and Nielsen, P. H. (2007) ‘Degradation of phthalate esters in an activated sludge wastewater treatment plant’, *Water Research*, 41(5), 969–976. doi: 10.1016/j.watres.2006.11.049.

Sánchez-Avila, J., Bonet, J., Velasco, G. and Lacorte, S. (2009) ‘Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant’, *Science of the Total Environment*. 407(13), 4157–4167. doi: 10.1016/j.scitotenv.2009.03.016.

Schiliro, T., Pignata, C., Rovere, R., Fea, E. and Gilli, G. (2009) ‘The endocrine disrupting activity of surface waters and of wastewater treatment plant effluents in relation to chlorination’, *Chemosphere*. 75(3), 335–340. doi: 10.1016/j.chemosphere.2008.12.028.

Silva, S. L., Alves, A., and Santos, L. (2009) ‘Uncertainty measurement of chlorophenols and PCBs analyzed in aqueous media by SPME-GC-ECD.’, *Journal of Chromatographic Science*, 47(2), 1 03-109.

U C T (2013) ‘Solid Phase Extraction of EPA 8270 Compounds in Water Procedure ’:, 15.

WHO-UNEP (2013) *State of the Science: Endocrine Disrupting Chemicals - 2012, Summary for Decision Makers*. Available at: http://apps.who.int/iris/bitstream/10665/78102/1/WHO_HSE_PHE_IHE_2013.1_eng.pdf?ua=1.accessed 17/02/2017.

Xue, W. C., Wu, C. Y., Xiao, K., Huang, X., Zhou, H. D., Tsuno, H. and Tanaka, H. (2010) ‘Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation’, *Water Research.*, 44, 5999–6010.

Zhou, F., Li, X., and Zeng, Z. (2005) ‘Determination of phenolic compounds in wastewater samples using a novel fiber by solid-phase microextraction coupled to gas chromatography’, *Analitica Chimica Acta*, 538, 63–70.

APPENDIX

Appendix 1

Calibration curves for some selected phthalate esters and surrogates

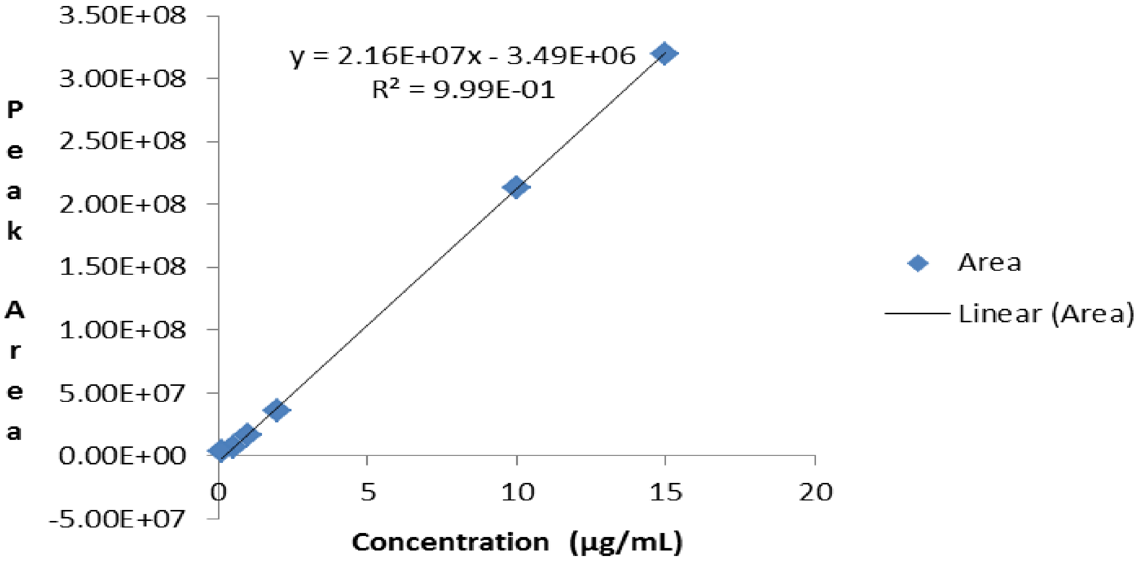


Figure A.1 Calibration curve for dimethyl phthalate.

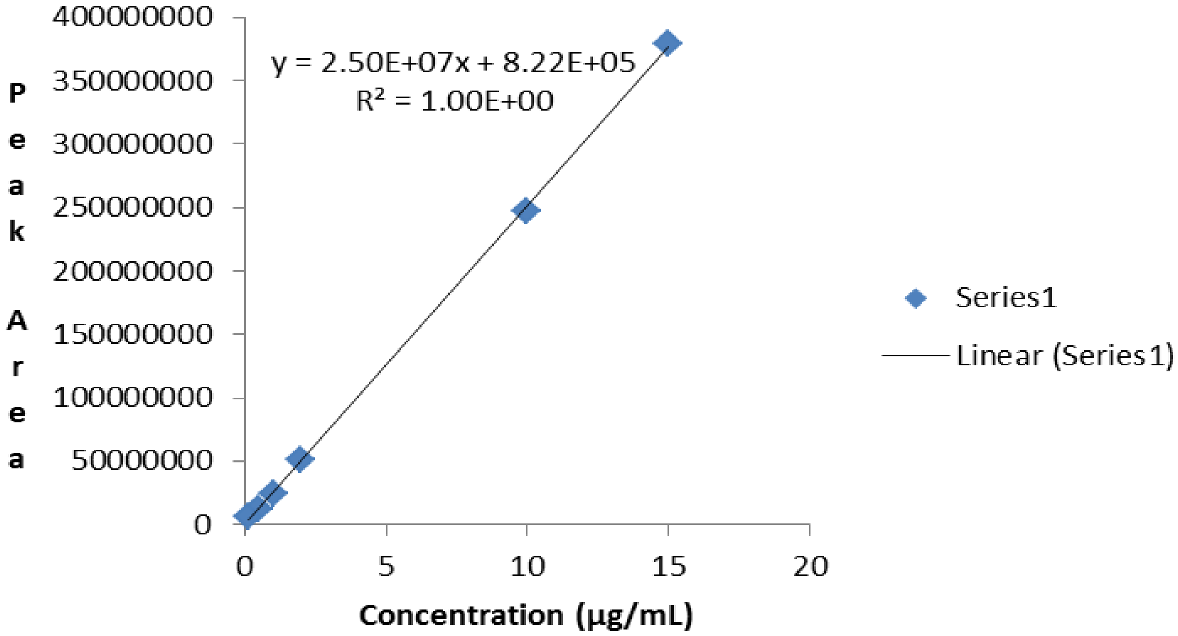


Figure A.2 Calibration curve for di-n-butyl phthalate (DBP).

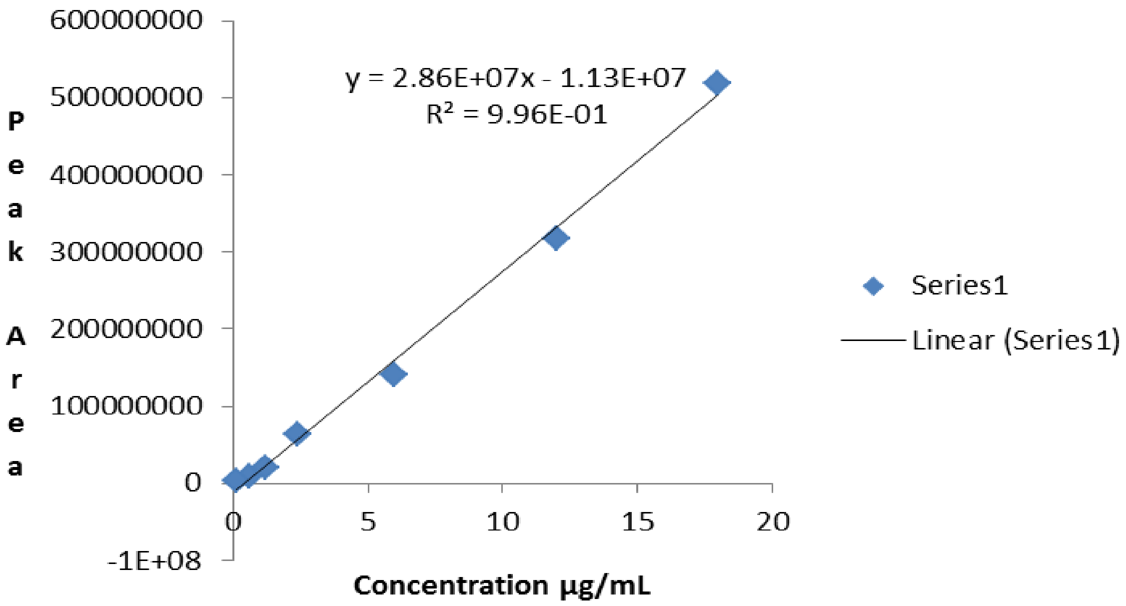


Figure A.3 Calibration curve for 2-fluorobiphenyl (surrogate).

1.2. Calibration curves for selected phenolic compounds and surrogate

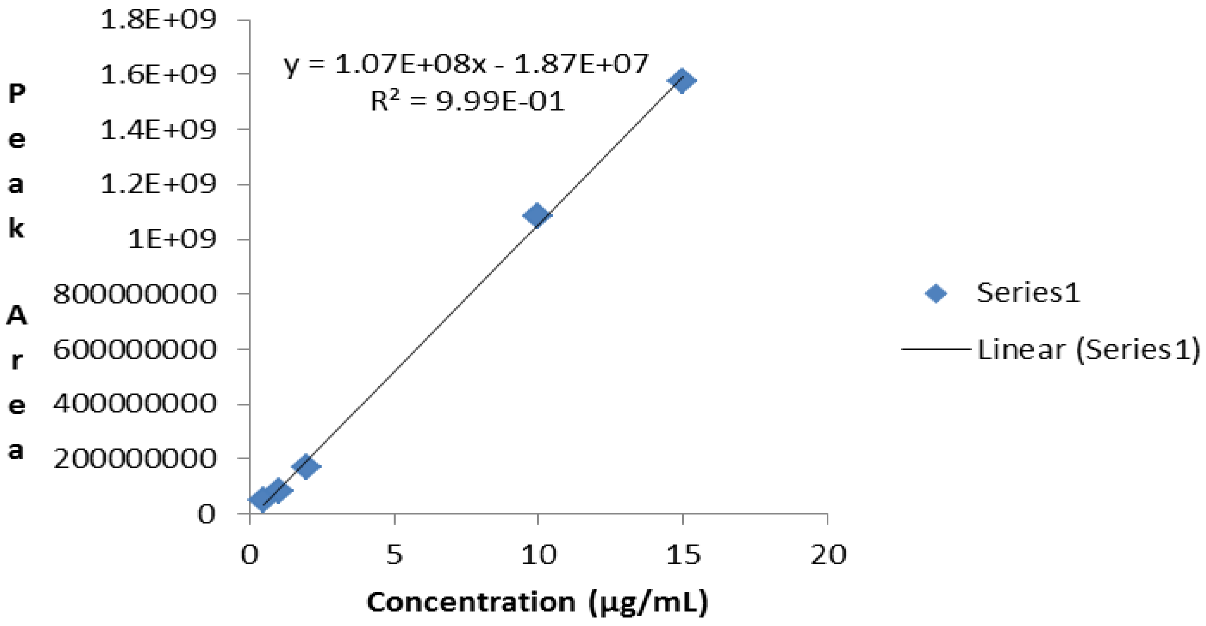


Figure A.4 Calibration curve for 4-chloro-3-methyl phenol.

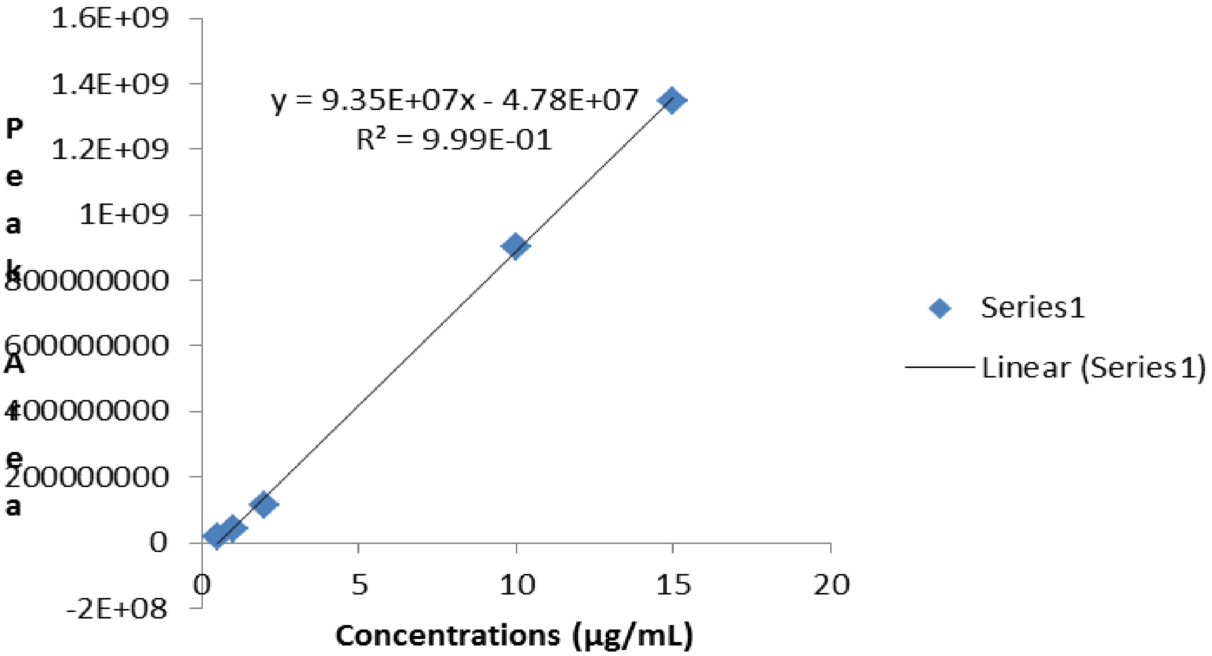


Figure A.5 Calibration curve for 2,4,6-trichlorophenol.

Appendix 2

Computations of analyte concentration from final extract volume and instrument response.

2.1. The same method was used for phthalate esters and phenolic compounds.

$$\text{Concentration of analyte } (\mu g/mL) = \frac{\text{instrument conc.} \mu g/mL \times \text{final extract volume } (mL)}{\text{Initial volume of water sample (usually 500mL)}}$$

*Where instrument concentrations were calculated from the calibration curve equation using instrument corresponding peak area for each analyte

$$\text{Concentration in } \mu g/L = (\text{Concentration in } \mu g/mL \text{ above}) \times 1000$$

2.2. For sludge samples

$$\text{Concentration of analyte } (\mu g/g.d.w.) = \frac{\text{instrument conc.} \mu g/mL \times \text{final extract volume } (mL)}{\text{Initial weight of sludge (usually 2g)}}$$

Where d.w. = dry weight

2.3. To calculate percentage recovery of spiked surrogate concentration;

$$\frac{\text{Concentration of surrogate recovered } \mu g/L \text{ (following method above)}}{\text{Spiked concentration (known)}} \times 100$$

2.4 Percentage recovery of laboratory fortified matrix samples ®

$$R = \frac{LFM - SM \text{ --------..}}{\text{Spiked concentration}} \times 100$$

Where LFM = Laboratory fortified matrix concentration; SM = concentration of sample matrix

Appendix 3

Chromatograms of phthalates and phenolic compounds in selected wastewater samples

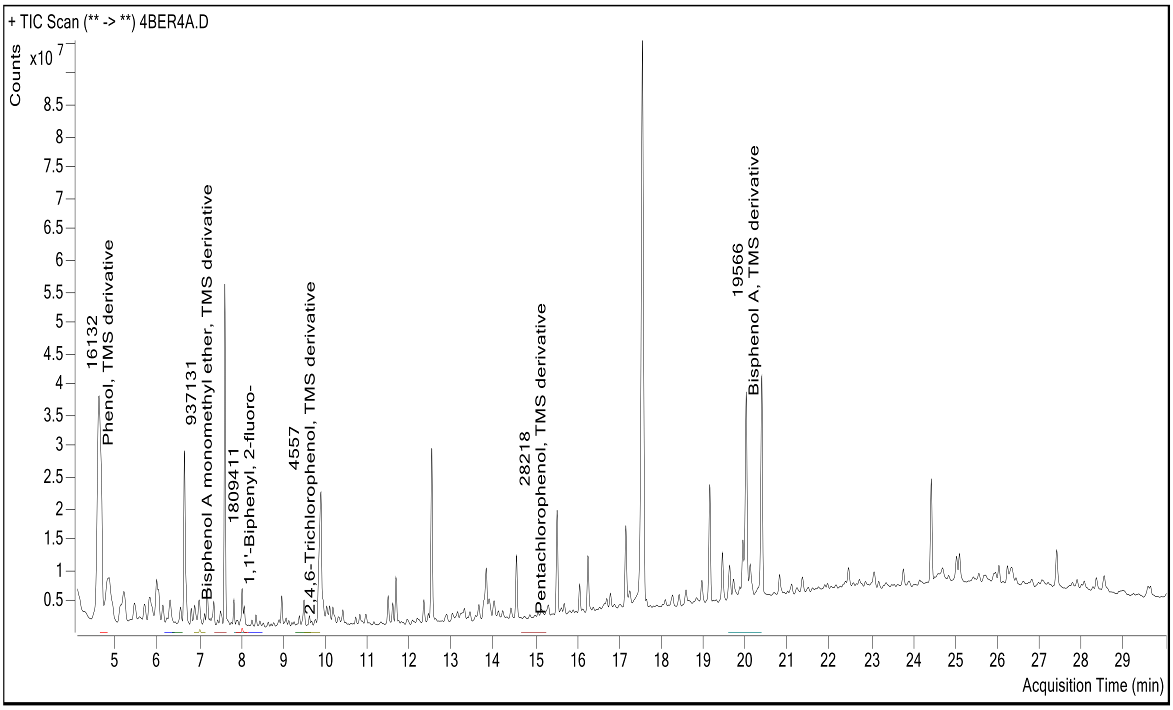


Figure B.1 Chromatogram of phenolic compounds in Berlin wastewater sample.

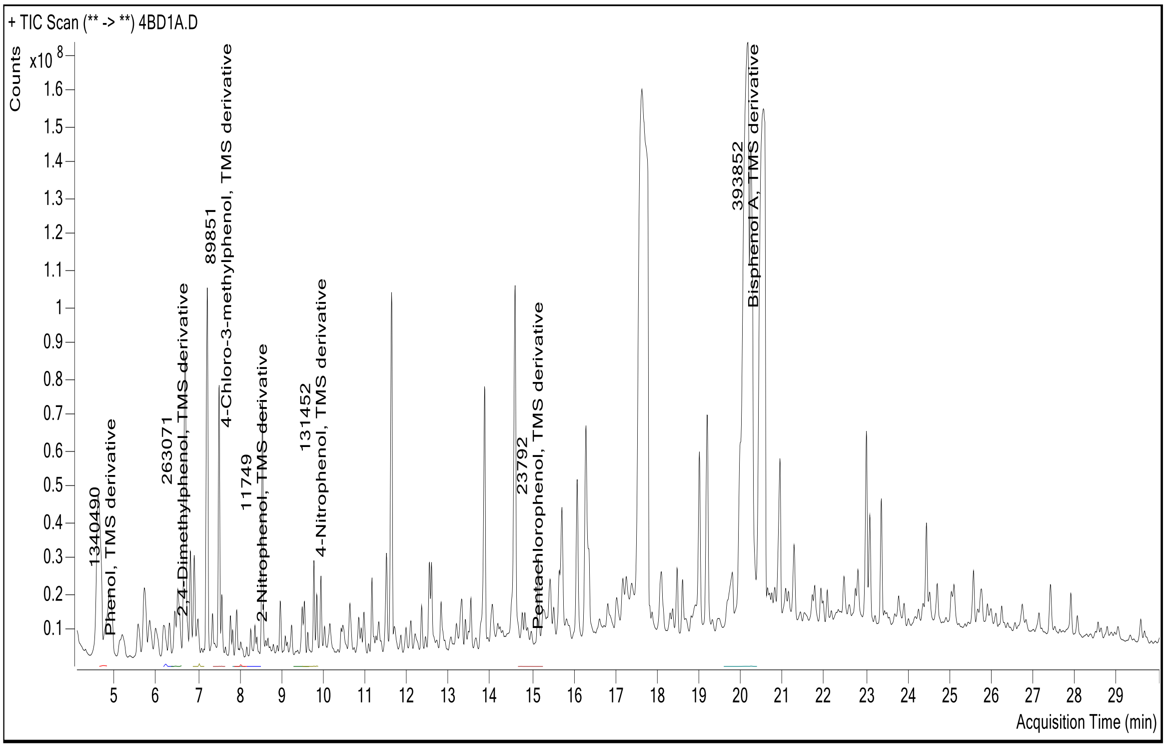


Figure B.2 Chromatogram of phenolic compounds in Bedford wastewater sample.

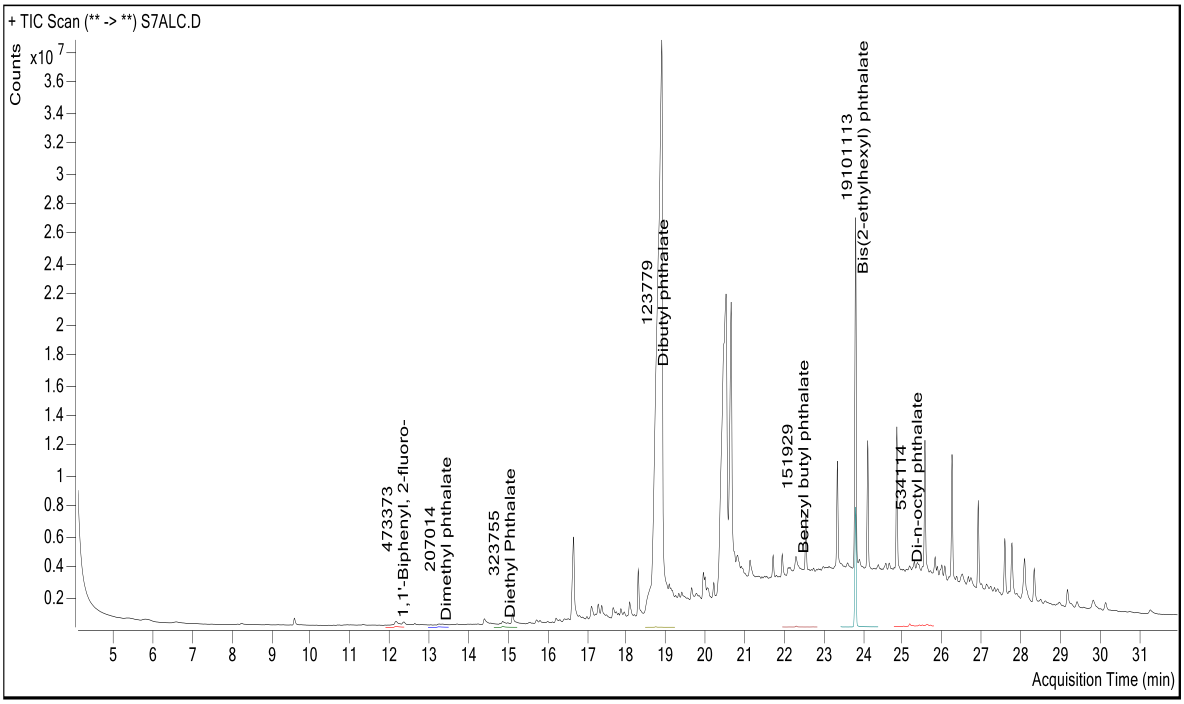


Figure B.3 Chromatogram of phthalate esters in Alice wastewater samples.

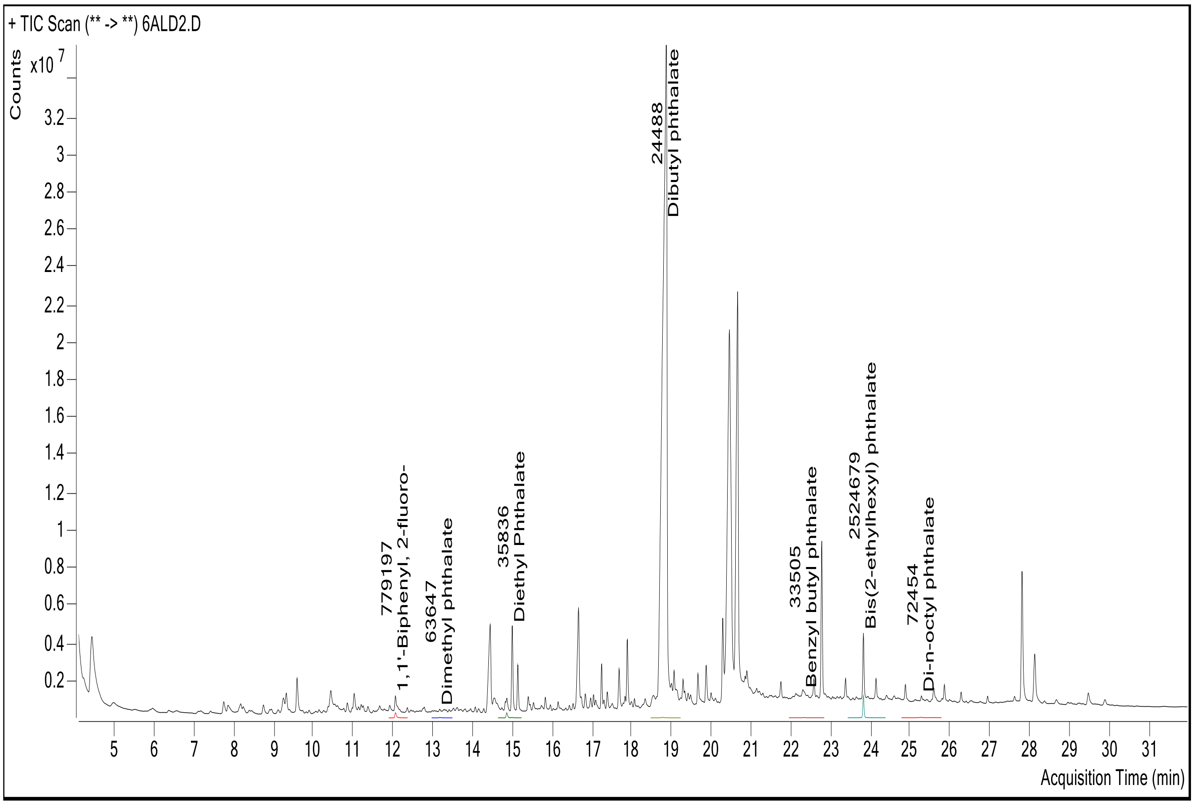


Figure B.4 Chromatogram of phthalate esters in Adelaide wastewater samples