Lancaster Environment Centre
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Application of a diffusive passive water sampler to investigate the fate of organic pollutants in wastewater treatment works and comparison of engineered and natural treatment systems

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This is dedicated to God Almighty

Abstract

Compliance with Environmental Quality Standards for organic pollutants is paramount to healthy living and sustainability of aquatic organisms. The European Commission expects that certain standards should be met by members' state although the procedure or operational approach to meeting the standards is left with each member state. For some members, the costs of monitoring their surface water basins are excessive and as such, meeting the expected standards has been a challenge. This study was designed to investigate and further validate the use of passive water as investigative tools to measure the presence of organic pollutants in our environment as well as a comparison of engineered and natural treatment systems. Various studies have been undertaken with the use of Diffusive Gradient in Thin-film passive samplers to validate their potential wide range applications including in wastewater treatment works. The study investigated nature-based treatment technology as a viable alternative to the conventional treatment works for the removal of personal care product ingredients and antibiotics from the environment. Removal efficiency rates and chemical partitioning in the treatment works were also considered between the sludge cakes that are used by the farmers as organic manure and the removal process.

DGT samplers containing HLB gels for personal care products and Amberlite XAD-18 for antibiotics were deployed for various aspect of this study. This study investigated the performance of passive sampler in the selected 6 nature-based treatment technologies and 3 conventional treatment works in Italy where eight antibiotics were detected out of 23 that were investigated. Removal of Clarithromycin ranged from < 1% to 100% for nature-based technologies and < 1% to 100% for the conventional systems. Removal rates of other pharmaceutical ingredients are up to 100% in both systems, but the SPD had an influent/effluent concentration of 91 ngL^{-1} to 810 ngL^{-1} in the Nature-based system. Removal of personal care products between the two systems was very comparative where average removals of preservatives in nature-based and conventional treatment was 72% to 58%, Antioxidants were 57% to 44% except for TCC in both systems and 47% to 57% except for Nonylphenol respectively. High concentrations of TCC and NP at the effluents may have resulted from the degradation of parents' compounds that were not detected at the influents channels. However, some low removal rates in some of the treatment systems may have resulted from low degradation or high sorption affinity to organic matter since DGT only measure dissolved compounds.

Sampling campaigns in wastewater treatment works in North West England focused more on DGT for long-term monitoring of organic pollutants as well as partitioning in the treatment works. A 52-week sampling campaign at the influent and effluent channels confirmed a high removal efficiency of personal care products. The overall average removal rates in this site over the 52 weeks ranged from < 1% 4-T-OP to 92% MEP. However, 28 days sampling to investigate the chemical partitioning at the same works confirmed less variability in the treatment works with PCPs removal efficiency of 14% to 100% compared to the long-term monitoring.

A further study was conducted on the sludge cake that is gaining a wide acceptance in agricultural use being the most economical method for disposal which ultimately reduces costs in terms of farmers investments on enhancing the productivity of their land. The major associated costs are the transportation and spreading/soil modification with the cakes which is far less than the costs of incineration which a common disposal method in many European countries. The laboratory study of 21 days deployment period compared the competing removal mechanisms of degradation and sorption of chemicals.

Current regulations reduce the considerable effect of biohazard on the agricultural land by leaving a substantial time of not less than 28 days after amending the soils before cultivation. Some compounds degrade swiftly while uptake of PHBA increased from 859 $\mathrm{ngL^{-1}}$ to 2,114 $\mathrm{ngL^{-1}}$ over the period, which shows that PHBA would need more than 21days to fully desorb and degrade. Having considered other passive samplers to include Chemcatcher, POCIS and grab sampling, the size of DGT and its simple operations procedures was very helpful in delivering this study.

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List of Abbreviations

A	Exposure window area, cm2
ACN	Acetonitrile
AMX	Amoxilin
AS	Activated sludge
BAF	Biological aeration filter
BEP	Benzylparaben
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BOD	Biochemical Oxygen Demand
BPA	Bisphenol-A
BPB	Bisphenol-B
BPF	Bisphenol-F
BPs	Bisphenols
BUP	Butylparaben
CAS	Chemical Abstracts Service
Cb	Analyte concentration in the bulk solution
C_{DGT}	Water concentration measured by DGT
COD	Chemical Oxygen Demand
C_S	Analyte concentration in the passive sampler
C_W	Analyte concentration in the aqueous environment
CFX	Ciprofloxacin
CLM	Clarithromycin
CTW	Conventional Treatment Works
δ	Thickness of diffusive boundary layer, mm
Δg	Thickness of the diffusive layer, mm
D_{25}	Diffusion coefficient of analyte at 25 °C, 10-6 cm^2 s^{-1}
DBL	Diffusive boundary layer
De	Diffusion coefficient of analyte, 10-6 cm^2 s^{-1}
DES	Diethylstilboestrol
DGT	Diffusive gradients in thin films
DOM	Dissolved organic matter
DT	Diffusion coefficient of analyte at temperature T, 10-6 cm^2 s^{-1}
E1	Estrone
E2	β-estradiol
E3	Estriol
EA	Ethyl acetate
EE2	17α-Ethinylestradiol
EFX	Enrofloxacin
EOCs	Emerging organic contaminants
EQSs	Environmental quality standards
ESI	Electrospray ionisation
ETP	Ethylparaben
ETM	Erythromycin
ETM-H20	Erythromycin-H_2O
h	Hour
HEP	Heptyl paraben
HLB	hydrophilic-lipophilic-balanced
HPLC	High performance liquid chromatography
i-PRP	Iso- Propylparaben
IS	Ionic strength

ISs	Internal standards
Kow	Octanol–water partition coefficient
LC-MS	Liquid chromatography- mass spectrometer
LIM	Lincomycin
LWWTP	Lancaster wastewater treatment plant
M	Analyte mass accumulated in the passive sampler
MFX	Marbofloxacin
MEP	Methylparaben
MQ	Milli-Q
NBT	Nature Based Technologies
NFX	Norfloxacin
NP	Nonylphenol
OFX	Ofloxacin
OPP	Ortho-phenylphenol
PES	Polyethenesulfone
PHBA	4-Hydroxybenzoic acid
POCIS	Polar organic chemical integrative sampler
POPs	Persistent organic pollutants
PRP	Propylparaben
PUF	Polyurethane foam
PWS	Passive water sampling
QA/QC	Quality assurance/quality control
REACH	Registration, Evaluation, Authorization, and Restriction of Chemicals
ROM	Roxithromycin
SDM	Sulfadimethoxine
SDX	Sulfadimethoxine
SDZ	Sulfadiazine
SMX	Sulfamethoxazole
SMZ	Sulfamethazine
SPE	Solid-phase extraction
SPD	Sulfapyridine
SPMDs	Semipermeable membrane devices
STZ	Sulfathiazole
RS	Sampling rate
t	Time
T	Temperature
TCC	Triclocarban
TCS	Triclosan
TMP	Trimethoprim
4-t-OP	4-tert-octylphenol
TBHQ	Tertiary butylhydroquinone
TWA	Time-weight average
WFD	Water Frameworks Directives
WWTPs	Wastewater treatment plants

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1. Introduction

1.1. Emerging contaminants in surface waters

Prevalence of Emerging Organic Pollutants (EOP) in the environment has raised environmental and public health concerns over the years(Pal *et al.*, 2014). Increasing industrial production and consumption contribute immensely to the concentration of these pollutants in the environment (Zhang *et al.*, 2012). Studies have revealed that many of the organic pollutants such as Antibiotics, Endocrine Disruption Compounds (EDC), and Personal Care Product ingredients (PCPs) have been detected in the effluents of most of the domestic Waste Water Treatment plants (WWTP) (Ifelebuegu and Ezenwa, 2010; Chen, 2016). This leaves us with the question of how do these organic pollutants find their ways into surface waters (Jiang et al. 2013) and what are their effects on biota either as single substances or as mixtures (Camargo and Alonso, 2006; Chen, 2013). Many of the previous studies on the Persistent Organic Pollutants (POPs) focused mainly on the concentrations of these chemicals in the environment (Kot, Zabiegała and Namieśnik, 2000; Kot-Wasik *et al.*, 2007; Seethapathy, Górecki and Li, 2008a; Chen *et al.,* 2012a), However, bioaccumulation is more important as it gives insight into the risk that these pollutants pose on the terrestrial organism (Kelly and Gobas, 2003), and in the aquatic faunas (Jamieson *et al.*, 2017).

The level of concerns about these organic contaminants has increased due to their presence in drinking water (Schriks *et al.*, 2010), and the likely toxicological implication of these compounds on human health (Schriks *et al.*, 2010). It is therefore important that more information is made available to the public to understand the danger and consequences of unscrupulous anthropogenic activities (Huibers, Redwood and Raschid-Sally, 2009), the behaviour of these organic pollutants such as transportability, non-biodegradation, and bioaccumulation (Fitzgerald and Wikoff, 2014) of some of their ingredients, and some measure that could be taken to reduce the dangers associated with uncontrolled disposal of PPCPs. A high concentration of POP has been reported in eggs (Pusch *et al.*, 2005), blood samples (Mitra *et al.*, 2011) and human breast milk from Yucatan Peninsula, Mexico (González-Navarrete *et al.*, 2010).

1.2. Sources to the Environment

Pharmaceuticals and Personal Care Products (PPCPs) release into the environment has been traced to various sources to include untreated sewage from consumer use and excretion (Dai *et al.*, 2015), agricultural use (Witte, 1998); improper disposal of expired and unused PPCPs (e.g. via the toilet or emptying down drains) (Kusturica *et al.*, 2012); wastewater from hospitals (Hocquet, Muller and Bertrand, 2016), manufacturers of cosmetic pharmaceutical drugs(Larsson, de Pedro and Paxeus, 2007). Other potential sources include landfill leachate, leakage from septic tanks (Brindha, Renganayaki and Elango, 2017) and agricultural waste-storage structures (Dai *et al.*, 2018), or non-localised sources to include runoff as well as unsaturated permeation in the groundwater. It is however effectively impractical to measure the level of inappropriate PPCP disposal into the environment, all that is understood is that source-receptor pathway can significantly influence the amount of contaminants influx into a treatment plant likewise the concentration level in the effluent water (Brooks et al., 2009).

1.3. Insight and relevance of Water Framework Directives (WFD)

In the year 2000, the EU Water Framework Directive (WFD) was adopted with the intention that groundwater and surface water in all the members' states should have a "good status" by the year 2015 (Kallis, 2001; Hering *et al.*, 2010). Three important categorizations of monitoring were agreed which include; Surveillance to create baseline water quality status and measure long term variations in the quality status, while the operational mode was to deliver extra data on water basins that are at risk of meeting environmental objectives set by the WFD; and the Investigative mode was to provide information on the cause of failure to meet environmental objectives where they are known. This target has been not achieved for many of the EC States with many priority chemicals routinely being reported about defined environmental quality standards (Moss, 2004, 2008). Following up on this, member states identified water basin management plans to effectively manage WFD compliance. However, they have not been able to meet with the requirements while has led to the compliance review being extended till 2021. Compliance with EQSs defined by the WFD has been challenging with appropriate measurement and sampling methods. Member States have, therefore, been left with the option of determining their methods for measurement and monitoring their River Basins.

The most common method for chemical concentrations by most of the EC members States is spot/active sampling (bottle/grab). this method is capital intensive for transportation and manpower, not representative of the entire fluctuations in the system conditions, inability to measure some very low Environmental Quality Standard Limit (EQS). These problems have been mitigated against by the passive sampling which is not dependent on the *in situ* power supply, non-mechanical devices that can be deployed for both short and long term sampling, which measures freely dissolved chemicals over some time (Time Weighted Average) and ultimately can concentrate chemicals in the gel compared to the active method. Adoption of passive sampling is more promising in compliance monitoring and as such, more validation data are still required considering several passive samplers that are available in the market. The first watchlist was introduced in 2015 under the EU Directives 2008/105/EC. The table below shows the chemicals under this list with their maximum acceptable method detection limit which is their predicted no effect concentration PNEC in any matrix.

Table 1: Substances on the first watchlist (Tavazzi et al., 2016)

Name of Substance	**CAS number**	**EU number**	**The maximum acceptable detection limit** (ngL^{-1})
17-α- Ethinylestradiol (EE2)	57-63-6	200-342-2	0.035
17-β-Estradiol (E2)	50-82-2	200-023-8	0.4
Estrone (E1)	53-16-7	200-023-8	0.4
Diclofenac	15307-79-6	239-348-5	10
2.6-Ditert-butyl-4-methylphenol (BHT)	128-37-0	204-881-4	3 160
2-Ethylhexyl 4-methoxycinnamate	5466-77-3	226-775-7	6 000
Erythromycin	114-07-8	204-040-1	90
Clarithromycin	81103-11-9		90
Azythromycin	83905-01-5	617-500-5	90
Methiocarb	2032-65-7	217-991-2	10

Acetamiprid	135410-20-7/ 160430-64-8		9
Clothianidin	210880-92-5	433-460-1	9
Imidacloprid	105827-78-9/ 138261-41-3	428-040-8	9
Thiacloprid	111988-49-9		9
Thiamethoxam	153719-23-4	428-650-4	9
Oxadiazon	19666-30-9	243-215-7	88
Triallate	2303-17-5	218-962-7	670

1.4. Organic contaminants of interest

The contaminants of interest out of which some have been selected for this study can be grouped into three basic categories. These are Antibiotics, Preservatives, Antioxidants and Endocrine disruptive compounds. Many of the compounds in these categories are on the watch list while some are used regularly most especially the preservatives. There are 23 personal care products ingredients (PCP) that will be considered together with some selected antibiotics. These have been selected as a result of our standardised methods and previous studies that have been conducted at Lancaster University. These are **Preservatives:** Methylparaben (MEP), Ethylparaben(ETP), 4-Hydroxybenzoic acid(PHBA), Propylparaben (PRP), IsoPropylparaben(i-PRP), Butylparaben(BUP), IsoButylparaben(i-BUP), Benzylparaben(BEP), Heptyl paraben(HEP); **Antioxidants:** Triclosan(TCS), Ortho-phenylphenol (OPP), Butylated hydroxyanisole(BHA), Triclocarban(TCC), Hydroxytoluene(BHT); **Endocrine disruptive compounds;** Estriol(E3), Bisphenol A (BPA), Estrone(E1), 17β-estradiol (E2), 17α-ethynylestradiol (Ethinylestradiol) (EE2), Nonylphenol(NP), Diethylstilboestrol(DES), 4-tert-octylphenol(4-t-OP) and **Antibiotics** group include; Sulfapyridine (SPD), Sulfamerazine (SMR), Lincomycin(LIM), Sulfadiazine (SDZ), Trimethoprim (TMP), Norfloxacin (NFX), Ofloxacin(OFX), Ciprofloxacin (CFX), Marbofloxacin (MFX), Enrofloxacin (EFX), Cefquinome(CFQ), Cefapirin(CFP), Oxytetracycline (OTC), Amoxilin (AMX), Sulfamethoxazole(SMX), Sulfadimethoxine (SDX), Clarithromycin (CLM), Roxithromycin (ROM), Tylosin (TYL), Erythromycin-H2O (ETM-H2O), Penicillin G(PEG), Chlortetracycline (CTC), Erythromycin (ETM)

1.5. Monitoring techniques

The current monitoring technique has been improved from the conventional method or active sampling to passive sampling method using various tools like POCIS, SPMD, Grab Sampling etc. The use of passive samplers has gained acceptance in the environmental monitoring of water, air and soil pollution (Mills *et al.*, 2007). This study has been designed to carry out most of the investigation on the field in the wastewater treatment works in Italy, and the United Kingdom using a validated sampling device. However, the sampling devices include polyethene mesh and sampling cages to secure the passive sampler most especially in the sampling medium wit high organic matters or water flow rate.

1.6. Aims and Objectives of the study

This study was intended to investigate the use of a passive sampler to determine the fate of organic contaminants in the wastewater, compare the Conventional treatment works with the nature-based technology with the hope of having performance data of both systems. Therefore, the study shall consider the use of a passive sampler to investigate chemicals behaviours and removal rates in a different works system viz natural wastewater treatment technology, conventional treatment work, the effectiveness of passive sampler as an investigative tool for a long term monitoring of chemicals in wastewater, chemical partitioning in the sludge that is being used for agricultural purposes as well as chemical behaviours in the activated sludge. These are summarised thus;

- Performance of the sampling device or technique
- Comparing the performance of Nature-based treatment technology with Conventional treatment work.
- Reducing the knowledge gap about nature-based treatment technology as a substitute for the Conventional treatment system
- To learn more on the long-term use of passive sampler for environmental monitoring of pollutants
- To acquire insight into the partitioning of organic contaminants in the wastewater

- To gain knowledge of chemical desorption from soil amended soil for safety assurance to the agricultural stakeholders

1.7. Introduction to passive sampling

The development and use of passive samplers for the determining trace chemical concentrations in air and water has undergone considerable expansion since the early 1970s (Kot-Wasik *et al.*, 2007a). The development of passive sampling techniques started in water with semi-permeable membrane device (SPMD) (Kot-Wasik *et al.*, 2007a), in air, polyurethane foam (PUF) (Shoeib and Harner, 2002) and more recently has been applied to soil(Chen *et al.*, 2015) The Interstate Technology Regulatory Council, (2006), a state-led alliance working to reduce barriers to the use of innovative remediation environmental technologies and processes, gave an outline of twelve various passive samplers including Semi-Permeable Membrane Devices (SPMD), Polar Organic Chemical Integrative Sampler (POCIS), PETREX Soil Sampling Tube, Passive In-Situ Concentration Extraction Sampler (PISCES), with comprehensive comparative performance data. Diffusive Gradients in Thin-films passive samplers (DGT) was invented with a world trademark in 1993 by Bill Davison and Hao Zhang. Most of the early passive samplers were unable to detect chemicals with low detection limit leaving out most of the dissolved bioavailable organic compounds unaccounted for in the environments. This left the early researchers with an inability to obtain signals of some of the hazardous chemicals in the environment (Mayer et al. 2003).

The development and application of Diffusive Gradients in Thin-films Passive Samplers (DGT) started with *in situ* monitoring of labile trace metal concentrations in aqueous systems (Zhang and Davison, 1995). It has been extensively tested and validated for inorganic chemicals. Research on the use of DGT for organic chemicals or contaminants is currently limited (o-DGT) (Chen, Zhang, & Jones 2012). Laboratory and field testing have confirmed that this precision plastic DGT device provides *in situ* concentrations of chemicals over the deployment time in a wide range of media. Dissolved chemical accumulations occur in a controlled form. Beyond its use in research, consideration is being given to advancing DGT's acceptability as a preferred passive sampler with its commercialization worldwide although, there is an urgent need to ensure that the product is validated for environmentally toxic chemicals. Following the WFD, Member States were expected to have a good water status by 2015, which has been extended till 2021. The absence of standardized universally accepted ways of achieving compliance with the WFD has stressed the need for extensive research aimed at the development of best techniques for determining trace chemical concentrations in water. Before the development of DGT, there have been various passive sampling techniques.

In general, there are various types of developed passive samplers. There are equilibrium samplers which have a low sampling capacity where chemical equilibrium partitioning condition is reached quickly by the chemical. Solid-phase microextraction (SPME) is a clear example of this sampler. However, Chemcatcher, Polar Organic Chemical Integrative Sampler (POCIS), Semipermeable Membrane Device (SPMD), Silicone strips are all classed as integrative sampler where the chemicals have greater samplers and water partitioning coefficient, suitable for a long-time deployment of up to 2 months. SPMDs and POCIS have been tested with the following compounds: Polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs), Dioxins and Furans, Emerging Contaminants (POCIS), Chlorinated pesticides (DDT, chlordane, etc), Fragrances, Hormones compounds, Pharmaceuticals / Illicit Drugs, Current-use pesticides, Antimicrobials (triclosan). However, the performance of passive sampler fundamentally requires the understanding of chemical behaviours and transportations. Over 350 chemicals have been sampled with an average sampling rate Rs ranging from 0.1 to 0.4L/day. The diffusive gradient in thin-film passive sampler is also an integrative sampler, suitable for sampling over a period, time-weighted average (TWA) as well as some of the other samplers like POCIS & SPMD.

POCIS as passive samplers for organic pollutants have been tested by deploying it in a hospital sewage pipe for four days. The outcomes confirmed that the assessed water concentrations were comparative to that obtained during twenty-four-hour composite samples. Rs are dependent on the flow velocity and temperature (Bailly *et al*., 2013). This suggests that POCIS, Chemcatcher, DGT are all cost-effective to use compare to the composite/grab sampling where TWA is vital to have an insight into the activities in the medium over the sampling period. POCIS has also been confirmed suitable by the French Water Agency with demonstrative capability for investigative and operational monitoring of pollutants in the environment(Poulier *et al.*, 2014). It was also confirmed in another study that POCIS measures dissolved fraction of dimethenamid and the whole water concentration for compounds like desethylatrazine, metolachlor and atrazine. However, a successive deployment over 14-days has shown an evidence of biofouling on the polyethersulfone (PES) membrane while in use for river sampling using HLB binding gel in rivers sampling in France, which could have a significant impact on the uptake concentrations or the R_s(Lissalde *et al.*, 2014). Polar Organic Chemical Integrative Samplers (POCIS) works well in the environmental condition and with Performance Reference Compound correction, it looks appropriate for estimating time-weighted average (TWA) concentrations of compounds. It is therefore important to consider the condition of the medium and the environment where this is deployed for effective performance.

The development and application of DGT timelines are shown below in Figure 1.

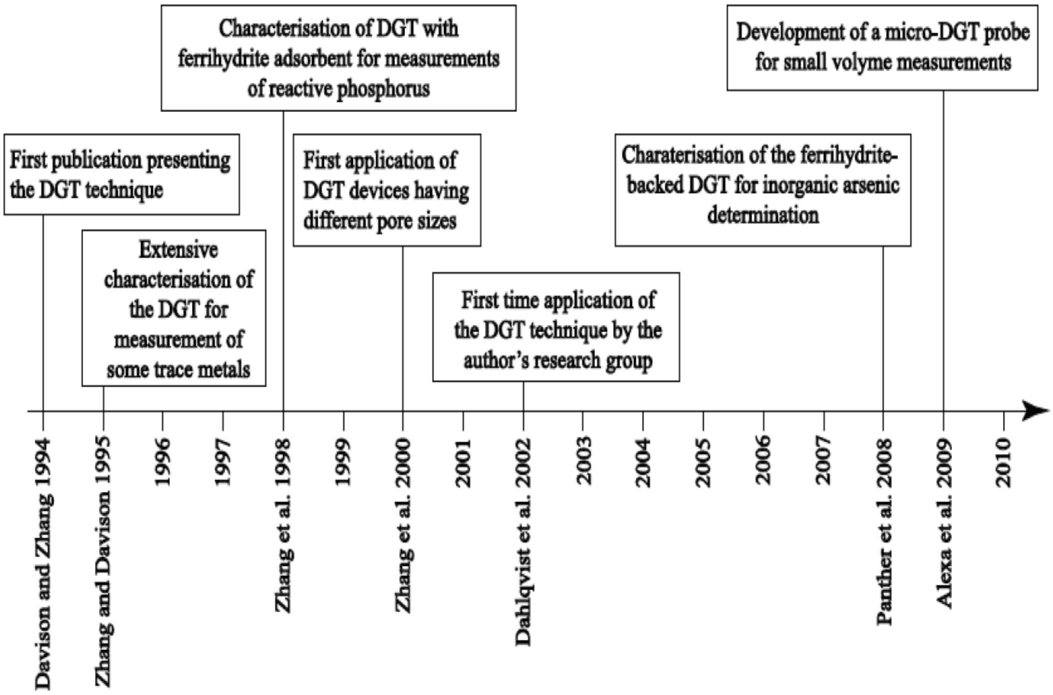


Figure 1: Development of DGT techniques (Österlund, 2010)

In addition to passive sampling techniques, there are various types of autosamplers specifically designed to meet regulatory requirements. These products are simple to operate, rugged, and reliable. Their areas of application include stormwater sampling, wastewater sampling and river water sampling. The units can be configured to operate on a time-weighted or flow proportional discrete or composite sampling. To prevent the chemicals, present in the samples from degrading several cooling options have been designed while some of the sampling kits now include refrigerated units, ice bags, and mobile coolers. However, the sampling kits require a power supply which is often through an internal rechargeable battery or external batteries and the whole set up could be of significant size. There is also a cost implication as hiring a unit could cost between £245 - £500 per week.

Some models like Campbell Scientific Inc Composite Portable Automatic Liquid Sampler PVS5120C uses an external vacuum pump for the water uptake rather than the conventional peristaltic pump which can sample less turbulent water with a better representation of the sampled water to the sampling medium. Cross-contamination can now be prevented using samplers that use air pressure (up to 28 psi) which is sufficient to purge the tubing of excess water. Security of the equipment is of great concern because of the high replacement costs.

1.8. Principles of Diffusive Gradients in thin-films passive sampler

The first passive water sampler was invented in about 30yrs ago and since then there has been considerable research into their design and performance (Kot-Wasik, Bożena Zabiegała, *et al.*, 2007). This study examined the use of Diffusive Gradient in thin-film (DGT) in a range of applications and uses grab sampling methods for comparison. This will also enable the researchers to identify the differences between this conventional method and DGT. Most previous studies have concluded that the reliability of grab sampling method is questionable with the reasons being that;

1. There are chances that chemical of interests or contamination episodes could be missed due to the timing of the sampling.
2. The ‘spot check’ method only captures the pollutants around the sampling horizon in the water column at the instant the samples are taken.
3. Collected samples usually must be extracted and pre-concentrated to overcome issues surrounding low limits of detection.

The Diffusive Gradients in thin-film passive sampler was originally designed for sampling metals or non-organic compounds (Chen, 2013). The sampling toolkit has been patented in Australia while efforts are on the way to have a worldwide trademark. DGT has been found useful in detecting some of the organic pollutants which have a very low detection limit with high accuracy (Clarisse and Hintelmann, 2006). The technique has also been successively used to monitor radionuclides in the environment and organic contaminants including pharmaceutical contaminants (Mengistu, 2009). Literature suggests that sources of pollutants could be monitored over a period using DGT more efficiently than grab sampling due to its ability to accumulate chemicals over time. This device accumulates pollutants during sampling period following the principles of Fick's first law of diffusion and providing a Time-Weighted Average (TWA) concentration of chemicals. This sampling toolkit has been extensively used in sampling inorganic compounds while there is paucity of data relating to organic chemicals such as EDCs. However, other passive samplers have been extensively used in investigating the presence and concentration of EDCs in the environment.

DGT provides a time-integrated sampling of dissolved chemicals but chemical uptake rates can be dependent upon factors such as the sampling medium, deployment methods, sampling duration and composition of the binding gels, diffusive gels and filter membranes. The device has three components; the base, gels with the membrane layers and the cap (Stuer-Lauridsen 2005). The gel and membrane layer includes a resin-impregnated binding gel layer with a hydrogel diffusive layer and the filter membrane. The choice of selection of the binding gel is dependent upon the chemical of interest (Gregusova & Docekal 2011). DGT theory is based on the diffusion characteristics of the chemicals of interests and the sorption properties of the binding gel (Zhang & Davison 1995; Zhang 2003).

The diffusive gel layer must be composed of a material that allows passage of the analyte of interest. The flux of an analyte from the solution medium to the binding gel observes the Fick's first diffusion law and the diffusive transportation continues until a state of equilibrium is attained between the binding gel and the solution phase. It implies that the concentration of an analyte in the solution can be quantified (Zhang and Davison, 1995; Denney, Sherwood and Leyden, 1999). The binding gel layer serves as the sink for the accumulated chemicals from the solution which passes through the diffusive layer (Degryse et al. 2009). The rate of diffusion can be influenced by pH, solution composition, and/or ionic strength by altering the behaviour of the functional groups of the diffusive gel polymers (Torre et al. 2000; Harper et al. 2000). The diffusive gel is protected by 0.45µm pore size filter against particles as well as preventing the diffusive and binding gel layers from being damaged.

Fick's First Law of diffusion is instrumental in understanding the operations of DGT (Thomas, 2009). Fick’s law of diffusion relates the diffusive flux to the concentration under a steady-state assumption. It means that solute migrates from the region of high concentration to that of low concentration across a concentration gradient. Upon determining the mass of analytes, the time-weighted averaged concentration of the analytes; C_{DGT} can be obtained using equation 1 below;

$$C_{\mathrm{DGT}} = M\Delta \mathrm{g} / DtA \tag{1}$$

The mass of the analytes in the resin can be obtained with a simple equation by changing the above equation to be represented as;

$$M = \frac{D_e C_b A_t}{\Delta g + \sigma} \tag{2}$$

where M stands for the analytes mass, Δg, the diffusive layer thickness with the filter membrane, the analytes diffusion coefficient is represented by D, while deployment time is represented by time t, Area of DGT window by A, and σ as the DBL, (Zhang, 2003). Figure 2 below shows various components of DGT and its interaction with the solution medium during deployment.

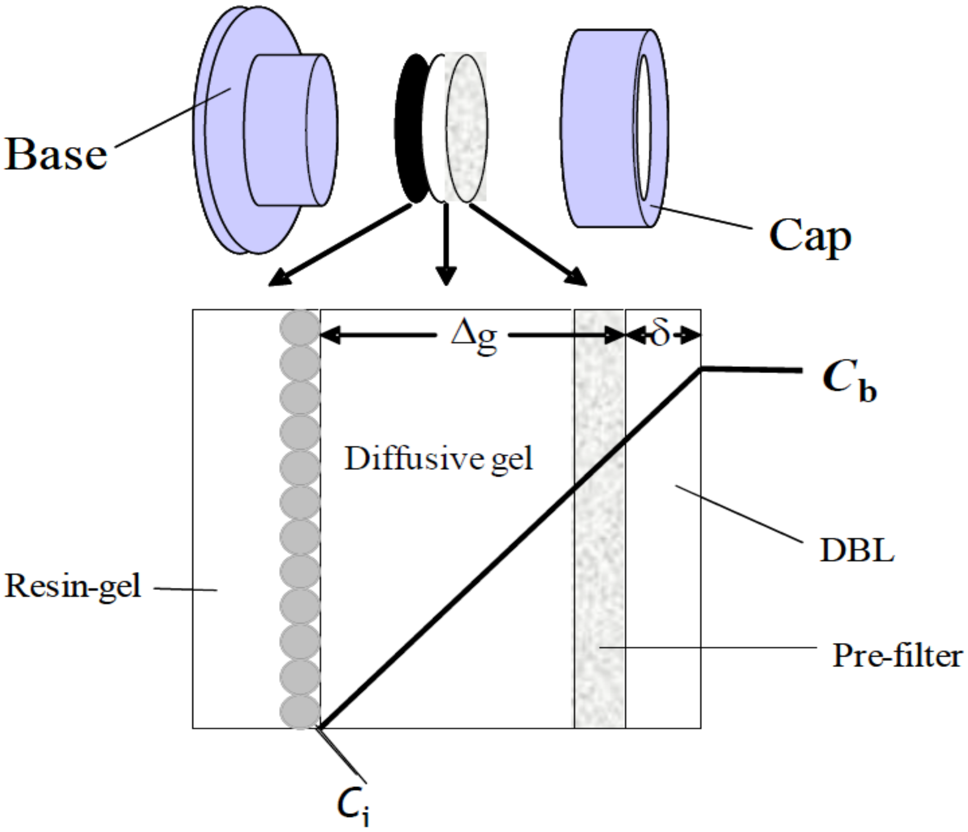


Figure 2: A combined representation of DGT components and the analytes concentration in the adjacent solution medium during deployment: DBL; diffusive boundary layer, Ci concentration at the diffusive gel-resin boundary, C_b *as the concentration in the aqueous medium (Source: Chen 2013)*

The diffusive gel thickness varies from 0.4mm to 2mm while the thickness of Diffusive Boundary Layer (DBL) in solution is dependent upon the rate of water movement (Zhang, 2003). In a high-velocity fluidal solution, the diffusive path length between the solution and the binding layer is insignificantly influenced as the DBL thickness and can be considered insignificant compared to the thickness of the diffusive gel layer. DBL thickness varies inversely with the flow, which is dependent upon time, and space, and determining the DBL is thereby very difficult. It can be determined mathematically by deploying multiple samplers with varying diffusive gel thicknesses simultaneously. It is important to note that if the thickness of the diffusive boundary layer (δ) is not negligible then the accuracy of the DGT sampling method can be affected (Garmo et al. 2006). It has been suggested that to avoid any loss of accuracy in the sampling process the DBL needs to be less than 0.5mm (Buzier *et al.*, 2014). Considering the above, further studies would be needed to have a better understanding of accurate determination or measurement of the DBL for any concentration estimation using DGT samplers.

Numerous field and laboratory tests have been conducted using DGT to sample inorganic pollutants in the environment. DGT has emerged as a sustainable monitoring device for labile components in solution (Davison & Zhang 2012) and some of the limitations and advantages realised in the previous studies are summarised in Table 1. Passive sampling also operates economically better than active samplers that need an energy supply (Conesa, Schulin and Nowack, 2010). The physicochemical properties of the study chemicals and the surface area of the diffusive gel and binding resins are the key parameters while the method is also affected by analyte permeation in the gel (Kot, Zabiegała and Namieśnik, 2000). Passive sampling methods can be classed as either absorptive or adsorptive (Kot et al., 2000; Vrana *et al.*, 2005; Chen., *et al.*, 2013). The possibility of chemical discrimination cannot be ruled out in the case of absorptive methods because of the physicochemical characteristics of the diffusion barrier or membrane. However, surface binding, physical or chemical retention by surfaces as well as surface area are very important parameters in adsorptive methods. The basic characteristics of DGT in an aquatic system are summarised in Table 2a.

Table 2a: Outline of advantages, limitations or considerations relative to the use of DGT in an aquatic environment (adapted from INAP, 2002)

Advantages	Limitations / Considerations
Time Integration: This is not a characteristic that is unique to DGT, but it is one of the features that make it comparative and preferable over some other samplers. It provides a time-integrated concentration of analytes.	**Mining-Impacted Systems:** It is often more difficult to obtain an accurate concentration in this type of environment. Therefore, more work will be required for its validation and application.
A multitude of Applications: • Metal speciation • Toxicity testing • Complexation kinetics of metal-ligand complexes • Complexation Capacity • Development of site-specific discharge criteria and assimilative capacity	**DGT vs. Toxicity:** Various studies are ongoing on bioavailability and more data would help build up a full picture of any relationship between DGT data and its interpretation to understand toxicity to living fauna.

User Friendliness: This is an easy to learn tool, prepare, deploy and retrieve without any need for specialist tools or training	The retrieved samples are to be well preserved to avoid cross contaminations and degradation of analytes

The membrane / diffusive barrier in the absorptive method consists of a diffusion barrier which is the most important component of permeation passive dosimeters. Vrana et al. (2005) suggested that samplers can be grouped into two categories based on the properties of their barriers which could either be a diffusion-based or permeation-based device. The membranes must meet certain specific conditions such as a large permeability coefficient for the analytes or sampled chemicals (Kot, Zabiegała and Namieśnik, 2000). Nonetheless, permeation is determined by dissolution in the membrane as well as the diffusion which is dependent on the materials components of the membrane.

Passive samplers can operate in either kinetic or equilibrium regimes. Under the kinetic regime, the concentration of analytes in the aqueous solution exceeds that of the binding gel and as the sampler is exposed for a longer period, the concentration of the analytes in the binding gel and that of the aqueous solution reaches a state of thermodynamic equilibrium. There is an assumption that the adsorption of chemicals into the resin gel is unidirectional from the aqueous medium (Demirbas *et al.*, 2005). It means that the adsorbed chemicals cannot deplete without the use of an extraction chemical. Both equilibrium and kinetic functions are schematically represented as shown below;

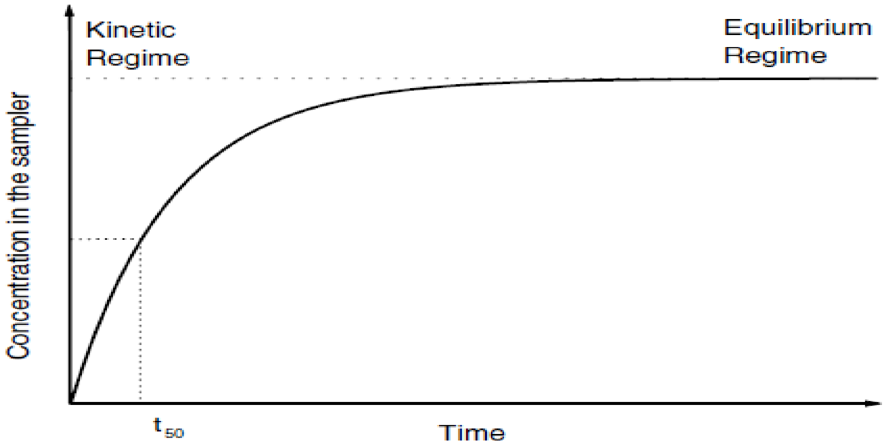


Figure 3: Passive samplers operate in Kinetic and Equilibrium regions (Source: Vrana et al., 2005)

The mass M of the accumulated chemical by the DGT passive sampler in a kinetic state can be represented as;

$$M\ (t) = DC_bAt\ /\ \Delta g \qquad (3)$$

While the concentration C_s of analytes in the passive sampler at equilibrium state can be represented as;

$$C_s=C_bK \qquad (4)$$

where K is the phase-water partition coefficient, C_b is the TWA concentration of pollutants in the water phase.

1.9. DGT and other passive sampling techniques

It is important to conduct a comparative analysis of the most used conventional passive sampling techniques with DGT. Previous comparative studies on Nickel and Cadmium using DGT and dialysis samplers (peepers) in oligotrophic and acidic Lake Tantare, Que. Canada revealed that DGT performance was better than dialysis samplers as DGT consistently measured higher concentrations of Cd and Ni (Torre *et al.,* 2000). However, the performance was not consistent when it was deployed into water that was low in major cation concentrations. To appropriately compare grab sampling techniques with *in situ* samplers such as DGT, system variability must be understood. It is pertinent to adopt high-frequency sampling using grab samples during the deployment of DGT to understand differences in performance. Comparative studies of DGT and grab sampling has revealed advantageous discrepancies in accuracy and uptake with the DGT as well as its ability to be used for a wider spectrum of chemicals (Mengistu, 2009). The outcome of the correlation between the two techniques was weak (Duan *et al*. 2014). Comparative studies between Grab sampling and DGT have shown that DGT is more accurate for many applications that require long-term environmental monitoring and Time Weighted Average (TWA) concentrations (Kot-Wasik *et al.*, 2007; Seethapathy *et al.*, 2008b). Importantly, in comparison with other passive samplers which require regular calibration due to variation in flow rates, DGT does not require calibration.

Another comparative study on DGT and Grab sampling technique to measure radiocerium in natural waters revealed that even though they performed similarly in terms of accuracy, grab sampling was not suitable to monitor the water for a long period of about 5months. In contrast, one of the limitations identified in DGT technique was the degradation of chemicals if left in the sampled medium for a longer period due to lack of preservation which can easily be overcome with grab and active sampling. The recalibration of DGT due to a wide temperature variation was also deemed to be a challenge (Murdock *et al.*, 2001). A comparative analysis is given in Table 2b for the active sampling technique and the passive sampling method most especially the DGT sampling techniques.

There has been an increasing trend in the acceptance of passive samplers to monitoring environmental pollutants(Gong *et al.*, 2018) while various studies have been conducted on the use of polar organic chemical integrative samplers. POCIS and other sampling tools to include spot sampling of PPCPs. In one of the studies, it was concluded that flowrate and hydrophobicity (LogK_{ow}) has no significant direct relationship as the uptake increases slightly with increasing flow rate. The study concluded that flow rate has little influence on the uptake of PPCPs and EDS by POCIS in the environment (Li *et al.*, 2010). However, POCIS has shown to be suitable for compounds with Log Kow <4, it was however concluded that DGT is less susceptible to environmental influences such as Temperature(Kot-Wasik *et al.*, 2007) etc. Meanwhile, grab sapling has shown to be an expensive sampling approach compared to POCIS for its TWAC inability (Mengistu, 2009). POCIS has proved to have a great edge over traditional sampling method in measuring fluctuating and trace concentrations of organic pollutants which are often missed by the traditional method (Harman *et al.*, 2012).

Table 2b: Comparison of Active and DGT sampling Techniques

	Active Sampling	**DGT/Passive Sampling**
Costs	This is not cost-effective compared with passive sampler if a small number of samples are required over a short period of times like 2-3days. The sampling costs could be huge most especially where the sampling sites are far away from the laboratory. Associated costs include costs of preserving the samples, power supply, and safety of equipment, rental or costs of buying the equipment.	The DGT sampling techniques involve high set up costs which can easily become insignificant once the sampling spans beyond 2-3days. The costs of deployment and removals are limited while there are no daily costs of sampling/transportations. Comparatively, passive sampling like Grab sampling could have a low set up costs and high daily operational costs. This technique is also very economical in a situation where multi-locational sampling campaigns are being carried out.
Time	It is time-consuming and it engages a lot of manpower where many samplers are needed most to do some studies on seasonal variations. Configuration and equipment calibrations are very sensitive, and this makes Active sampling more time consuming, unlike the Passive sampling technique.	DGT sampling technique operates on Time-weighted Average (TWA) which implies that the chemical concentrations are accumulated over time. This gives a clear reflection of variations over the sampled time. However, Grab sampling is more of a spot sampling technique.
Accuracy	The technique could have some challenges capturing	DGT accumulates chemicals over time. Variations in chemical

	daily or hourly variations in the chemical concentrations. However, the variations are dependent on factors such as temperature, pH, dilution rate, flow rate etc.	concentrations are well accounted for. It is also good in studying chemical characteristics such as ecotoxicological characteristics should also be considered (Buzier, Tusseau-Vuillemin and Mouchel, 2006). However, Grab sampling approach is imperfect in accounting for variations in chemical concentrations over a large space of time.
Detection Limit	Sampling could be cumbersome because of the detection limit of these environmental pollutants.	Time-integrated samples are more representative of fluctuations and other chemical activities in the aqueous medium than Grab sampling techniques most especially for a chemical with sizes below 0.45um.

1.10. Components of DGT Samplers

The DGT moulding contains a Piston/Base, Resins, Diffusive gel, Membrane filter and the moulding cap.

1.10.1. Gel discs

Gel discs are made to 2.5cm diameter for soil or solution DGT with a shelf life of 12months whilst a loaded solution DGT moulding has a shelf life of 6 months if kept refrigerated under 4^{o}C. A gel sheet, which is approximately 13cm by 13cm, is used to make diffusive gel only with gel strips approximately 7cm by 22cm used for various purposes. Some of the commonly used gels are; AGL gel *(for Sulphide)*, AMP gel *(for Cs)*, Fe-oxide gel *(for phosphorus)*, Chelex gel *(for metal)*, Diffusive gel *(open pore)*

However, this study used Hydrophilic Lipophilic Balance (HLB) for the PCPs and Amberlite XAD– 18 for the antibiotics binding gels together with Agarose gel for the diffusive layer.

1.10.2. Mouldings

The surface area of a standard solution moulding is 3.14cm^2 while soil mouldings have a surface area of 2. 54 cm^2. These mouldings are customised to take diffusive gels of varying thicknesses. Details are given in Table 3;

Table 3: Spacers for making varying Gels thicknesses

Spacers' thicknesses	**Diffusive gel**	**Binding Gel**	**Filter membrane**
0.25mm	0.40mm	0.40mm	0.45um
0.35mm	0.56mm	**0.56mm**	**0.45um**
0.50mm	**0.80mm**	0.80mm	0.45um
0.25+0.50mm	1.20mm	1.20mm	0.45um
0.5mm+0.5mm	1.60mm	1.60mm	0.45um
Spacers for 1.60 + 0.40mm gels	2.00mm	2.00mm	0.45um

It is important to note that the mouldings have been designed to allow combinations of gels and filter membranes totalling 1.335mm i.e. 0.4mm resin, 0.8mm diffusive gel, and 0.135mm filter (Davison *et al.*, 1993).

1.10.3. Components of Diffusive gel and Binding gel

This aspect will focus on the preparation and extraction of the o- DGT sampler only as the inorganic DGT passive sampler has been extensively tested and validated both in the lab and field. The following steps and chemicals are needed to prepare Gel solutions for DGT

- Acrylamide solution (40%)

This chemical is suspected to be a human carcinogen. It's however recommended that care should be exercised while handling the chemical

- DGT gel cross-linked (2%)

  (from **DGT Research Ltd**, Skelmorlie, Bay Horse Rd, Quernmore, Lancaster LA2 0QJ, UK.)

Gel solution preparation requires (15% acrylamide and 0.3% cross-linker) Using 40% Acrylamide Solutions:

100 ml gel solution is prepared by mixing 15 ml of DGT gel cross-linked (15 gram of the solution) with 47.5 ml Milli Q or deionized water thoroughly in a clean plastic beaker (or bottle) while 37.5 ml of acrylamide solution (40%) is added to the mixture. The gel solution is mixed thoroughly by shaking or stirring. The gel solution can be stored in a refrigerator (4ºC) for at least three months.

Other chemical components of binding gel are;

- ammonium persulphate –APS (10%, or 0.1 g of APS in 1 g of water) This was prepared on the day it was used
- N,N,N'N'-Tetramethylethylenediamine (TEMED), 99%
- XAD-18 (65-150um) and/or Hydrophilic-Lipophilic Balance (HLB) (30um-100um) resin. There is a full process of preparing the XAD-18 or the HLB from its powdery state. The resins have to be stored in methanol (MeOH) for at least 30mins to soften before the cleaning process (Rohm and Haas, 2006)

2. DGT Applications, Performance Data and Methodologies

2.1. Diffusive and binding gels preparation

The technical procedures for the preparation of various gels for organic chemicals including antibiotics and Personal Care Products (PCPs) are stated below.

2.1.1. Agarose diffusive gel

Decon (90) was used to wash glass plates while spacers and plastics were not rinsed with Decon (90) as they would degrade easily. They were washed using Milli-Q water trice as well as all apparatus. Spacers were dried in the fume hood/cupboard while glass plates were dried in the oven at about 60 °C for about half an hour. 0.9g agarose powder was dissolved in 60 mL MQ water (i.e. 1.5%) and heated in the microwave at 80 °C until all the powder dissolved to obtain transparent agarose solution. Glass plates were assembled using the 0.8mm spacers to interface them on three edges to make 0.8mm diffusive gel. Hot agarose solution was injected using pipette through 1 mm overlap offset that has created at the open edge between the two plates. These assembled glass plates were cooled down in a room temperature while a 2.5 mm diameter gel cutter was used to cut out the gel disc. Gel discs were stored in (0.01-0.1M) NaCl solution for use and stored in the refrigerator at 4°C.

2.1.2. Resin gel preparation

Hydrophilic Lipophilic Balance (HLB) and Amberlite XAD-18 preparation are the same only that different organic solvents were used. These powders were stored in glass bottles once they have been pre-washed with methanol most especially because XAD-18 powder as it was supplied imbibed in NaCl and Na_2CO_3 to inhibit bacterial growth. Cleaning of glass plates and spacers with other apparatus used the same principles as in the case of diffusive gel. 10 ml of gel solution was measured into a glass bottle and added 4g (wet weight, depends on the size of the beads) of HLB resin (63µm) or XAD-18 (63 µm -150 µm) and mixed thoroughly. 60 µl of ammonium persulphate solution was added and then 15µl of TEMED. The steps can be summarised as follows;

10ml gel Solution + 4g resin + 60 µl APS + 15µl of TEMED.

The solution was mixed thoroughly to ensure and injected into the interface between the two glass plates. The assembled plates with the resin were heated in the oven under 42 ~ 46°C for a minimum of 1 hour till the gel is completely set (without liquid). However, the resins settled by gravity on one side of the gel as the plates were laid flat while in the furnace. The resin gel was hydrated in MQ water or deionised water and changed 1~2 times before storing in 0.01M NaCl solution. 0.35mm spacer was used for casting 0.56mm binding gels. In the DGT assembly, the resin gel was placed on the DGT base, while the side with the settled resin was upside and superimposed with the diffusion gel. Assembled DGT Samplers were stored in a zip-lock bags at 4°C in the fridge (not freezer), with some Milli-Q water (0.01M NaCl) to keep the hydrogel hydrated as dryness will damage the gels. Below is a typical setup or gels arrangements.

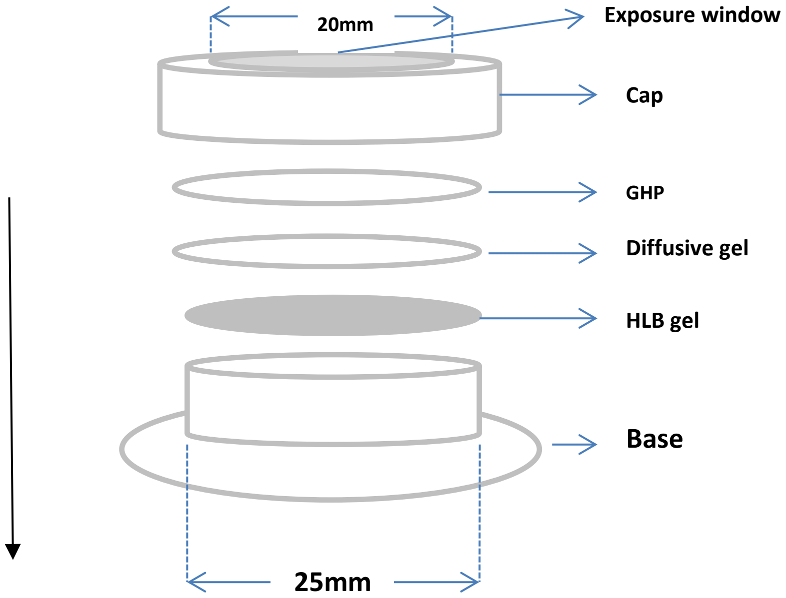


Figure 4: Dimension and components of o-DGT passive Sampler

2.2. Field Deployment of the Samplers

DGT handling

1) DGT units are stored in a refrigerator if not being used immediately.
2) These are not to be removed from the sealed plastic bag where they are stored until the deployment time to avoid contaminations. If exposed, it will dry off and be damaged.
3) While handling the DGT units, it is important to wear gloves to avoid contamination.
4) The filter membrane on the DGT must be well protected to avoid damages and contaminations. The filter membrane section of the unit should also not be touched so as not to damage the underlying gel layers.

DGT deployment

DGT units can be deployed using a range of methods, devices and can be suspended using a fishing line fixed into the small hole that is in the base unit. For duplicate deployment, the two DGT units can be bound together back by back. Alternatively, DGTs can be attached to a suitable holder or deployment device such as the 'Octopus' (Figure 6) which is designed for multiple deployments. Also, they can be protected by the polythene mesh or aluminium cages. They must be deployed immediately after removing them from the polythene bags.

The units could be deployed in flowing water or contained water most especially in the laboratory, while excessive turbulence, particularly bubbles should be avoided. For example, suspending DGT units at about 30 cm (depending on the river situation) below the water surface is ideal but avoid deploying too close to the sediment. The surface area of the DGT units must be fully submerged into the water or sampling medium during the deployment period or sampling campaign.

An accurate record of the deployment time (when do you put in the water, such as 2014.07.08, 10:05) and the daily temperature of the sampling medium or water during the entire deployment period most especially at deployment and removals. If temperature variation falls within ± 2℃, an average (start and end temperature) will be adequate. If the variation is more than ± 2℃, mean temperature could be taken from an integrated record of temperature (chart recorder or data logger). Measuring the temperature and the sampled medium pH is essential to understanding the physical and some activities going on in the medium.

Below is the summary of some of the vital information that needed to be collected while on the field.

Table 4: Sampling medium information

Location	**Required**	**Value**
Name of the locations including information about the location/sampling. It is a good practice to include a map of the location. Please note that the information should also include the samples' name. eg BR1-3| 10-17/5/2016:1015am -XAD BR1-3| 10-17/5/2016:1015am -HLB	pH at Deployment	
	pH at Removal	
	Temp at deployment	
	Temp at Removal	
	Water Flow rate	
	Water level/depth	
	The population of the locality	
	Receptor	
	Year of starting	
	Receptor Capacity	
	Average BOD load	
	Average COD load	
	Typology of the wastewater plant	
	Chemical for disinfection	

2.3. Deployment Devices

To this date, there has not been any detailed study on the effect of deployment devices. Studies are underway to test the performance of various deployment devices such as the 'Octopus' (Figure 6), sampling cage and the polyethene sampling bag/mesh. This will help to determine any variation in their uptake rates and the impact of each device on the exposed area of the DGT. Also, the impacts of DGT sampler's orientation on DGT uptake rates need to be assessed at the same time. A preliminary study suggested that the uptake rate of samplers in the cage is higher than that of the Octopus. This cannot be substantiated until further tests on the devices are undertaken. These devices host the DGT and they also help in ensuring that the samplers are not washed away by the sampling medium's current/turbulence.

Figure 5: Octopus with DGT sampling disks at the WWTP effluent

To keep the orientation of the sampling disks in a similar direction while using the sampling mesh or the cage, the samplers should be lined up in a holder similar to that shown in the Octopus or using the fishing lines as shown in Figure 6.

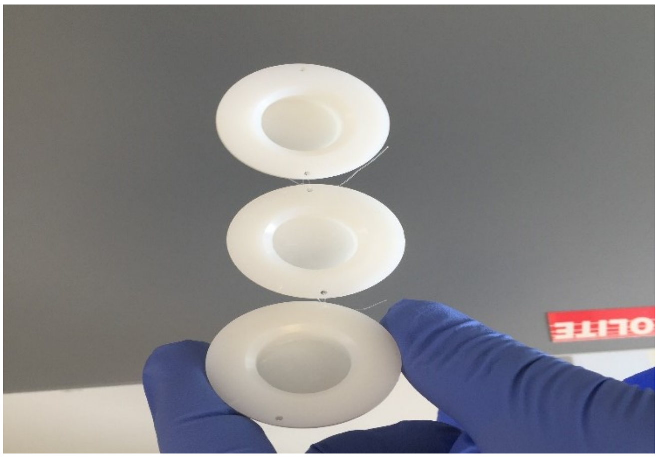


Figure 6: DGT sampling disks connected using the fishing lines

2.4. DGT Samplers retrieval

On retrieval of the deployed DGT holding and the device, DGT unit was immediately (minutes) removed with utmost care to ensure that the face of the filter is not tampered with. The time was recorded as well as the water temperature (and pH if possible). DGT unit was rinsed with distilled or deionized water (Milli Q water) from a wash bottle prepared in the laboratory for the filed exercise. The DGT was placed in an individual clean polythene bag and sealed with least air space. After recording the location details on the bags, they were transported to the laboratory for further analysis/. However, in instances where the DGT could not be analysed immediately, they were stored in the refrigerator at 4ºC.

2.5. Target chemicals extraction from binding gel

The resin gel was removed by inserting a screwdriver into the groove/space in the cap and twist it to break the cap. The broken cap was peeled off to expose the filter and the diffusive gel layer. These layers were removed to reveal the bottom resin-gel layer. Resin gels were placed in 15mL prebaked clean amber vials spiking with an appropriate amount of internal standard (IS). 5 mL of methanol (MeOH) was added to XAD-18 resin for antibiotics extraction, to ensure the resin gel was fully immersed in the organic solvent. This was put in an ultrasonic bath for 30mins, decanted and repeated the process but without IS. The vials were rinsed with 2ml appropriate organic solvent and each of the extracts contained 12ml organic solvents. The extract was concentrated under gentle N_2 flow to dryness (nearly dry) and was reconstructed by spiking with 1 mL MeOH. The concentrated extract was filtered with 0.2um PTFE syringe filter to 2mL amber GC vials and stored for further processing for LCMS analysis. This processed was also used to extract PCPs from the HLB binding gel with organic solvent Acetonitrile (ACN). Various quality assurance samples were also prepared to include field blanks, laboratory blanks and equipment blanks.

2.6. Limit of Detection and Quantification

There are various methods of determining LOD which includes a visual method, Standard Deviation of the Response, Signal-to-Noise Approach, and Slope approach. This is also known as the linear regression method. LOD (limit of detection) of any compound is the lowest amount or concentrations of detectable analyte in any given sample at a given time which does not necessarily imply that they can be quantified reliably. This can be expressed thus;

$$\boldsymbol{LOD} = \frac{\textbf{3 x SD of lowest known concentration}}{\textbf{Slope of the calibration Curve}} \qquad (1)$$

However, this study adopted a visual limit of detection of 0.5ng/ml. The LOQ (limit of quantification) is the quantification limit of an individual analytical procedure which is the lowest quantifiable concentration of analytes in a sample that could be relied upon with appropriate precision and accuracy. The same methods to determine the Limit of Detection (LOD) can be applied to the Limit of Quantification (LOQ) with a typical signal-to-noise ratio of 10:1 (Gulkowska *et al.*, 2008). Using the Standard Deviation of the Response and the Slope approach as well, the quantitation limit (LOQ) may be expressed as:

$$\boldsymbol{LOQ} = \frac{\textbf{10 x SD of lowest known concentration}}{\textbf{Slope of the calibration Curve}} \qquad (2)$$

2.7. DGT Analytical method

Some of the Lancaster samples were analysed using the Agilent 1100 series HPLC system connected to a Quattro Micro triple-quadrupole mass spectrometer (QqQ MS, Micromass, Manchester, UK). The HPLC system comprised of a binary pump, an auto-sampler, thermostatic column compartment and a vacuum micro-degasser. The Quattro Micro triple-quadruple mass spectrometer was equipped with an electrospray ionisation (ESI) source. The high-purity nitrogen gas was supplied by Peak Scientific, UK which was used as desolvation and nebulising gas, 99.999% bottled argon was used as the collision gas. The instrument data and controlled was managed by Masslynx 4.1 software.

However, the majority of the samples were analysed using the Schimadzu LCMS-8040 Triple Quadrupole Liquid Chromatograph Mass Spectrometer. There are two separate configurations for the analytical machines as shown below. An internal standard calibration curve was obtained using 2.5, 5.0, 10, 25, 50, 100, 250, 500µg L^{-1} calibration standards and 50 µg L^{-1} for each internal standard in Lancaster samples but with 100 µg L^{-1} for the Italian samples.

2.7.1. Antibiotics

Mobile phase A: 100 % MQ water, with 0.2% Formic Acid; mobile phase B: 100 % MeOH, with 0.2% Formic Acid. LC separation was carried out on an Xbridge BEH C18 column (100 mm × 2.1mm, 2.5 µm, Waters, UK). The optimised gradient window was: 0 - 5 min 20 % B, then increased to 60% B within 9mins, 80 % B within 10 min, followed by reaching to 100 % B in 10 min, held for 2 mins, then back to the initial condition (20 % B) in 13 mins, finally, with 4 mins of post-run to ensure equilibration leading to a total of 17 mins per sample. The total pump flow was set at 0.2mL/min with a nebulizing gas flow of 2 L/min. The sampling speed was 5µL/sec with a needle stroke of 52mm while the rinse time was 2sec externally. The column oven temperature was 25oC with a pump pressure limits of 300bar. The injection volume was 10 µL. Above was the standard setting for the antibiotics with little or few variations when needed.

2.7.2. Personal care products

Mobile phase A: 100 % MQ water, with 5 mM NH4OH; mobile phase B: 100 % ACN, with 5 mM NH4OH. LC separation was carried out on an Xbridge BEH C18 column (100 mm × 2.1mm, 2.5 µm, Waters, UK). The optimised gradient window was: 0 - 4 min 15 % B, then increased to 80% B within 13mins, followed by reaching to 100 % B in 18 min, held for 4.50 mins, then back to the initial condition (15 % B) in 23 mins and 30mins, finally, with 3mins of post-run to ensure equilibration leading to a total of 33mins per sample. The total pump flow was set at 0.2mL/min with a nebulizing gas flow of 2L/min. The sampling speed was 5µL/sec with a needle stroke of 52mm while the rinse time was 2sec externally. The column oven temperature was 25oC with a pump pressure limits of 300bar. The injection volume was 10 µL. Above was the standard setting for the antibiotics with little or few variations when needed.

2.8. Quality Control

Various blanks were analysed for each stage of the experiments. The Italian samples were analysed with a travel blank (undeployed DGT Sampler). While laboratory blanks (spiking the organic mobile phases with the internal standard) were also analysed for the analytes of interest. Targeted compounds were not detected. This process was also conducted for all the samples that were taken in the United Kingdom as well as Ireland before the site was abandoned. The samples were analysed in parallel with the calibration standard solutions for instrumental performance.

2.9. Application of DGT Passive Sampler

DGT is an *in situ* samplers that can be used for sediments, soils, and water especially in the mining and other sectors (Zhang, 2003). It has been used to determine the concentration of metals, nutrients and organic chemicals in rivers and bioavailability of contaminants in soils. It has also been used to validate the effectiveness of WWTPs to remove emerging contaminants or bioavailable pollutants in the water such as antibiotics and personal care product ingredients before releasing into the environment (Chen et al. 2012b; Chen et al. 2015; Chen 2013; Vrana et al. 2005). Various studies have also been conducted on rivers across the UK and other parts of the world on labile metals (Thomas, 2009; Han *et al.*, 2013). Some of the other areas of DGT application include; monitoring and prediction of bioavailability and formation kinetics of various pollutants in the environment (Bade, Oh and Shin, 2012; Yin *et al.*, 2014) and predicting plant-available nutrients in the soil (Menzies, Kusumo and Moody, 2005; Tandy *et al.*, 2011). It is also useful in determining the chemical toxicity of effluent discharged into rivers over time. DGT can also be used to determine the (bio)available fraction of soil contamination with field testing on chemicals like Cu, Zn and Cd (Senila, Levei and Senila, 2012).

Studies have shown that Dissolved Organic Matter (DOM) could impact the uptake rate of the DGT. Persistent organic pollutants (POPs) have a strong affinity to DOM (Li *et al.*, 2011; Chen *et al.*, 2015). Also, the hydrophobicity of some organic pollutants is a factor to be considered while determining the types of DGT sampler and the sampling procedures in the sampling campaign. In an instance where the ionic strength of water is low and with or where the organic matter concentration is significant, DGT might not work (Yabuki *et al.*, 2013). This method involves the development of apparent diffusion coefficient (D_{ap}). This is carried out in the laboratory using the field sample collection and the obtained value is thereby used for in situ determinations. The equation given below can be used to calculate the apparent diffusion coefficient (D_{ap});

$$D_{ap} = (M\Delta g).\ (ACt)^{-1}. \quad (4)$$

Where deployment time (in seconds) is represented as t while $\sigma = (M/t)$. The equation could be written differently with "σ" being the angular coefficient of the deployment curve;

$$D_{ap} = (\sigma\Delta g).\ (CA)^{-1}. \quad (5)$$

There have been several studies using the DGT passive samplers where field validation tests are still required to validate some of the measurable parameters that have been extensively measured in the laboratory. Some of the areas where DGT can now be deployed includes organic pollutants bioavailability monitoring (effective concentration of analytes), fluxes in sediments and soils (Davison and Zhang, 2012), thermodynamic and Kinetic constants, speciation (a measure of labile organic and or inorganic species), time-averaged concentrations of chemicals and in situ chemical concentrations measurements.

The application and prospect of DGT in monitoring chemical bioaccumulation in the environment are summarised in Table 5.

Table 5: DGT applications and areas of interest in environmental monitoring

Chemicals	**Research update**	**Remarks**
Inorganic Chemicals • Metals • Nutrients ○ Phosphorus ○ Nitrates	Various Chemicals have been studied both in the lab and on the field	
Organic Chemicals • Pesticides • Personal care products • Pharmaceuticals • Anticancer drugs • Illicit drugs	Very little researches have been done and more will be required. Some of the missing data include	EDCs are to be intensely studied as there seems to be a paucity of information on this using the DGT

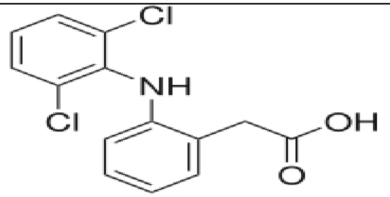
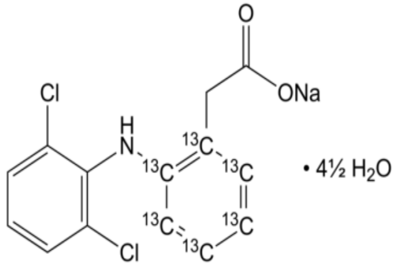
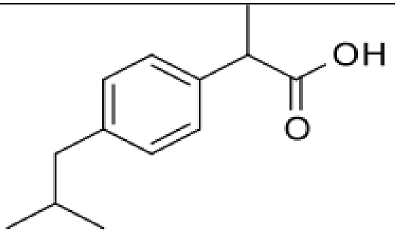
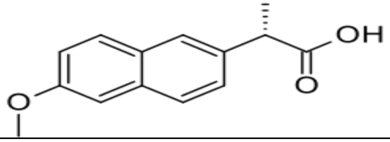
• Hormonal drugs • Endocrine Disrupting Compounds (EDC)	performance information on the deployment devices, -post-retrieval storage etc.	
PAH: Polycyclic aromatic hydrocarbons **Perfluorinated chemicals** **PFOS**: Perfluorooctane sulfonate **PFOA**: Perfluorooctanoic acid		

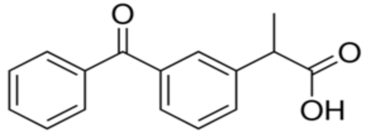
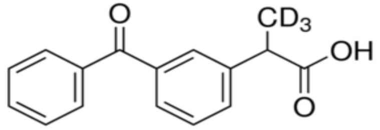
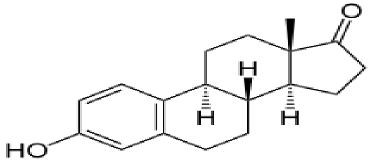
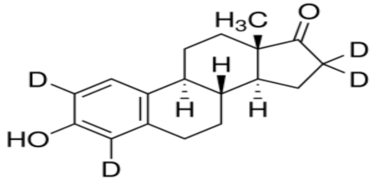
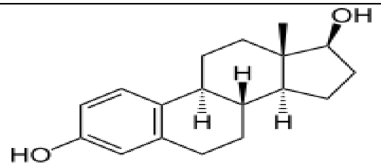
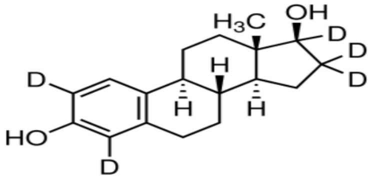
2.10. Organic chemicals

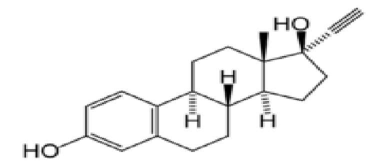
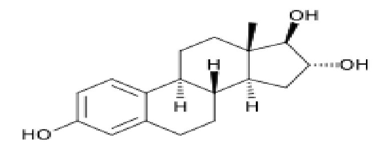
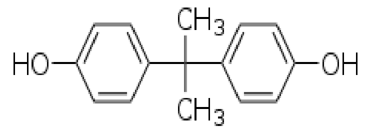
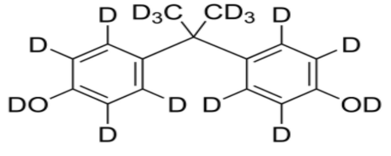
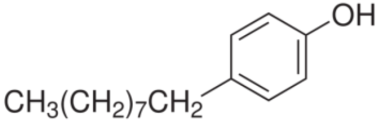
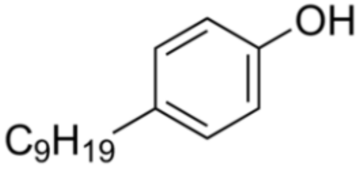
Various categories of chemicals have been sampled with DGT. At the time of this study, the following categories of organic chemicals have been studied using DGT, antibiotics, personal care product ingredients, pesticides and organophosphate flame retardants. The chemicals listed below have been effectively sampled and analysed in the laboratory.

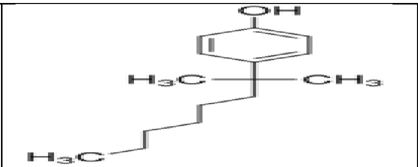
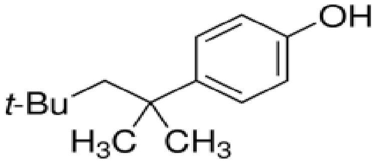
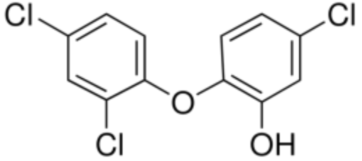
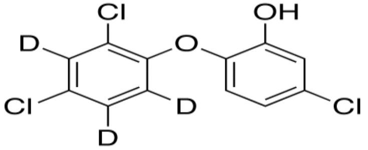
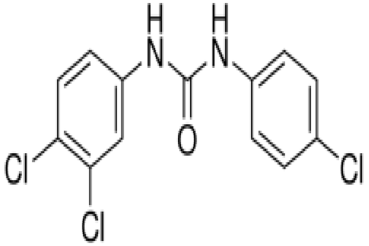
Few of the wide spectrum of organic chemicals have been selected for this study following up on their behaviours in the environment. Laboratory studies have been carried out on these groups of chemicals with a few field studies. These selected group of chemicals were investigated in the later chapters to give us insight into their behaviours in the environment as well as their behaviours in the removal process. Personal care products are used daily and formed part of human life while a selection of Antibiotics has been based on the ones appear frequently in WWTP. Properties of these compounds are shown in the table below.

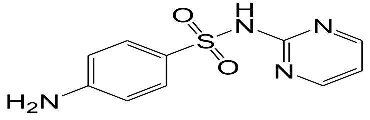
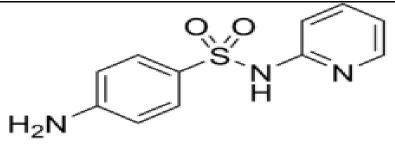
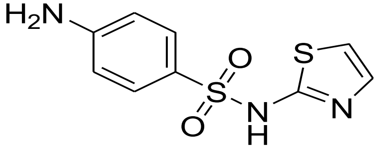
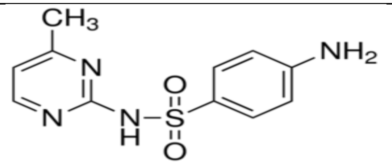
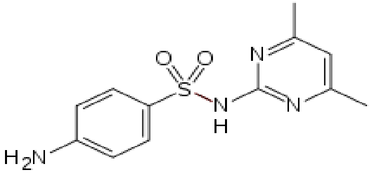
Table 5a: Pharmaceuticals / Personal Care Products

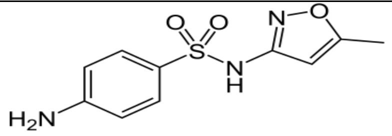
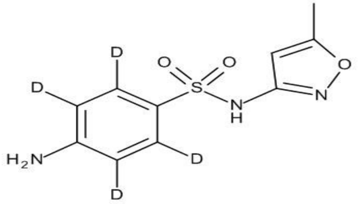
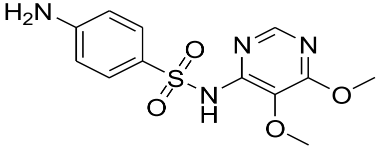
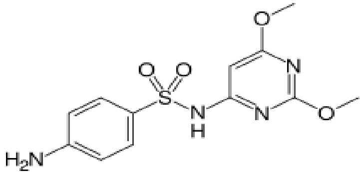
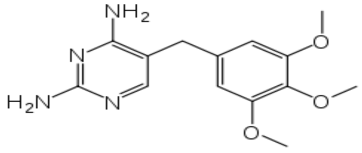
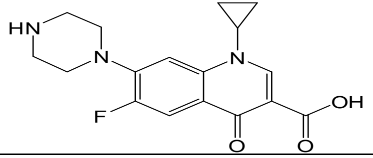
Group	**Name (abbrev)**	**CAS no.**	**Chemical structure**	**Mw (g/mol)**	**S_w (mg/L) at 25 °C**	**logK_{ow} (or logP)**	**pK_a**	**Analytical method**
Pharmaceuticals: non-steroidal anti-inflammatory drug (NSAID)	Diclofenac	15307-86-5		296.15	2.37	4.51	4.15	LC-MS
Internal standard	Diclofenac-(acetophenyl ring-$^{13}C_6$) sodium salt hemi (nonahydrate)	• 1261393-73-0 (anhydrous)		13C6C8H10Cl2NNaO2 · 4.5H2O 405.16				LC-MS negative
Pharmaceuticals NSAID	Ibuprofen	15687-27-1		206.29	21	3.97	4.91 [Drug Bank]	LC-MS negative
NSAID	Naproxen	22204-53-1		230.27	15.9	3.18	4.8	LC-MS negative

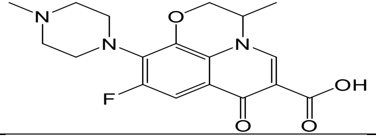
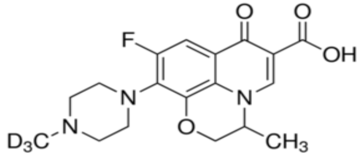
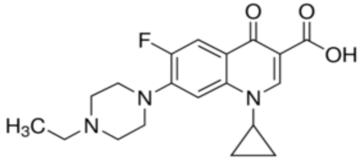
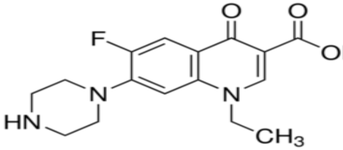
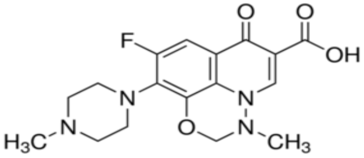
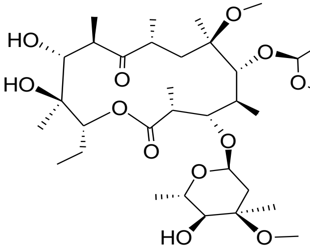
NSAID	ketoprofen	• 22071-15-4		254.28	51 (22 ºC)	3.12	4.45	LC-MS negative
Internal standard	Ketoprofen-d3	• 159490-55-8		257.30				
Hormones Estrogens	Estrone (E1)	53-16-7		270.37	30	3.13	10.3	LC-MS negative
Internal standard	E1-d4			274.39				
Hormones Estrogens	17-beta-estradiol (E2)	50-28-2		272.39	3.9	4.01	10.3	LC-MS negative
Internal standard	E2-d5			277.41				

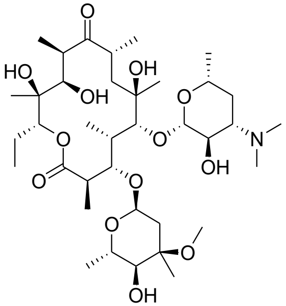
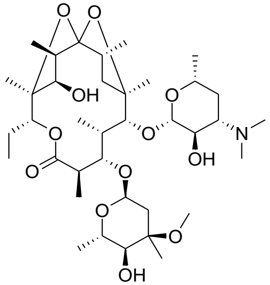
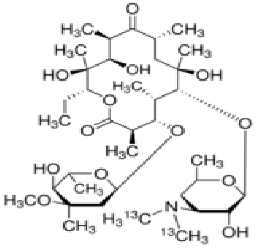
Hormones Synthetic Estrogens	17-alpha-ethinylestradiol (EE2)	57-63-6		296.41	11.3 (27 ºC)	3.67	10.3	LC-MS negative
Hormones Estrogens	Estriol (E3)	50-27-1		288.39	440 (est)	2.45	10.313.6	LC-MS Negative
Estrogenic chemical	Bisphenol A (BPA)	80-05-7		228.29	120	3.32	9.65, 10.4	LC-MS Negative
Internal standard	BPA-d16			244.38				
Alkylphenols	4-n-nonylphenol (4nNP)	104-40-5		220.36	7	5.99	10.3	LC-MS negative
Alkylphenols	Nonylphenols (a mixture of ring and chain isomers) (NPs)	• 84852-15-3		220.36				

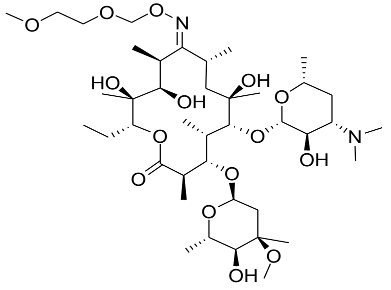
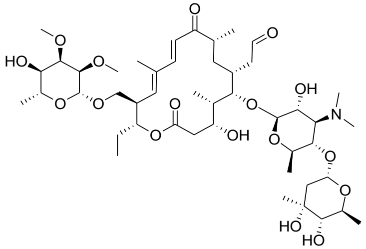
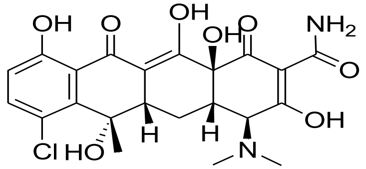
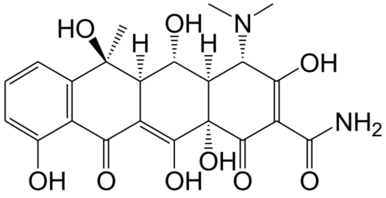
Alkylphenols	4-tert-nonylphenol (4tNP)	68152-92-1		220.36				
Alkylphenols	4-n-octylphenol (4nOP)							
Alkylphenols	4-tert-octylphenol (4tOP)			206.33	4.8	5.28	10.2	LC-MS negative
Disinfectant	Triclosan (TCS)			289.54	10	4.66	7.68	LC-MS Negative
Internal standard	TCS-d3			292.56				LC-MS negative
Anti-bacterial agent	Triclocarban (TCC)			315.19	0.65	4.90	11.4	LC-MS Negative

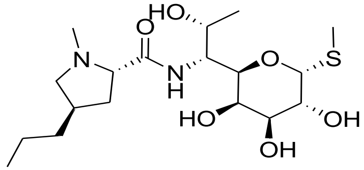
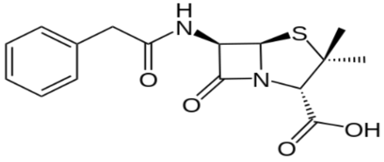
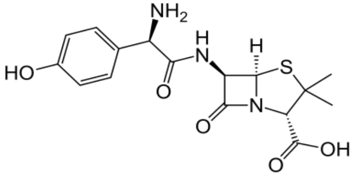
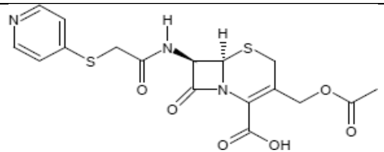
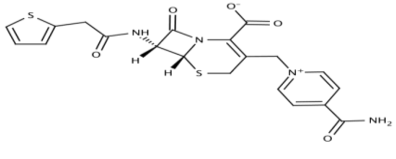
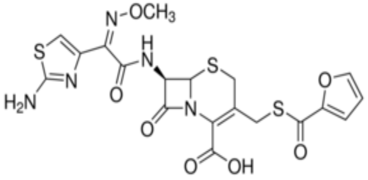
Group	**Name (abbr)**	**CAS no.**	**Chemical structure**	**Mw (g/mol)**	**S_w (mg/L) at 25 °C**	**logK_{ow} (or logP)**	**pK_a**	**Analytical method**
Sulfonamides (SAs)	Sulfadiazine (SDZ)	• 68-35-9		250.3	77	-0.09	1.87, 5.45	LC-MS positive
SAs	Sulfapyridine (SPD)	144-83-2		249.3	270	0.35	2.58, 8.43	LC-MS positive
SAs	Sulfathiazole (STZ)	• 72-14-0		255.32	373	0.05	2.01, 7.11	LC-MS positive
Can be Internal standard	Sulfamerazine (SMR)	• 127-79-7		264.30	202 (20 °C)	0.14	2.01, 6.99	LC-MS positive
SAs	Sulfamethazine (SMZ)	57-68-1		278.34	1500	0.9	2.07, 7.49	LC-MS positive

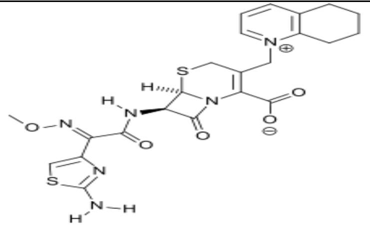
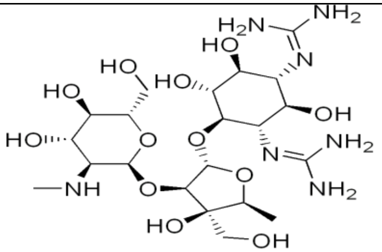
SAs	Sulfamethoxazole (SMX)	723-46-6		253.28	610	0.89	1.9, 5.6	LC-MS positive
Internal standard	SMX-d4	1020719-86-1		257.30				LC-MS positive
SAs	Sulfadoxine (SDX)	2447-57-6		310.33	2700	0.7		LC-MS positive
SAs	Sulfadimethoxine (SDM)	122-11-2		310.33	343	1.6	2.13, 6.08	LC-MS positive
Diaminopyrimidines	Trimethoprim (TMP)	738-70-5		290.32	400	0.91	3.23, 6.76	LC-MS positive
Fluoroquinolones (FQs)	Ciprofloxacin (CFX)	85721-33-1		331.30	30000	0.4	3.01, 6.14, 8.70, 10.58	LC-MS positive

FQs	Ofloxacin (OFX)	82419-36-1		361.3	2830	0.36	5.97, 8.28	LC-MS positive
Internal standard	Ofloxacin-d3 (OFX-d3)	1173147-91-5		364.39				LC-MS positive
FQs	Enrofloxacin (EFX)	93106-60-6		359.39	130000	1.1	3.85, 6.19, 7.59, 9.86	LC-MS positive
FQs	Norfloxacin (NFX)	• 70458-96-7		319.33	17800	-1.03	3.11, 6.1, 8.6, 10.56	LC-MS positive
FQs	Marbofloxacin (MFX)	• 115550-35-1		362.36				LC-MS positive
Macrolides (MLs)	Clarithromycin (CLM)	81103-11-9		748.00	0.342	3.16	8.99	LC-MS positive

MLs	Erythromycin (ETM)	114-07-8		733.90	2000	3.06	8.9	LC-MS positive
MLs	Erythromycin-H2O	23893-13-2		• • 715.9				
Internal Standard	Erythromycin-(N,N-dimethyl-13C2) (ETM-13C2)			• 735.91				

MLs	Roxithromycin (ROM)	80214-83-1		837.0		2.75	9.17	LC-MS positive
MLs	Tylosin (TYL)	1401-69-0		916.1	5	1.63	7.73	LC-MS positive
Tetracyclines (TCs)	Chlortetracycline (CTC)	57-62-5		478.9	630	-0.62	3.33, 7.55, 9.33	LC-MS positive
TCs	Oxytetracycline (OTC)	79-57-2		460.4	1000	-1.22	3.22, 7.46, 8.94	positive

Lincosamides	Lincomycin (LIM)	154-21-2		406.5	927	0.56	7.6	LC-MS positive
β-Lactams	Penicillin G (PEG)	• 61-33-6		•			2.8	
β-Lactams	Amoxicillin (AMO)	•		•			2.4 7.4 9.6	
β-Lactams	Cefapirin (CFP)	•		•			2.2	
β-Lactams	Cefalonium (CFL)	•		•				
β-Lactams	Ceftiofur (CEF)	80370-57-6		• 523.56 •				LC-MS Positive

β-Lactams	Cefquinome (CFQ)	•		•				
Aminoglycoside（AGS）	Dihydrostreptomycin (DHS)	•		• 583.59			7.8	

2.11. Performance data

With increasing field testing of DGT passive samplers, an increasing amount of data has been collected which has examined the effects of various environmental factors on their performance (Seethapathy *et al.*, 2008b). There is a range of parameters to be considered when deploying DGT. These include pH, salinity, temperature, dissolved organic matter, water flow rate and turbulence, biofouling, and structure/polarity of the analytes of interest. All these factors can potentially impact on the sampling rate R_s (Alexa *et al.*, 2009; C. Chen, 2013; Söderström *et al.*, 2009). Uptake behaviour of POPs and pharmaceutical ingredients are influenced by the pH of the aqueous medium/phase which affects speciation and potentially R_s. Biofouling is both dependent on the sampling time and the type of aqueous medium (Chen *et al.*, 2013). Several studies have suggested that keeping DGT in the aqueous medium for a long period leads to biofouling which ultimately reduces the uptake rate (Seethapathy et al. 2008; Zhang 2003). Reduction in the uptake could result from biofilms covering the surface area of the sampler thereby preventing the influx of analytes. Studies have suggested that a minimal deployment period less than 10days show less risk of biofouling(Chen *et al.*, 2013) while a similar study also suggested that DGT can provide time-integrated concentrations for up to 18 days in situ deployments with marginal biofouling effect on the diffusive boundary layer.

To integrate ambient concentrations over time to provide a time-integrated signal and sufficient sensitivity, deployment of o-DGT for 7 days is recommended to avoid the risks of significant biofouling and reaching the accumulation capacity of the binding gel. This is also a factor to be considered in the sampling design. There have been some experimental approaches to prevent biofilm formation on DGT. Deployment in the laboratory and field testing revealed that pH within the range 3 to 7.5 and ionic strength did not affect the response of DGT to various anti-biofilm agents. Adding silver iodide to the membrane filter was the most effective approach to the prevention of algal growth while Chloramphenicol and copper iodide were partially effective for over 14 days (Pichette *et al.*, 2007).

2.12. Resins (Binding gels)

Some viable studies have been undertaken by W.Chen, (2016) in the development and validation of the DGT technique both in the Laboratory and on the field. He suggested that suitability of resins is dependent on the compounds to be investigated and this led him to conclude that there is no single resin that is suitable for all types of pollutants. However, each of the pollutant groups due to their having similar chemical characteristics can be measured together using the same binding gels. It is important to determine which resin will have a better affinity to the chemical of interest, and elution performances. Some of the key factors to be considered while selecting suitable resins are not limited to ensuring that chemicals of interest do not have chemical interactions with the binding gel or receiving. It has been shown in previous studies that chemical accumulation by DGT increase with the length of deployment time for up to 10 days. HLB is a suitable resin for PCPs with good linearity while XAD-18 has been shown more consistent performance for antibiotics(Chen, 2016). This study was conducted in the laboratory and below are the result for various compounds that were adsorbed by HLB which affirmed its suitability for PCP sampling.

HLB binding gels' DGT mass uptake per time showed strong linearity for all the compounds investigated which are represented in Figure 7 below.

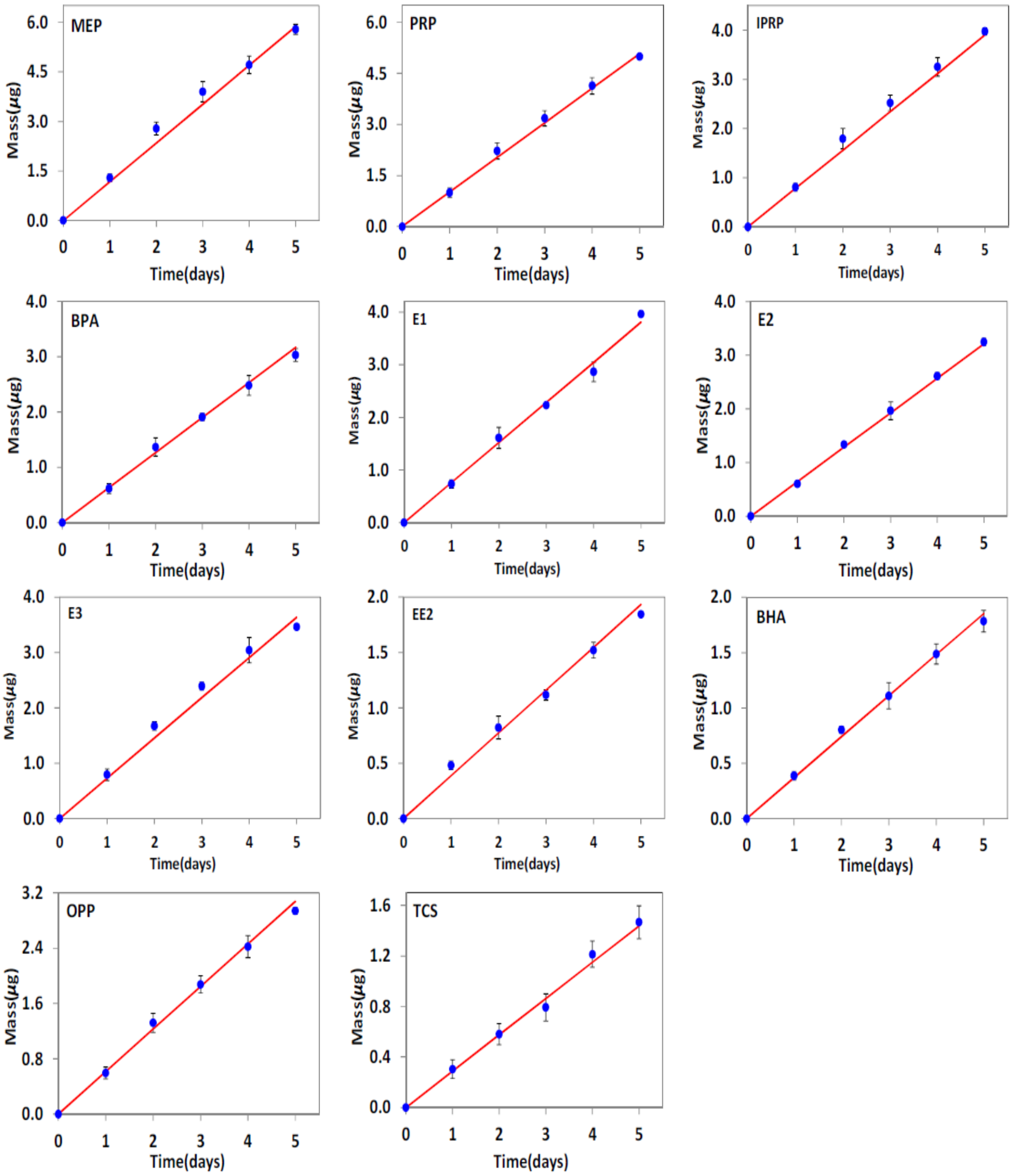


Figure 7: The linearity of measured masses (M, µg) of test chemicals in HLB-DGT deployments for with times (Laboratory testing at IS =0.01M, pH=6.8 ±0.2, T=24 ±2oC, n=3) (Chen, 2016)

Having considered various conditions that can influence chemical uptake and sampler performances, previous studies have concluded that certain resins are more suitable for some chemical classes than others. The table below shows a summary of some commonly used resins.

Table 6: Most commonly used resins and their suitability

Resin name	**Acronyms**	**Suitability**	**Reference**
Chelating ion exchange	Chelex 100	Inorganic compounds ○ Metals	Chelex-resin's DGT has a limitation with the reduced performance at both high and low pH ranges. Even though it has high selectivity for metal ions, it is pH-dependent (Drozdzak *et al.*, 2015)
Ion exchange	Amberlite XAD-2,4,7,8	Inorganic compounds ✓ Metals	
Amberlite XAD	Amberlite XAD-18	• It is a highly absorbent resin. • Organic compounds ○ Antibiotic	
Hydrophilic Lipophilic Balance	HLB	• Pharmaceuticals • Personal care products	
Strong anion exchange	SAX		
Cationic exchange	Microlite PrCH		

Table 7: Performance data of binding gels on various chemicals

Binding Gels	Analytes of Interest	Extraction Time	Performance /efficiency	Best performance PH	Reference
Chelex	Metal – Cu, Ni, Zn, Fe, Pb	24hrs	89% with 1mol/L HNO_3 and 96.2% with conc HNO_3 Uptake of Uranium on Chelex-100 was 94.9±1.3%	pH 5-9 (PH <5, the adsorptive capacity shrinks and PH>9, the resin is prone to swelling)	(Hutchins et al., 2012; Conesa, Schulin, & Nowack, 2010)
Diphonix resin gel	Metal - e.g. Uranium	24hrs	Accumulation was 97.4±1.5% while the elution efficiency was	pH (6-9) Diphonix resin gel performed better with Uranium compared to Chelex and Metsorb adsorbent gel	(Drozdzak *et al.*, 2015)
Metsorb adsorbent gel	Metal - e.g. Uranium Aluminium	24hrs	95.2±0.4% with 1mol L^{-1} NaOH /1mol L^{-1} H_2O_2	pH (5-9). For Al, it is more accurate than Chelex-100 at high pH	(Hutchins *et al.*, 2012) (Panther *et al.*, 2012)
Microlite PrCH cation exchange	NH4-N	24hrs	Uptake is >92.5 %, with elution efficiency 87.2% using 2molL-1NaCl. Detection limit was 0.63mgL-1 based on 24hrs deployment	It is appropriate for a broad range of pH (3.5-8.5), ionic strength (up to 0.012molL-1, NaCl)	(Huang *et al.*, 2016)
XAD-18	Antibiotics	24hrs			
HLB	PCPs	24hrs			

2.13. Reed Beds Nature-Based Technology (NBT)

Reed Beds Nature-Based Technology (NBT) has over the past few years garnered interest in wastewater treatment. Highly mechanised wastewater treatment technologies have played important roles treatment process while the operational costs to include energy costs, labour and set up costs have given way to Constructed Treatment Wetland (CTW) technology (Sundaravadivel and Vigneswaran, 2001). This technology use more of natural coagulants such as tannin and cactus, Moringa oleifera and nirmali seeds (Strychnos potatorum) which has been appraised for its environmental friendliness even in the removal of dye from the textile wastewater (Verma *et al.*, 2012) rather than the chemical coagulants such as Polyferric chloride (PFCl), Polyaluminium chloride (PACl), Polyferrous sulphate (PFS) and Polyaluminium ferric chloride (PAFCl)(Moussas et al., 2011). Reed beds technology was considered by the United States Army Construction Engineering Research Laboratories (USACERL) for sludge dewatering with many advantages to including low investment and operating costs, effective sludge removal costs, and other inherent values being a simple and economical technology compare to mechanical methods. However, most systems do have their limitations and the most prevalent limitation is the large land requirements and little scientific models of this pragmatic technology which is still at the developing stage(Kim and Smith, 1997).

There have been various researches on the use of this technology with over 167 sites in 1997 and environmental positive outcomes and performance data such as removal of ~10.7 mg/l of total nitrogen have further justified its potential use in the place of Conventional treatment technology (Griffin and Pamplin, 1998). This technology has always been commonly used for a small settlement of probably 1 to 2000 population equivalent where it would have ordinarily not economical constructing a mechanized treatment works (Rousseau *et al.,* 2004), however, its set up mas made it possible to be used in combination with the conventional treatment system as a secondary or tertiary stage system in the treatment process while it is being used in many European countries as for the treatment of sludge, Sludge Treatment Reed Beds (STRBs) (Kołecka et al., 2018). Integrated systems have been suggested for operations in some of the European Countries and eutrophication is one of the challenges surrounding the system. The free-water-surface (FWS) reed beds have been suggested to be lower in the removal rate compared to vertical flow (VF) wetlands (Rousseau et al., 2004). It has also been confirmed that Sludge Treatment Reed Bed (STRB), being a nature-based technology is also efficient in eliminating polar pollutants in addition to nutrient removal (primary purpose) and sludge dewatering with 99% removal rate of pharmaceuticals compared to Conventional WWTP (Kołecka *et al.*, 2019). A caution is to be exercised as the pollutants could be trapped or stored in the beds by a sorption process which is also to be considered in details while recommending the use of the NBT as an alternative to mechanical WWTP.

2.14. Introduction and summary of papers

The outcome of the study has been subdivided into four segments or papers. Paper 1 focused on an investigation into the effectiveness of nature-based technologies for the removal of pharmaceutical and personal /household products ingredients

The possibility of nature-based treatment technology as an alternative to the conventional treatment works was investigated using DGT as the sampling tool kit for Personal care product ingredients. This study was conducted in some sites around Florence in Italy for a period of 7-10days and the study suggested that nature-based technologies removal rates ranged from 41% to 100% while the conventional treatment works ranged from 40% to 100%. Performance of compounds varied across all the systems, but it was suggested based on the available data that nature-based technology would be a good alternative to the conventional treatment works. A further study was also conducted on the removal of antibiotics in both systems to examine the removal rates across both nature-based treatment technologies and conventional treatment works.

Paper 2 considered Effective removal of some selected pharmaceutical products in the wastewater. Samplers were deployed in six nature-based treatment works and three conventional treatment works. SPD, LIM, TMP, NFX, OFX, CFX, SMX and CLM were detected. Removal rates of detected chemicals were high except for SPD, SMX, and CLM. The poor removal rate could be attributable to various processes within the system and degradation of parents' chemicals which are outside our study. It implies that even though the systems might have been effective in the removal of these 3 compounds, but this study has not been able to substantiate this unless a full study with a full scan of varieties of antibiotics is conducted which will helps to know the sources of these chemicals in the process chain. The concentrations of the above-detected compounds at the conventional treatment works ranged from 6 ngL^{-1} to 960ngL^{-1} in the influent and 4.5 ngL^{-1} to 410 ngL^{-1} in the effluents while the concentrations at the nature-based were 6.6 ngL^{-1} to 11,600 ngL^{-1} in the influents and 3.7 ngL^{-1} - 1,295 ngL^{-1} in the effluents. This suggests that nature-based treatment technology is a viable alternative to conventional treatment works.

Following up on the Water Frameworks Directive, there is a huge financial implication of the practicality of the European Commission's expectations. It is therefore important to have a viable and economical way of monitoring river basins over a long period using a passive sampler. A study was designed to address the suitability of o-DGT as a monitoring tool kit for organic contaminants in the wastewater treatments plants. This third study focused on the Long-term monitoring of removal efficiency of selected pollutants during Wastewater Treatment

The removal efficiency of a wastewater treatment works in the Northwest of England was investigated for a consecutive 52weeks. This study focused on 23 personal care products being that they regularly used out of which 21 were effectively quantified. Samplers were deployed in the influents and effluents in triplicates. Due to different seasons of the year, there was high variability in removal rates for HEP and 4-T-0P showing a low removal efficiency with influent and effluent concentrations of 1 ngL^{-1} to 32 ngL^{-1} % in HEP and 11 ngL^{-1} – 325 ngL^{-1} 4-T-0P in the week 14. This suggests heterogeneity of contaminants sources over the year. The samplers at the effluent channels detected a higher concentration of compounds compared to the influent channels. Although an extensive work involving a full scan of personal care products ingredients spectrum would be helpful to understand all the various ingredients in the system which could have as a result of various processes given rise to the high concentration at the effluent. The study also suggested further that the removal of organic compounds from the treatment work could either be by sorption or biodegradation. It was however concluded that DGT could be deployed for long-term monitoring but having the same batch of binding gels or resins could reduce the variability to an acceptable limit.

The fourth paper was designed to investigate Fate and availability of emerging contaminants in sewage sludge amended soil Considering the impact of organic contaminants from sludge cakes application to farmlands and the disposition of the consumers and the landowners, 15 g of sludge cakes were mixed with MQ water in a ratio 3:37 to make sludge slurry. Many of these stakeholders are not predisposed to using sludge cakes to improve soil nutrients because of the risk to the health of both terrestrial and aquatic faunas. The initial study showed that 14 days would be ideal to leave farmland after applying sludge cakes. However, a further study was initiated to investigate this further and the behaviours of many chemicals. The study revealed that though some compounds degrade easily, a period not lesser than 28days was suggested to enable desorption of compounds from the sludge.

The importance of monitoring water basins to ensure good water status is important to the wellbeing of human existence. Various studies that were conducted as highlighted above confirmed that DGT is a reliable tool for long term pollutants monitoring. More also, nature-based treatment technology is a viable substitute for conventional treatments works. However, the downside of DGT is its inability to measure non-dissolved components of chemicals in the sampled medium. It means any non-fully soluble compounds would be difficult to measure using o-DGT.

3. Paper One: An investigation into the effectiveness of nature-based treatment technologies for the removal of personal care/household products ingredients

An investigation into the effectiveness of nature-based treatment technologies for the removal of personal care/household products ingredients

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Abstract

The advancement in technology and the need for sustainable wastewater treatment systems costs has led to the invention of environmentally driven treatment technologies to ensure that environmental hazards are kept under control. Conventional technology has proven to be capital intensive but efficient in the removal of wastewater pollutants, energy-intensive, and highly mechanised with high demand for manpower. This has since informed of a more robust and less expensive technology which utilises natural plants, solar energy and living organisms(Oller, Malato and Sánchez-Pérez, 2011). This study was able to investigate the prevalence of organic contaminants and compared the removal efficiencies of both the conventional treatment system and the nature-based technologies around Florence in Italy, with the aid of Diffusive Gradient in thin Film passive sampler containing Hydrophilic Lipophilic Balance (HLB) binding gels for the removals of personal care products ingredients. The concept behind the study was to investigate the performance of Diffusive Gradient in thin Film passive sampler in nature-based treatment technology to establish its viability as a replacement for the conventional treatment system. 23 Personal Care Products ingredients (PCPs) were investigated, and the study revealed average removal rates of 41 % to 100 % for preservatives in the nature-based technologies and 40 % to 100 % in the conventional treatment works. However, Heptyl paraben (HEP) had the lowest removal rates in both technologies. On the contrary, antioxidants were found to range from 131 ngL^{-1} – 6441 ngL^{-1} Butylated hydroxytoluene (BHT) at the effluent channel of nature-based technology and up to 100% removal Butylated hydroxyanisole (BHA) in the natured based technology while up to 56% Butylated hydroxyanisole (BHA) to 97% Ortho-phenylphenol were recorded in the

conventional systems. Endocrine disruptive compounds removal rates ranged from under 1% Nonylphenol to 100% β-estradiol in the nature-based and up to 100% 17α-Ethinylestradiol in the conventional treatment systems. Statistical analysis of some of the compounds, Methylparaben, Ethylparaben, Propylparaben, Butylparaben, IsoButylparaben, Triclosan also confirmed a week correlation between removal rates and log K_{oc} in both systems. The study thence suggested that even though the selection and design of the treatment system should be chemically driven, removal rates of these compounds show that nature-based technology is a viable alternative for the conventional wastewater treatment works. The concentration of Nonylphenol was high at the effluent which could be attributable to biodegradation of alkylphenols and other conjugates such as nonylphenol ethoxylates in the treatment system. This study did not investigate the entire group and as such, there may be many of the compounds in this group at the effluent channels which were not detected giving rise to the concentration of Nonylphenol (NP) at the effluent. It is hereby reiterated that NP concentration at the effluent cannot be construed to imply the performance of the treatment system neither the experimental process. Nonylphenol Ethoxylates (NPEs) have various uses beyond their primary use as surfactants in cleaning chemical formulations, they are also used as wetting agents and as emulsifiers or dispersants in the chemical formulations of some pesticides.

Introduction

The occurrence of Emerging Organic Contaminants (EOCs) released into water bodies via the discharge of waste-water effluents is raising concerns due to their potential persistence, toxicity, and bioaccumulation in biota. Waste Water Treatment Plants (WWTPs) are designed for pre-processing of wastewaters (Subedi *et al.*, 2014) before discharge into receiving water bodies. However, conventional treatment systems have been shown not to completely (or sufficiently) remove EOCs, such as some antibiotics, Endocrine Disruption Compounds (EDCs), Pharmaceuticals and Personal Care Product ingredients (PPCPs) from wastewater (Miao *et al.*, 2004; Chen *et al.*, 2006; Gómez-Canela *et al.*, 2014). The occasional upsurge in hydraulic loads of wastewater influents (Molander *et al.*, 2000; Kasprzyk-Hordern *et al.*, 2009) has also been confirmed to infrequently exceed the design capacities of the plants, which often lead to direct discharge of wastewater into receiving water bodies without or with only minimal treatment. Thus, providing new and effective wastewater treatment strategies for removing EOCs is still a challenge, as there is a need to reduce their environmental impact particularly on aquatic organisms and food chains.

Recently, nature-based technologies which are also called Integrated Constructed Wetlands (ICWs) have demonstrated to be a promising alternative to conventional WWTPs for removing or reducing levels of EOCs in wastewaters (Chen *et al.*, 2006; Farooqi, Basheer and Chaudhari, 2008; Wu *et al.*, 2015). The high running costs of conventional WWTPs (Friedler and Pisanty, 2006) and the considerable costs of constructing additional storage for occasional use during an upsurge in wastewater influx have highlighted nature-based technologies as a possible solution to wastewater treatment challenges. This method improves water quality at a reduced cost and utilizes interactions between a range of plant species and the constructed ponds where wastewaters are treated (Ntengwe, 2005; Struck, Selvakumar and Borst, 2008; Vymazal, 2014).

Among EOCs, the presence of PPCPs in wastewaters has received increased concern over the last decade due to their presence in different compartments of the aquatic environment i.e. water, sediments, biota (Bu *et al.*, 2013), their transformation products, fate and potential risks to humans and wildlife. The European Union (EU) and the United States Environmental Protection Agency (USEPA) have identified a list of priority pollutants present in wastewater and stormwater runoff that may cause a risk to the receiving water bodies as well as the surface water. An initial list identified 33 priority substances in 2000 under the EU Water Framework Directives (WFD) 2000/60/EC, out of which 13 were classed as priority hazardous substances which include Nonylphenols. However, in 2007, some PPCPs such as diclofenac was identified as future emerging priority substances which are now being removed, whereas bisphenol A, ibuprofen, triclosan, clofibric acid, and phthalates were proposed to be added to the list.

Further to previous investigations, this study considered removal efficiency as relevant to this research to investigate the suitability of nature-based technology as a viable alternative to conventional Wastewater Treatment Plants (WWTPs). However, the cost-effectiveness of any treatment system is very important and nature-based systems have proven to be cost-effective in removing nutrients that are discharged into the environment from arable land (Dunne *et al.*, 2012; Strand and Weisner, 2013). Nature-based treatment technology has been described as a successful sustainable wastewater treatment technology most especially for small to medium populated communities and for developing countries (Sundaravadivel and Vigneswaran, 2017). It is important to include land costs in the determination of total technology cost (Vymazal, 1996) as the nature-based technologies require a lot of landmass for their construction with reed beds. Vymazal, (1996) cited a typical example in Czech republic with vegetated beds covering between 18m^2 and 4493 m^2 with a population equivalent between 4 and 1100. The design is in such a way that the organic matter (BOD or COD) is removed by sedimentation and filtration together with the suspended matter. Nutrients such as Nitrogen, nitrates are removed by denitrification and nitrification in biofilms as well as plant uptakes. However, phosphorus nutrients are purified through precipitations with calcium, iron, and aluminium while some including nitrogen could also be removed by adsorption into soil. The system also makes room for pathogens to naturally die off, filtered or adsorbed (Sundaravadivel and Vigneswaran, 2001; Yin, 2010). This study has been centred on the organic pollutants which in effect get removed by degradation over a period or sorption into the soil or clay particles.

The commonly used reed beds plants in Europe is *Phragmites australis* (Decamp and Warren, 1998; Begg *et al.*, 2001). It is important that if the plants are not harvested and allowed to decompose, nutrients that have been taken up by the plants would be reintroduced into the treatment medium through decomposition (Brix, 1994, 1997). It is therefore suggested that the plants be harvested seasonally.

Objectives of the study

Following the demand for alternative treatment systems to conventional wastewater treatment works most especially in local settlements, nature-based technologies have been suggested as viable alternatives. However, there is not enough data yet to conclude on its ability to replace Conventional treatment works in the removal of Emerging Organic Contaminants. This study investigated the removal efficiency of personal care/household care product ingredients in six nature-based wastewater treatment systems and three conventional WWTPs in Italy. An evaluation of the removal rates would assist in deciding along with consideration for other factors, the possibility of nature-based systems being a viable alternative to the conventional WWTPs. This study would also assist in comparing the performance of both systems as well as their characteristics.

Materials and Methods

Chemicals and Reagents

Table 1 includes the sources of all chemicals used in the preparation of DGTs which were made at Lancaster University. Table 1 below provides details.

Table 1: Reagent Information

Name	Abbreviation	Purity	Supplier
Acetonitrile	ACN	HPLC	Fisher Scientific (UK)
Agarose	AG	Bio-analysis	Bio-Rad Laboratories (UK)
Ammonia solution	NH_4OH	5M, analytical	Sigma-Aldrich (UK)
Ammonium persulfate	APS	≥ 99%, analytical	Sigma-Aldrich (UK)
Gel solution	-	-	DGT Research Ltd (UK)
Hydrophilic-Lipophilic-Balanced	HLB	-	Waters Corporation (UK)
Milli-Q water	MQ water	(> 18.2 MΩ cm^{-1}	Waters Corporation (UK)
Methanol	MeOH	HPLC	Fisher Scientific (UK)
N,N,N′,N′-Tetramethyl ethylenediamine	TEMED	≥ 99%, analytical	Sigma-Aldrich (UK)
Sodium chloride	NaCl	≥ 99%, analytical	Sigma-Aldrich (UK)

DGT Samplers Preparation

HLB binding gels were prepared to a thickness of 0.56mm using 0.35 mm spacer, superimposed with agarose diffusive gel (0.80 mm thickness) as a diffusive layer. In preparing the binding gel, the principal ingredients are a mix of 4 g (wet weight, 63 µm beads size resin) HLB, with 10ml of gel solution. After mixing thoroughly, 60 µl of ammonium persulphate solution and 15 µl of N, N, N, N,-Tetramethyl ethylenediamine (TEMED) 99 % were added to create a solution. This solution was injected into glass plates using 0.35mm spacers and oven-dried at 42-46 ^{O}C for at least 1 hour until the gel was completely set. The gels were rehydrated in the MQ water (or deionised water) to obtain 0.56mm thickness. Diffusive gels were prepared by dissolving 0.9 g of agarose powder in 60 ml of Milli Q water (i.e. 1.5 %v/v) at around 80 °C in the oven to form a transparent agarose solution. This was also made to a thickness of 0.8mm and allowed to cool down under room temperature. Both the binding gels and the diffusive gels were cut to 25 mm diameter disc and stored in a saline solution NaCl solution (0.01-0.1M) pending their use. Studies have shown that most binding gels can be stored for over a year in a well-sealed container containing 0.01-0.03 M NaCl or $NaNO_3$ solution[1]. This was also investigated, and it was established that assembled DGT samplers could be stored for over 6 months with 95% uptake performance accuracy compared to those that were prepared and deployed immediately.

Sampling

Six nature-based WWTPs and three conventional WWTPs located in Italy were selected for this study. Table 2 summarizes information about the investigated WWTPs, including temperature, pH and the treatment processes within them. The six-nature based WWTP's have capacities to treat wastewater for 60 to 60,000 population equivalents with daily wastewater loads ranging from 7.5 to 525 m^3/day. DGT samplers were deployed in triplicate at the influent, intermediate and effluent points of the WWTPs for a period of about 7 – 10 days, in June 2016, with a total of 54 DGT analysed. Water sampling was performed using Diffusive gradients in thin films (DGT) passive samplers (Zhang and Davison, 2001; Chen *et al.*, 2017), which have been recognised to be a low-tech and cost-effective technique to monitor PPCPs in the influent and effluents of wastewater treatment plants (Foster and Shala, 2006; Chen *et al.*, 2017).

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[1] https://www.dgtresearch.com/faq/

Chemicals of Interest

Three groups of chemicals were investigated which include preservatives, antioxidants, and Endocrine Disrupting Compounds (EDCs). The compounds are; Methylparaben (MEP), 4-Hydroxybenzoic acid (PHBA), Propylparaben (PRP), Isopropylparaben (i-PRP), Ethylparaben (ETP), Butylparaben (BUP), Isobutylparaben (i-BUP), Benzylparaben (BEP), Triclosan (TCS), Heptylparaben (HEP), Ortho-phenylphenol (OPP), Butylated hydroxyanisole (BHA), Triclocarban (TCC), Butylated hydroxytoluene (BHT), Estriol (E3), Bisphenol-A (BPA), Diethylstilboestrol (DES), Estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (Ethinylestradiol) (EE2), 4-tert-octylphenol (4-t-OP), Nonylphenol (NP) and tert-butyl hydroquinone (TBHQ).

These compounds were investigated using DGT passive sampler rather than spot sampling due because of its advantages and better performance over the spot sampling. These performance characteristics include cheap costs of operations, spot samples have shown not to be effective to detect chemicals with low detection limit and as such, dissolved bioavailable organic compounds could be left unaccounted for in the environment. More also, considering the logistics surrounding this project in terms of spatial distributions of the sites, the spot sample would not be sustainable and cost-effective for this project. Also, the spot sampling data may not be the true reflection of the state of the sampled medium due to transportation and storage of samples.

Quality Control

All the samples were prepared in a single batch in the University of Lancaster Laboratory together with the internal standards. Upon preparation, the samplers together with the internal standards and sampling devices (polyethene mesh and weight) were sent to Department of Chemistry, via Della Lastruccia Italy in a cooler using air freight (DHL) to preserve and ensure that the samplers are not exposed to the environment or sea environment. Samplers were deployed and collected as designed while the extractions were done in the laboratory with travel blanks and filed blanks to monitor and check the quality of the process. The extracts were sent to Lancaster University Laboratory where the instrumentation analyses were conducted, and laboratory blanks were also run on the machine as part of the quality control measures.

Table 2 Italian treatment works classifications based on types and capacities.

S/N	Type	Influent loads (m^3/day)	Wastewater Type/ Constructed Wetland Scheme	Population equivalent	Description
CV1	Conv[2]	26,173	Urban & Industrial	120,000	Wastewater influx is a mix of Urban and Industrial waste. It was commissioned in 1984 having Olona River as the receptor. The treatment works have an average daily flow of 26,173 m^3/day with Average BOD load 3,328kg BOD/day and Average COD load 7,355 kg COD/day. However, the disinfectant compound is Sodium hypochlorite
CV2	Conv	34,524	Urban & Industrial	110,000	This treatment work receives mixed wastewater from Urban and Industrial. It was commissioned in 1986 having Bardello River as the receptor. The treatment works have an average daily flow of 34,524 m^3/day with Average BOD load 4,050 kg BOD/day and Average COD load 7,774 kg COD/day. However, the disinfectant compound is Sodium hypochlorite
CV3	Conv	30,348	Urban & Industrial	200,000	The information obtained for this system suggested that the treatment works must have been fully operational since 2002 with an average wastewater influx of 19,850 m^3/day mixture of urban and industrial wastewater. Average BOD load 2,058 kg BOD/day and Average COD load 6,229 kg COD/day. However, the disinfectant compound is Sodium hypochlorite
NB1	Nat[3]_ Based	75	Pre-treatment & 3 Stages	1,000	This Urban typology technology covers a land area of 2,014 m^2 as a replacement to an old activated sludge system which was no longer adequate to meet the demand of the community. Although the decision to set up the unit was also motivated by the fluctuations in the number of residents most especially during the winter period. This follows a French system where the system lacks a sedimentation stage but rather composed of gridded equalization tanks. The plant typology follows; RBF + VF + FWS were at the vertical flow reed bed filter, the accumulated solids are removed after 15-20yrs for agricultural uses as fertilizer. The second stage has two vertical-subsurface flow systems which promote the evapotranspiration process

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[2] *Conv. denotes Conventional wastewater treatment Works*

[3] *Nat_Based denotes Nature based wastewater treatment technologies*

					of the aquatic plants while the effluents water is used to recharge the groundwater through infiltration and then reused for irrigation purposes.
NB2	Nat_ Based	525	Urban Wastewater Pre-Treatment & 4stages	3,500	This Constructed wetland was designed as the largest secondary treatment plant in Italy. The wastewater flows into a horizontal subsurface treatment stage 1 after the primary pre-treatment stage. There are 16 Tuscany's autoctone vegetation species around this system. The system covers a landmass of 6,080m^2 and has been very effective in the removal of nitrogen most especially during the summer period. The plant typology is SFS-h + SFS-v + SFS-h + FWS. SFS-subsurface flow system, h-horizontal, v-vertical, and FWS—Free water system at the tertiary stage. This has been configured to work in all the seasons.
NB3	Nat_ Based	7.5	Urban Wastewater Pre-treatment & 1 stage(2beds)	125	This is an urban wastewater treatment system combining of pre-treatment: grid and Imhoff settling tank with a single-stage Horizontal flow (filter/HF) having 2 beds in series covering a total area of 420 m^2
NB4	Nat_ Based	16	Urban Wastewater Pre-treatment & 1 stage(1bed)	60	This is an urban wastewater treatment system combining of pre-treatment: grid and Imhoff settling tank with a single-stage Horizontal flow (filter/HF covering a total area of 201 m^2
NB5	Nat_ Based	55,296	Combined sewer Overflow Pre-treatment & 2stages	2,017	This is a combined sewer treatment technology designed to collect domestic sewages, rainwater runoff with industrial wastewater for transportation to the treatment plant. This is designed to discharge its excess wastewater to the nearby water body in the time of heavy rainfall which is called combined sewer overflows (CSOs). There are 4 sand filters vertical beds aimed to treat flood protection and control of several pollutants
NB6	Nat_ Based	19,465	Urban Wastewater Tertiary_ Pre-treatment & 2 stages	60,000	The need for an increase in depuration capability from 15000 to 60000 population equivalents led to the upgrade of the system containing two compartments of nitrification/denitrification technologies and Constructed wetland as the final stage. The CW was created as sedimentation being the tertiary treatment system with 10,000m^2 horizontal subsurface flow SFS-h and 50,000m^2 free water systems stages FWS respectively. Parts of the treated water are reused by the nearby industrial units.

[1] *Conv. denotes Conventional Treatment plants and Nat_Based denotes Constructed Wetland technologies*

DGT Extraction

Before extraction, DGT moulding surfaces were rinsed with Milli Q water to remove worms and organic deposits. HLB binding gels were removed from the DGT mouldings and placed in 15 ml vials. Samples were spiked with a freshly prepared mixed internal standard (50 µg/ml) per sample containing Parabens, PHBA-D4, E3-D2, TCS-D3, BPA-D16, E2-D5, EE2-D4, E1-D4, OPP ^{13}C, BHA-D3, 4-T-OP-D17, NP-D4, and BHT-D24. 5 ml of acetonitrile from Fisher Scientific (UK) was added to each sample and the 15ml vials were arranged in an ultrasonic bath for 30 minutes. The organic solvents were decanted into separate bottles and the extractions were repeated with new 5 ml vials. The vials were washed with 2 ml of acetonitrile and the samples were blown down to dry under a flow of ultra-pure nitrogen at 40 °C. Analytes were reconstructed using 1 ml acetonitrile and the samples were transferred into 2.5 ml vials for storage in the refrigerator at 4 ^{0}C. 100 µl of samples were taken and mixed with 400 µl of MQ water (20/80). The resultant volume of 500 µl was filtered into vials using a 0.22 mm PTFE syringe filter. Finally, 500 µl aliquot of each sample was transferred to the LC-MS for instrumentation analysis.

Chemical analysis

A Shimadzu Liquid Chromatograph Mass Spectrometer 8040 was used to analyse the selected Personal Care Product ingredients. A 10 µl of each extract was injected into an XBridge^{R} C18 column (Waters Corporation., 3.5 µm, 2.1 mm. 100 mm length) fitted with a guard column (Waters Corp., XBridge^{R} BEH C18, 3.5 µm, 2.1 mm, 5 mm length). The mobile phase was 5 mM NH_4OH in acetonitrile and 5 mM NH_4OH in Milli-Q water. The gradient procedure consisted of 4min 15% of 5mM NH_4OH ACN, held for 9 min, then increased to 80% of 5mM NH_4OH ACN, held for 5 mins before increasing to 100% of 5mM NH_4OH ACN and then sustained for 4.5 mins. Finally, 7.5 min of post-run to ensure the re-equilibration of the column before the next injection. The temperature of the column was set at 25 °C. The internal standard method was used for analytes' quantification. However, the analytes of interest were identified using the retention time of each of the compounds while many of them were manually integrated. This method has previously been used by Chen *et al, (*2017).

Quantification

Upon completion of the instrumental analyses, chromatograms of analytes of interest were selected and integrated. Internal calibration curves of nine concentrations (1, 2.5, 5, 10, 25, 50 100, 250, and 500ng/ml) were constructed for the quantification of target analytes in the samples. Good standard calibration curves were constructed with strong linearity and correlation coefficients of 0.999[4].

The DGT equation is often used to calculate analytes concentration in a sampled medium. Determining the bulk concentration C_b, diffusion coefficient D_e of all the compounds was required at the various sampling temperature for all the sites. Although, the laboratory concentrations were measured at 25^{O}C using 0.88mm thickness diffusive agarose gel layer and the D_e at various temperatures were calculated using equation 2 (Chen, Zhang and Jones, 2012; Chen, 2013). Diffusion coefficient at the average temperature in the field at the time of deployment and removal of the samplers were calculated to obtain the analytes bulk concentrations for each sample.

$$\mathbf{C_b} = \frac{\mathbf{M(\Delta g+\delta)}}{\mathbf{D_e At}} \qquad (1)$$

$$\boldsymbol{Log\ D_{e(t)}} = \frac{\mathbf{1.37023(\boldsymbol{t}-25)+8.36\times10^{-4}(\boldsymbol{t}-25)^2}}{\mathbf{109+\boldsymbol{t}}} + \boldsymbol{log}\frac{\boldsymbol{D_{e25}}\mathbf{(273+\boldsymbol{t})}}{\mathbf{298}} \qquad (2)$$

Where t stands for the temperature at the deployment site, D_e is the Diffusion coefficient at 25°C and $D_{e\,(t)}$ is the calculated diffusion coefficient at temperature t.

Results and Discussion

Tables 3 and 4 below show the DGT concentration data for the selected analytes present in the influents and effluents of the conventional and nature-based wastewater treatment plants in Italy environs. The percentage occurrence defines the prevalence of these compounds in each of the sampled media as evidenced by the uptake mass in the binding gel.

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[4] See supplementary information for details.

Table 3 Concentrations of PPCPs ingredients in the Influents of conventional and nature-based wastewater plants in Italy

Chemical of Interest	Nature-Based Treatment Plants		Conventional Treatment Plants	
	% Occurrence[5]	Range (ngL^{-1})	% Occurrence	Range (ngL^{-1})
PRESERVATIVES				
Methylparaben (MEP)	100	161 - 872	100	302 - 966
Ethylparaben (ETP)	100	113 – 2340	100	263 - 734
Propylparaben (PRP)	100	38.2 – 242	100	64 – 407
Propylparaben (i-PRP)	33	3.02 – 35.5	33.3	3 – 516
Butylparaben (BUP)	100	2.40 – 1593	100	9 – 31
Butylparaben (i-BUP)	33	3.02 – 511	33.3	3 – 4
Benzylparaben(BEP)	17	2 – 3	nd	nd
Heptylparaben (HEP)	100	3 – 332	33.33	3 – 9
4-Hydroxybenzoic acid (PHBA)	33	2 – 24	33.33	2 – 6
ANTIOXIDANTS				
Butylated hydroxytoluene (BHT)	83	5 – 6441	100	1335 - 6707
Butylated hydroxyanisole (BHA)	100	14 – 30	100	19 – 25
Ortho-phenylphenol (OPP)	100	224 - 4199.	100	415 – 3919
Triclosan (TCS)	100	16 – 684	100	53 – 127
Triclocarban (TCC)	nd	nd	nd	nd
ENDOCRINE DISRUPTIVE COMPOUNDS				
Bisphenol-A (BPA)	100	138 – 869	100	197 - 8311
Diethylstilboestrol (DES)	100	5 – 12	67	3 – 8
Estrone (E1)	100	16 – 95	100	10 – 45
β-estradiol (E2)	83	5 – 52	100	4 – 10
Estriol (E3)	100	13 - 395	100	14 – 57

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[5] This represents the % of treatments works where the chemicals are detected. For instance, if four systems were investigated for a compound and it is detected in one out of four, the % Occurrence would be 25%

17α-Ethinylestradiol (EE2)	17	5 – 11	33	3 – 5
4-Tert-Octylphenol (4-t-OP)	100	16 – 65	100	31 – 446
Nonylphenol (NP)	100	2440 – 185	100	2154 – 4315

Table 4: Concentrations of PPCPs ingredients in the effluents of conventional and nature-based wastewater plants in Italy

Chemical of Interest	Nature-Based Treatment Plants		Conventional Treatment Plants	
	% Occurrence	Range (ngL^{-1})	% Occurrence	Range (ngL^{-1})
PRESERVATIVES				
Methylparaben (MEP)	100	29 – 57	100	19 – 68
Ethylparaben (ETP)	100	21 – 89	100	35 – 84
Propylparaben (PRP)	83	3 – 54	100	3 – 27
Propylparaben (i-PRP)	nd	nd	nd	nd
Butylparaben (BUP)	nd	nd	33	2 – 3
Butylparaben (i-BUP)	nd	nd	nd	nd
Benzylparaben(BEP)	17	1 – 4	nd	nd
Heptylparaben (HEP)	100	2 – 1008	33	3 – 6
4-Hydroxybenzoic acid (PHBA)	17	2 – 3	nd	nd
ANTIOXIDANTS				
Butylated hydroxytoluene (BHT)	100	131– 4001	100	646 – 2059
Butylated hydroxyanisole (BHA)	nd	nd	67	4 – 18
Ortho-phenylphenol (OPP)	100	6 – 87	100	26 – 36
Triclosan (TCS)	67	4 – 76	100	8 – 17
Triclocarban (TCC)	nd	nd	nd	nd
ENDOCRINE DISRUPTIVE COMPOUNDS				
Bisphenol-A (BPA)	100	53 – 209	100	34 – 203
Diethylstilboestrol (DES)	83.3	3 – 11	100	3 – 3
Estrone (E1)	50	3 – 19	67	3 – 16

β-estradiol (E2)	nd	nd	33	4 – 5
Estriol (E3)	33.3	4 – 24	33	3 – 4
17α-Ethinylestradiol (EE2)	16. 7	5 – 6	nd	nd
4-Tert-Octylphenol (4-t-OP)	100	9 – 396	100	45 – 391
Nonylphenol (NP)	100	2595 –23828	100	4218 –27444

The selected analytes were grouped into 3 major categories viz preservatives, antioxidants, and endocrine-disrupting compounds (EDCs). Tert-butyl hydroquinone (TBHQ) was found only in the influent of the nature-based and conventional WWTP 2 at concentrations 17.4 ngL^{-1} and 11.1 ngL^{-1}, respectively. No trace of this compound at the effluents of these plants, evidencing that both wastewater treatment systems were sufficiently efficient in the removal of Tert-butyl hydroquinone. It also shows that the compound is not prevalent in the area, TBHQ has not been reported in Tables 3 and 4. On the contrary, the highest concentrations were detected for nonylphenol (NP), which ranged from 19000 ngL^{-1} to 24000 ngL^{-1} in the influents and from 2600 ngL^{-1} to 24000 ngL^{-1} in the effluents of the nature-based technologies. Similar patterns were detected for the conventional treatment plants with a range of concentrations between 2200 ngL^{-1} and 4300 ngL^{-1} in the influent and from 4200 ngL^{-1} to 27000 ngL^{-1} in the effluent. This compound was found to be highly prevalent in all treatment plants investigated and it reflects that both technologies are not capable of removing this compound. But on the hindsight, Nonylphenol prevalence at the effluent could have resulted from degradation of other forms such as nonylphenol ethoxylates which were not investigated at the influents resulting in higher concentration at the effluents(Ho and Watanabe, 2017; Liu *et al.*, 2017)

Nonylphenol

Nonylphenol belongs to a family of organic compounds called “long chain alkyl phenols" (LCAPs)(Liu and Abreu, 2006). They are commonly used in the production of antioxidants, laundry, and dish detergents, solubilizers and emulsifiers. They are also used as lubricating oil additives (Soaresa *et al.*, 2008). They have however developed much attention in the environmental study because of their prevalence in the environment with their potential active roles as endocrine disruptors and xenoestrogen. Nonylphenol is commonly found in river water, sediments, soils effluents from sewage or wastewater treatment works and sewage sludge.

Nonylphenol ethoxylates (NPEs) are very common surfactants in the group used in industrial formulations in textiles and clothing which degrade in the environment to Nonylphenol (Soaresa *et al.*, 2008). This gives us an insight into why there is an increase in its concentration at the effluents. This compound is banned (Julis *et al.*, 2014), and its presence in European wastewater cannot be overlooked (Havranek *et al.*, 2016) based on the fact that some of the products manufactured overseas which contain this additive end up in Europe. It is also a precursor to commercial compound Alkylphenol and Nonylphenol Ethoxylates which are used in detergents, pesticides, paints, personal care products, and plastics etc. It can be inferred from the results obtained in this study and by other researchers that there is a high possibility of occurrence of NP in our environment. This study measured NP which is a breakdown product of Nonylphenol polyethoxylate surfactants while the parent compound was not measured. This, however, explains why there were higher concentrations in the effluent channels which they might have got into the wastewater treatment system as parent compounds and thence degrade into Nonylphenol compounds Detailed concentrations of the investigated chemicals are shown in the above Table 3 and 4.

Removal rates

Six WWTP technologies were investigated along with the removal rates across the study compounds. Figures 1- 4a pictorially depict the removal rate of these compounds across the various treatment processes. Nature-based and conventional treatment works show that both treatments technologies have 88% and 87% removal rates respectively for the investigated preservatives. However, the removal rate at the individual nature-based technologies ranged from 5% HEP to 100% BUP across all the nature-based technology while the conventional treatment system ranged from 40% HEP to 100% BUP. These compounds Nonylphenol, 4-tert-octylphenol, Estrone, Diethylstilboestrol, Triclosan, Butylated hydroxytoluene had some negative removals as shown below. These were because of higher concentrations at the effluent channels compared to the influent channels. These outliers have no direct connotations in terms of the performance of the removal system or the treatment process. The increase could have resulted from many of the parent compounds coming in through the influents without being measured which biodegrade in the treatment process to liberate the analytes of interests. An example is nonylphenol which belongs to the alkyl phenol group(Rice *et al.*, 2003; Priac *et al.*, 2017). Many compounds in the group Alkylphenol and alkylphenol polyethoxylates could have been received into the treatment works giving rise to nonylphenol as they are subjected to biological processes. The only effective way of investigating the sources of these compounds is a full scan for their conjugate and metabolites which will be an extensive but sources targeted study on its own.

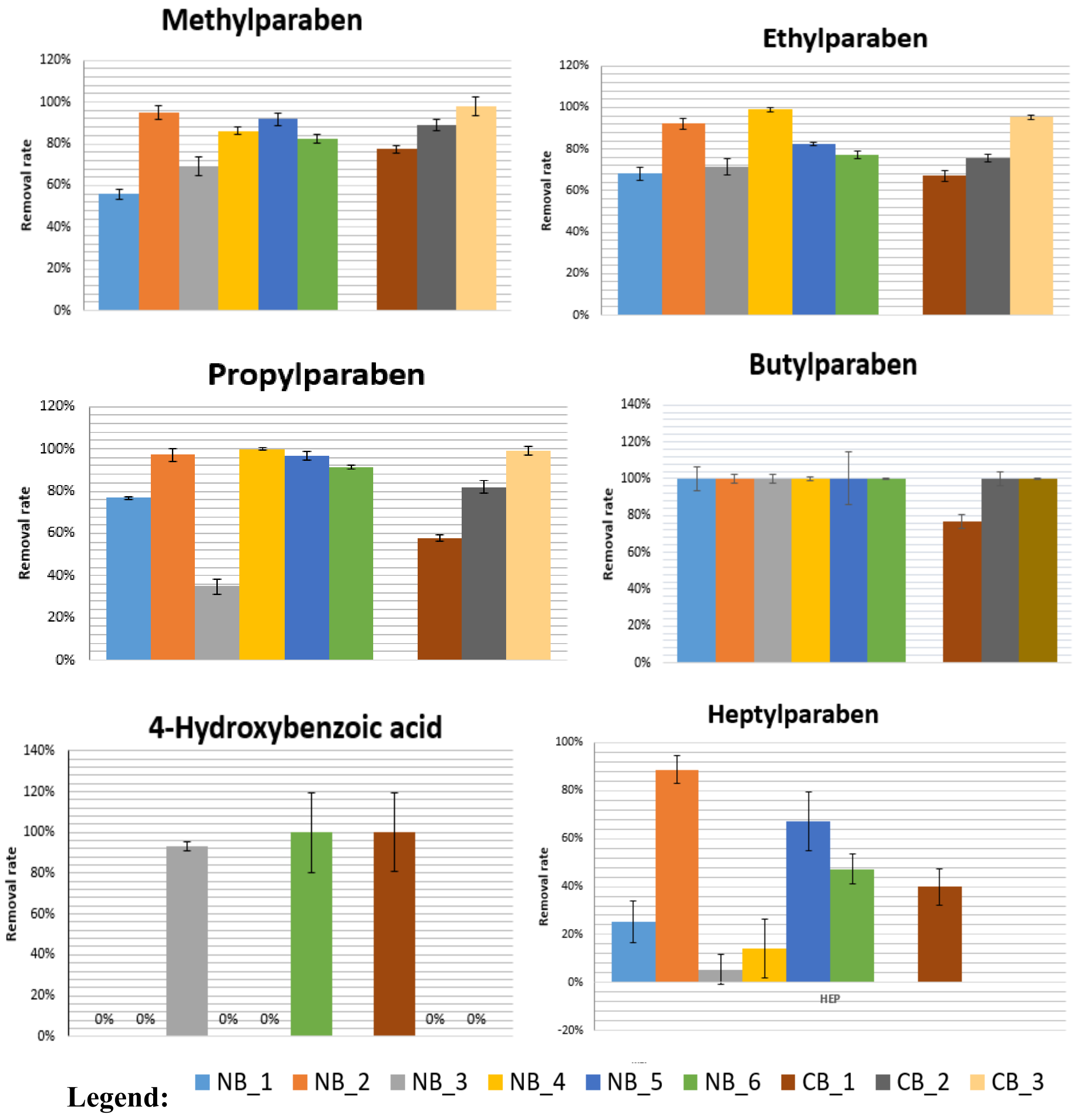
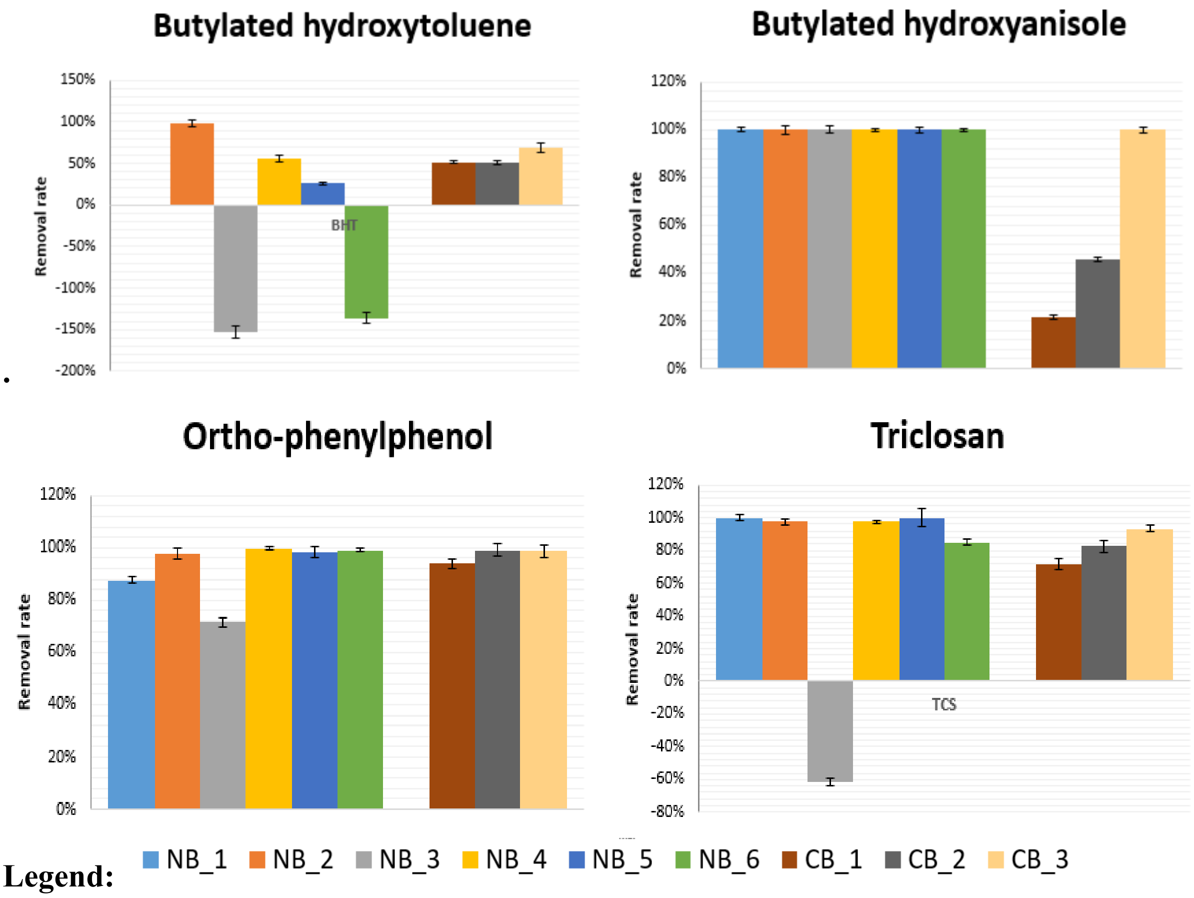


Figure 1: Removal rate of Preservatives in both Nature-based (NB) treatment technologies and Conventional (CB)Wastewater treatment works

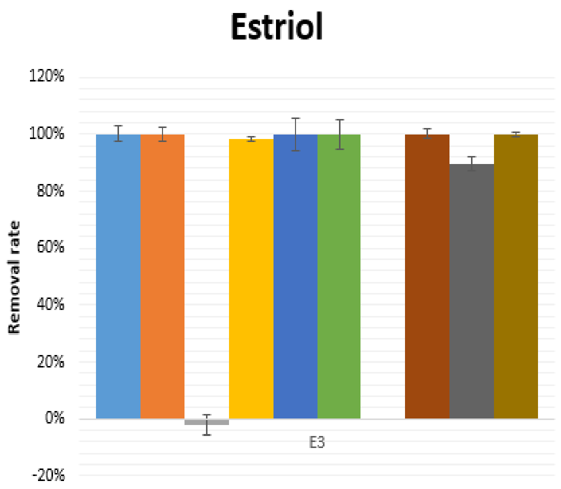
The results also show that IsoButylparaben and IsoPropylparaben were detected in two of the nature-based technologies and conventional works with 100% removal rate. However, Benzylparaben had 100% removal rates in a nature-based technology while Tert-Butylhydroquinone also recorded a 100% removal rate in a nature-based technology and a conventional treatment works. Figure 1 above depicts a promising removal potential of the above compounds of interest in both systems (Nature-based technology and Conventional treatment works) which are very ubiquitous in our environment due to the extensive use and high consumption of personal care products across all ages.

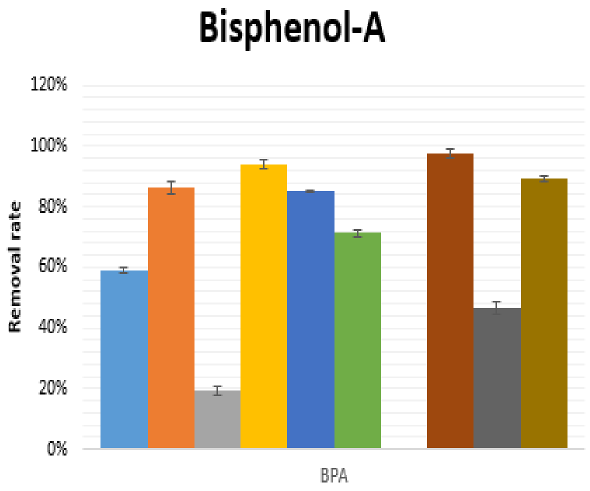


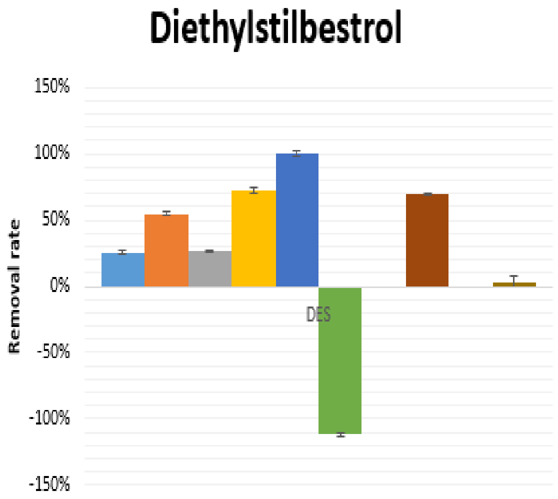
Legend:

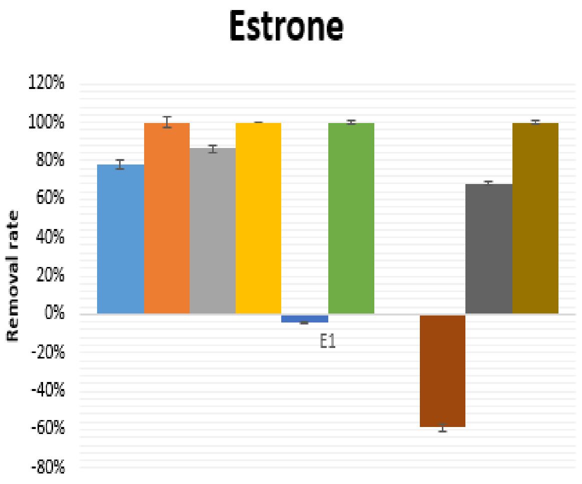
Figure 2: Removal rate of Antioxidants in both Nature-based (NB) treatment technologies and Conventional (CB) Wastewater treatment works

Both treatment technologies also demonstrated that effective removal rates of antioxidants. the nature-based technologies had removal rates ranged range from below 1% (5 ngL^{-1} - 217 ngL^{-1} at the effluent) BHT to 100% removal rates for BHA in all the NB technologies while the conventional treatment works demonstrated removal rates ranging from 22% BHA to 100% BHA. However, the average effective removal rates for both nature-based technologies and conventional treatment systems ranged from <1% BHT to 100% BHA and 56% BHA to 97% OPP respectively. Meanwhile, the overall average removal rate for antioxidants across all the investigated nature-based technologies stood at 60% while that of conventional treatment works was 73%. It is known that DGT only measures the dissolved phases and some of the compounds that show negative removal rates may have been removed in the adsorbed phase. Both dissolved and adsorbed phases must be measured should a conclusion be required to determine the efficiency of the removal systems. (Gao *et al.*, 2016). In addition to this, the measured compounds could have also existed in various conjugates which were not our targeted compounds for this study. It implies that some of these compounds may have to be investigated individually in these treatment works to be able to understand further their partitioning and removals phases.









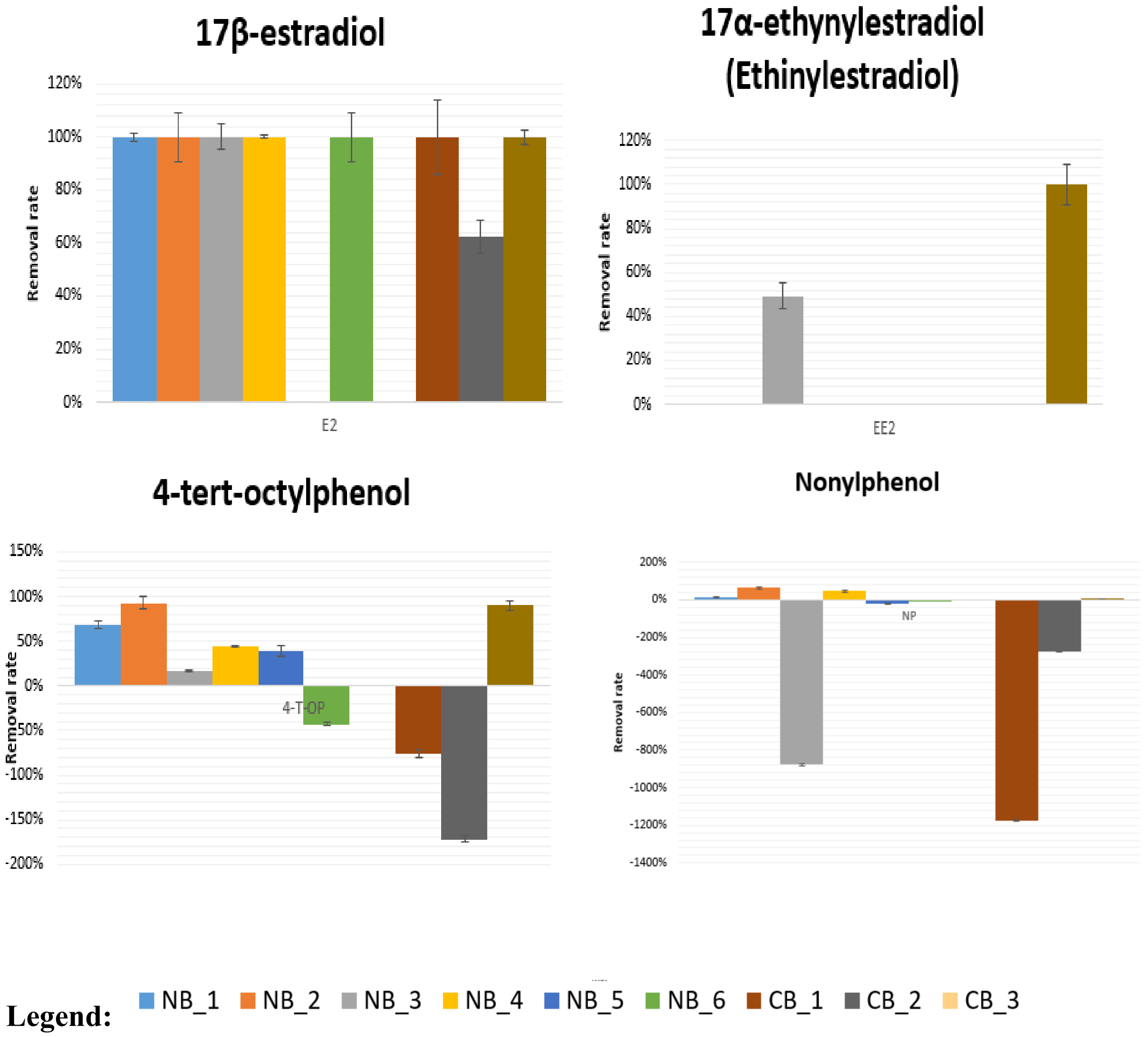


Figure 3: Removal rate of Endocrine Disruptive Compounds in both Nature-based (NB) treatment technologies and Conventional (CB) Wastewater treatment works

The removal rates of the EDCs was at the lowest in NP to 100% E2 in the natured based technologies and up to 100% EE2 in the conventional treatment works. However, considering the individual nature-based technologies and the conventional treatment works, the study showed a low removal rate of NP to 100% in many of the systems. The remaining data have been grouped into three categories viz Figures 4(a-j), which represents the preservatives mostly the parabens, Figures 5(a-d) for the antioxidants and Figures 6(a-h) for the Endocrine Distributive compounds. Figure 4a below depicts a comparison of the removal efficiencies of WWTPs for methylparaben.

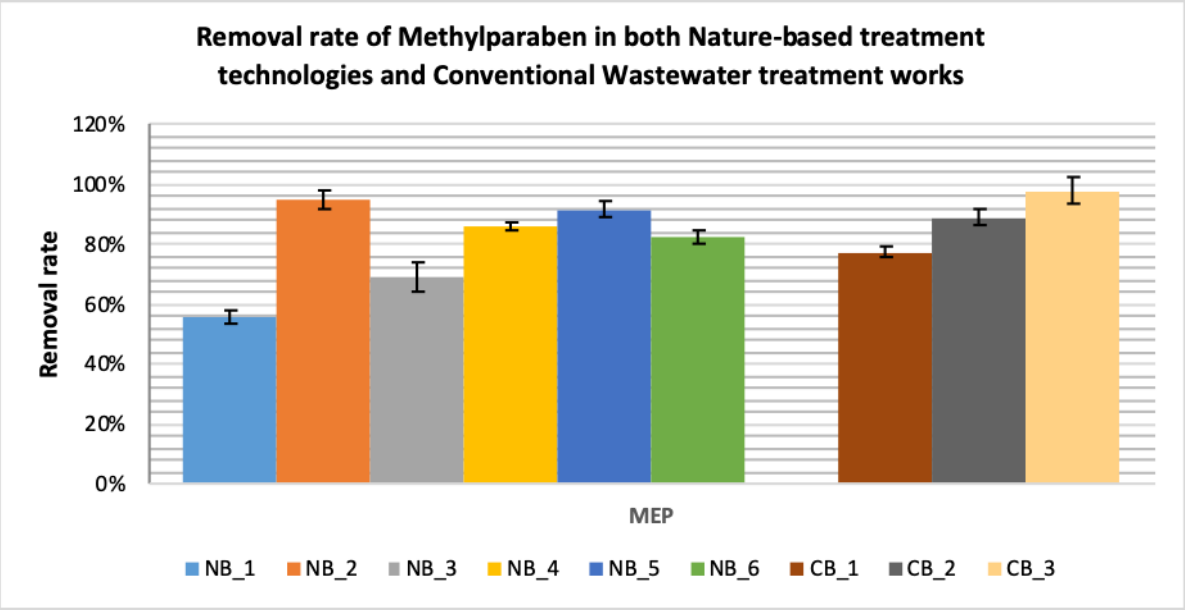


Figure 4a: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of MEP

The use of parabens as a preservative in cosmetics is regulated by the European Regulation on Cosmetic Products (2009)(The European Parliament, 2009). The maximum allowable concentrations is 0.8% (w/w) "weight for weight" for parabens mixtures and 0.4% (w/w) for single esters. However, Annex V of Regulation (EU) No 1223/2009 has recently been amended under Regulation (EU) No 358/2014 to prohibit the use of isobutyl paraben, isopropyl paraben, phenyl paraben, methyl paraben, and benzyl paraben in cosmetics(European Commission, 2014) while the use of propylparaben and butylparaben in children cosmetics has also been prohibited and as well as the maximum concertation being lowered under Regulation (EU) No 1004/2014 (Commission Regulation (EU) No 1004/2014, 2014).

From the above results, it is clear that both systems are suitable for the removal of methylparaben although there are variations within the technologies and across the systems. There is a range of possible factors that could be responsible for these variations viz the daily influent loads, the population sizes that are being served and the type of the system in terms of the treatment stages. Nature-based 2 technology is an Urban pre-treatment with 4 treatment stages technology which serves around 3,500 population with daily wastewater loads of 525 m^3/day. The other nature-based technologies treat wastewater for smaller communities except for nature-based 6 that serving around 60,000 people. Nature-based (5) technology has 55,296 m^3/day and NB_6 with 19,465 m^3 daily loads. Their designs are different as NB_5 was designed for a combined sewer overflow system that goes through a pre-treatment stage into two treatment stages while nature-based 6 treatment technology was designed for Urban wastewater to includes a tertiary treatment stage. It can, therefore, be suggested that removal rates have no direct relationship with daily loads or the population equivalents but their impact could affect the efficiency of the treatment system.

The BOD/ COD ratio in treatment works can be used to some degree to determine treatability and degradability of organic matter of waste-water (Contrera *et al.*, 2015). In practice, wastewater with a BOD to COD ratio between 0.4 and 0.6 is deemed treatable. For the three conventional works, it could be suggested that they are treatable due to their BOD: COD ratio being within the range of 0.4 – 0.6 which suggest high removal rates for most of the compounds. However, other parameters in the conventional wastewater treatment work such as the Hydraulic Retention times also have a direct relationship with the removal rate and biodegradability in the system. Zhang et al, (2015) suggested that by reducing the HRT on the biodegradation of trichloroethylene wastewater from 25hours to 5hours, the removal rate also decrease from 95% to 84.15%.

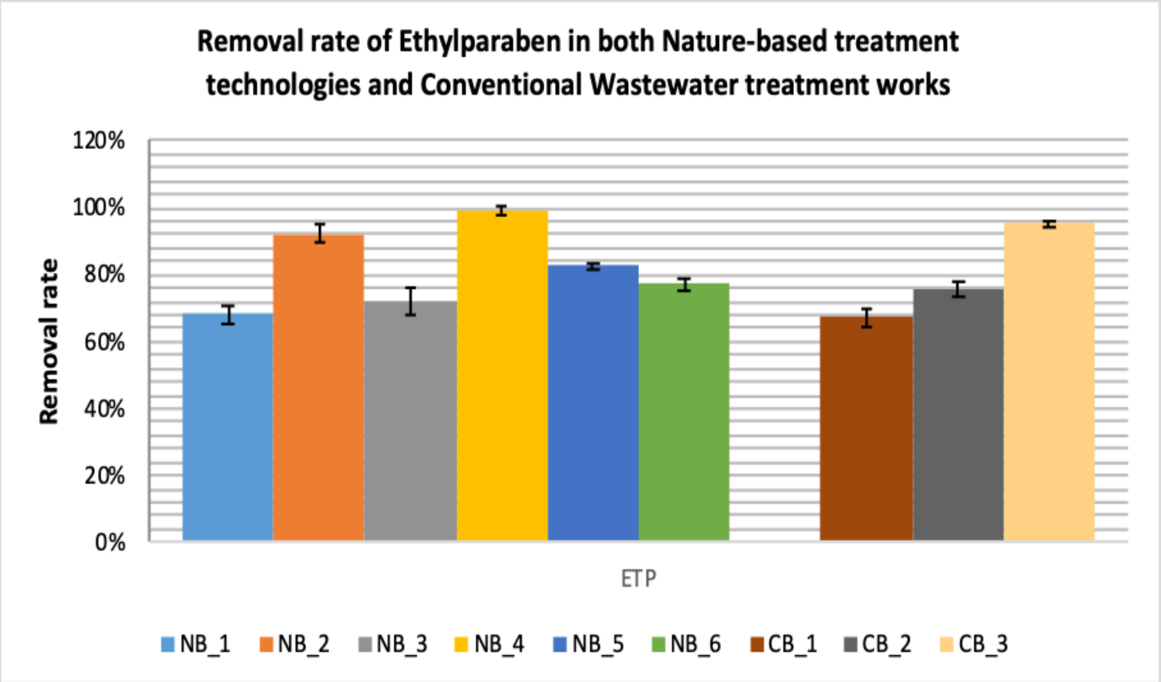


Figure 4b: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of ETP

Figure 4b shows the removal efficiency of ethylparaben which is used as a preservative in many skin care products, deodorants, skin cream, essential oils for dry skin and in some anti-septic oil such as primrose oil. Although parabens are usually assumed to be safe as they are included in products at very low percentages (0.04% - 0.08%) some have been reportedly linked to breast cancer (Crinnion, 2010; Darbre and Harvey, 2014). Methyl, n-propyl, n-butyl and ethyl parabens, are the most commonly used preservatives in personal care, pharmaceuticals, food and cosmetic products (Cowan-Ellsberry and Robison, 2009). The study shows that Ethylparaben had a removal rate of 68% to 99% in nature-based technologies and 67% to 95% in the Conventional system. t-Test: Two-Sample Assuming Unequal Variances confirmed that both Conventional and nature-based treatment systems have a mean value of 0.37 and 0.38 respectively Our p-value is larger than our Alpha (0.05) and as such, we can conclude that there is no significant difference between the means of each system. This study shows that the removal efficiency in both systems are comparative and as such, it is suggested that both systems have similar performance efficiency.

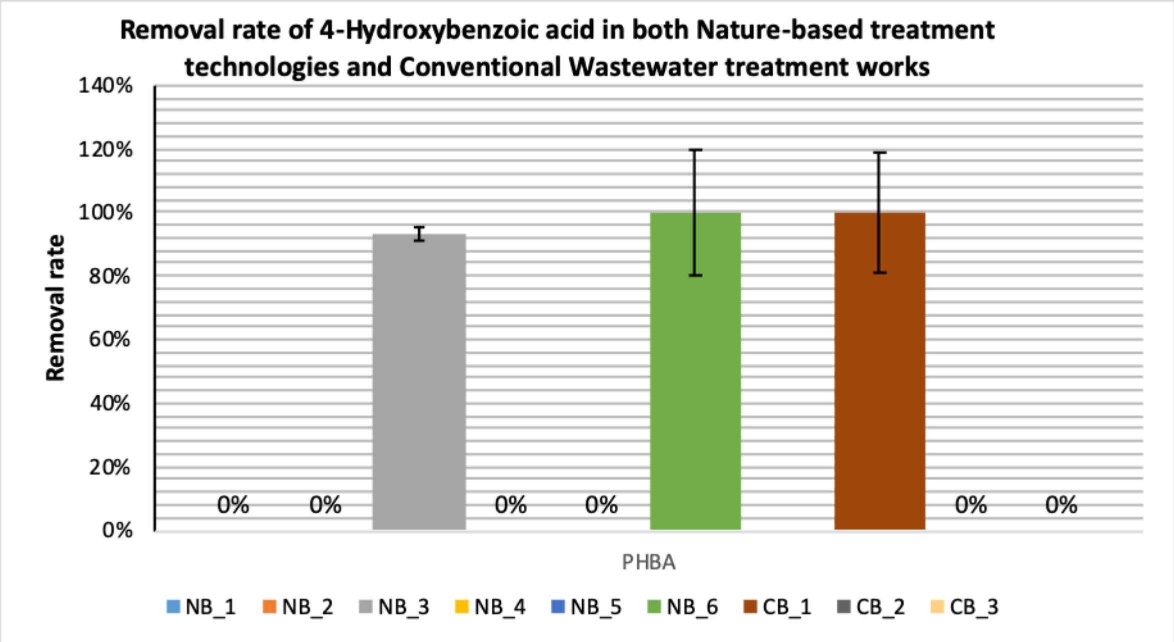


Figure 4c: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of PHBA

Figure 4c shows the results for 4-Hydroxybenzoic acid which is also referred to as *p*-hydroxybenzoic acid (PHBA). This is present in two nature-based systems and one conventional WWTP. PHBA is used as a preservative in cosmetics and it is slightly soluble in water but more soluble in polar organic solvents and can be found in humans due to its varied sources ranging from natural sources eg. edible mushroom (*Basidiomycete mushroom*), Acai oil which could be obtained from Acai palm, *Cocos nucifera* and biosynthesis of chorismic acid. 4-Hydroxybenzoic acid can also be commercially produced in the laboratory in Kolbe-Schmitt reaction from potassium phenoxide and carbon dioxide (Hirao, 1976) as well as by heating potassium carbonate with potassium salicylate to 240 °C, before treating with acid. Even though the compound was not detected across all the treatment systems, the removal efficiency is similar on an average with an average removal rate of 97% in the nature-based system and 100% in the conventional treatment system. This also suggests that both systems are suitable and the nature-based system could be used as a substitute for the conventional treatment works in the removal of *p*-hydroxybenzoic acid (PHBA).

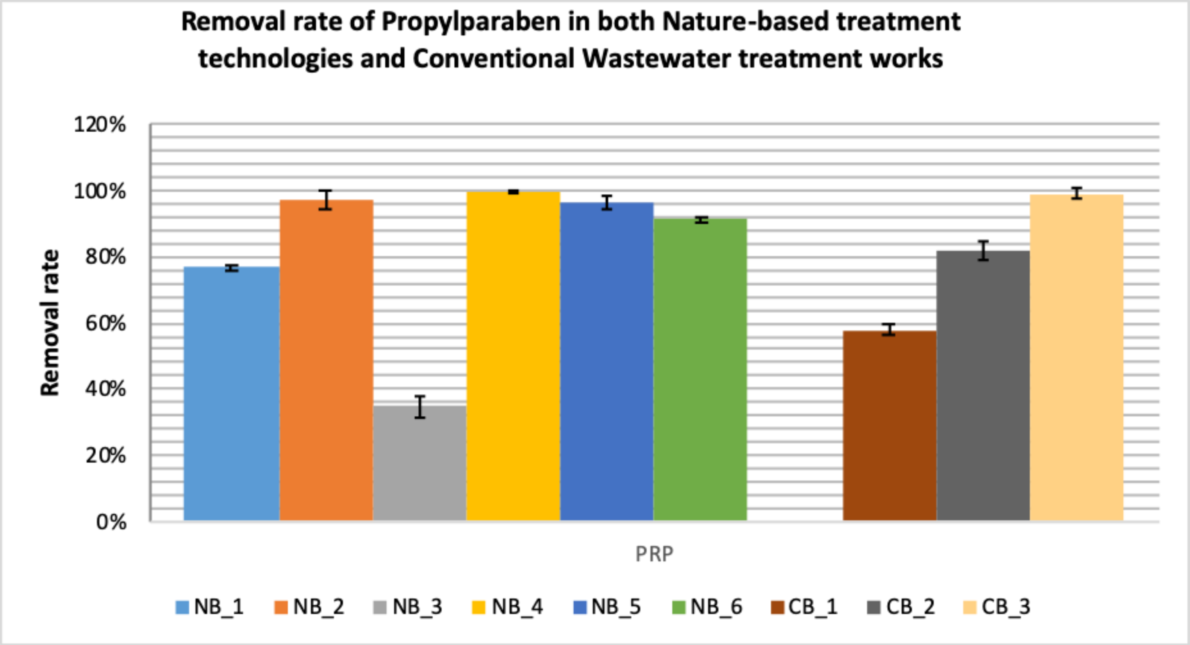


Figure 4d: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of PRP

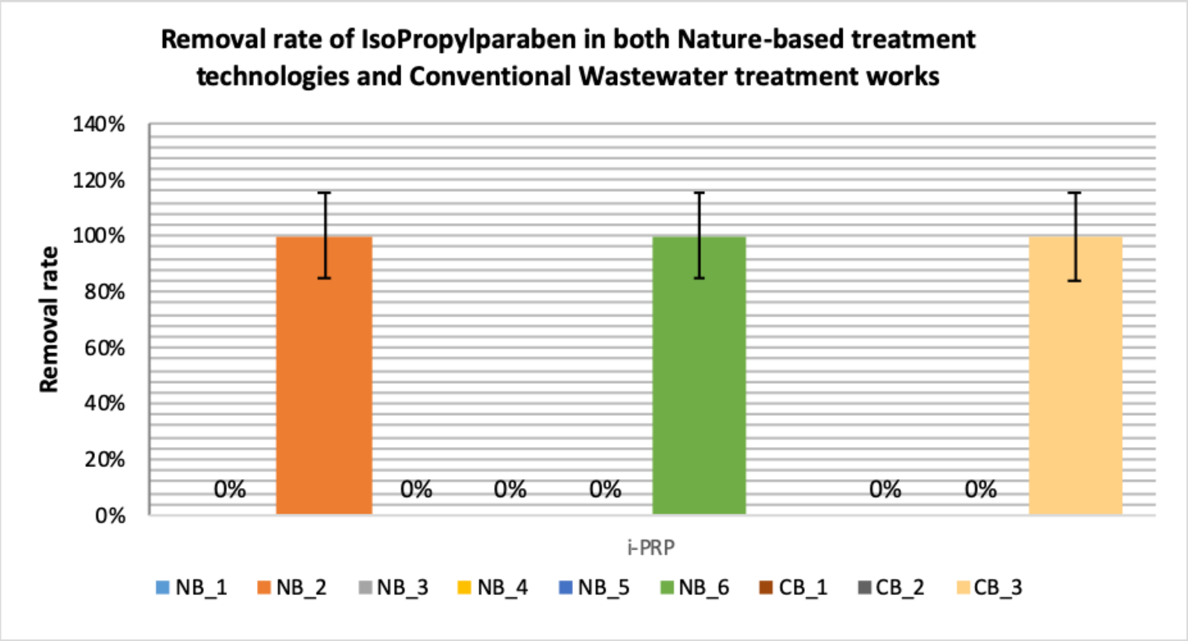


Figure 4e: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of i-PRP

Figures 4d and 4e show the removal efficiencies of propyl parabens and isopropyl parabens at the studied WWTPs. Propylparaben is one of the main parabens (p-hydroxybenzoic acid esters) that acts as an antimicrobial agent, commonly used as a preservative in personal care products (PCPs), foods, beverages, and pharmaceuticals. Even though many of the parabens have been classified as non-carcinogenic, studies have also shown that propylparaben affects sperm counts at all levels from 0.01% to 1.0% when tested on male rats (Anderson, 2008). The average removal rates of propyl parabens in these treatment systems are 83% in nature-based technologies and 80% in the Conventional wastewater treatment works. However, the concentration of PRP in the influent of the nature-based system ranged from 38 ngL^{-1} to 242 ngL^{-1} and 64 ngL^{-1} – 407 ngL^{-1} at the conventional WTP compared to 3 ngL^{-1} – 54 ngL^{-1} and 3 ngL^{-1} -27 ngL^{-1} in the effluents respectively. The prevalence of this compound is attributed to its widespread use. Considering the concentrations, it is conceivable that both systems are comparatively effective in the removal of PRP while advanced or targeted design technology could improve the removal rate in the nature-based WWTP technologies. Comparatively, isopropyl parabens concentrations in both the conventional and nature-based treatment systems were identical with the concentrations at the influent loads ranging from 3 ngL^{-1} -36 ngL^{-1} and 3 ngL^{-1} – 52 ngL^{-1} respectively. The occurrence at the influent loads stood at 33% for both systems while both systems have demonstrated effective removal of isopropyl parabens.

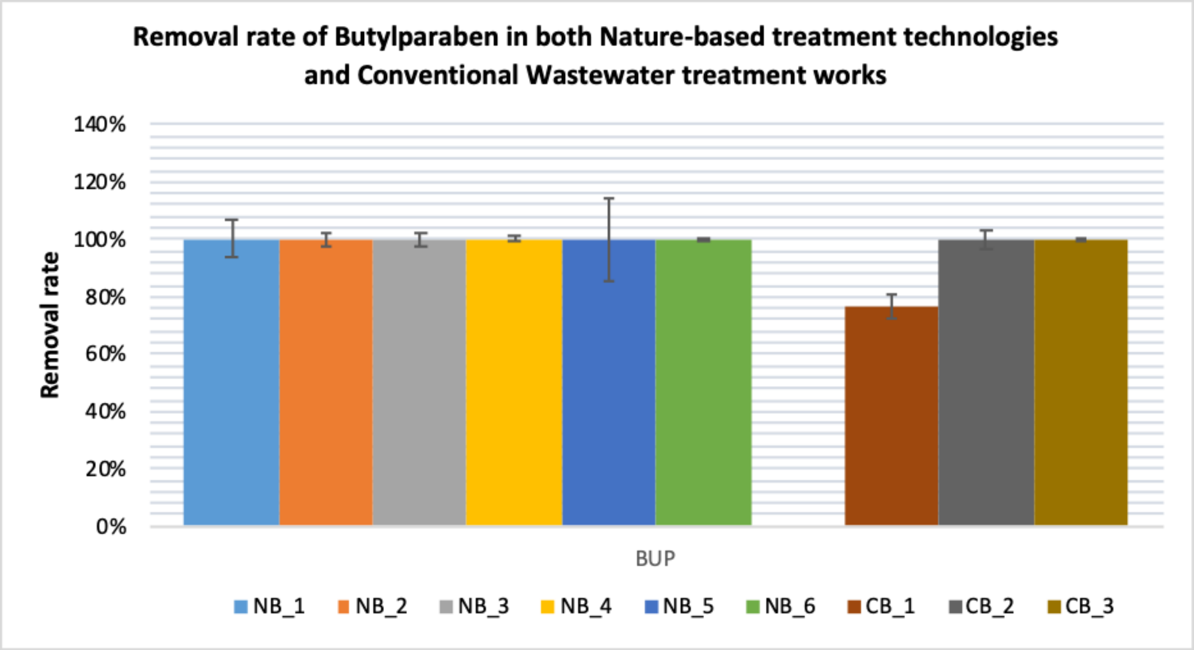


Figure 4f: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of BUP

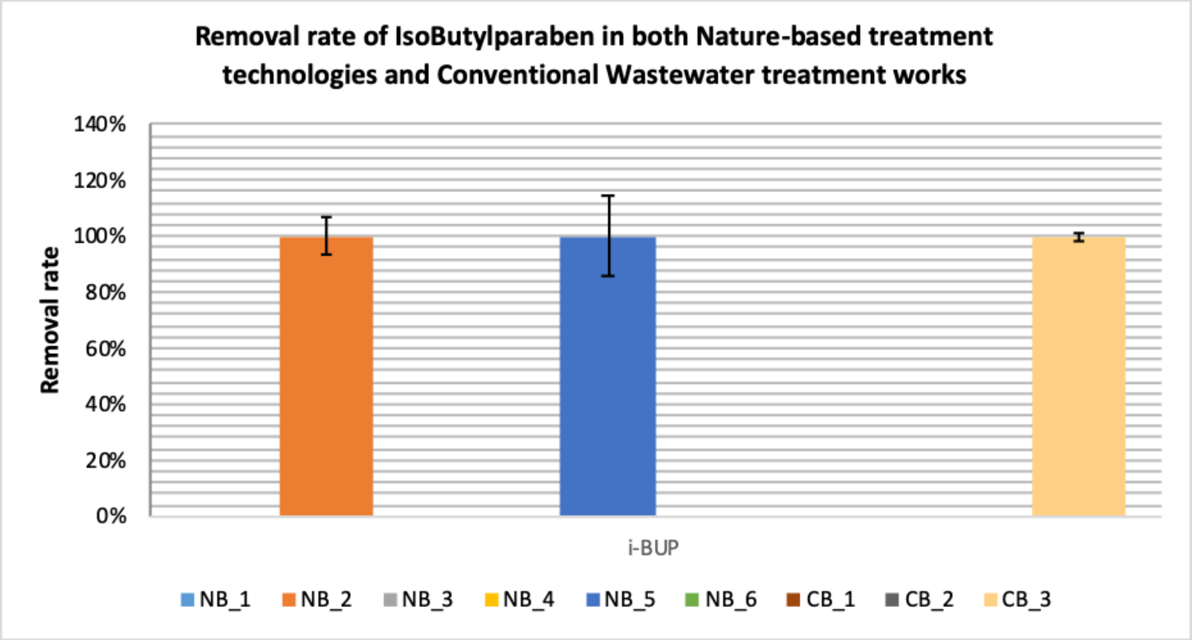


Figure 4g: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of i-BUP

Figures 4f and 4g above illustrate the removal efficiencies of WWTPs for butylparaben. Previous research on the exposure of rats to ButylParaben suggests that it tends to reduce sperm counts due to its antiandrogenic and oestrogenic properties (Boberg *et al.*, 2018). However, this compound is often added to foods, cosmetics and consumer products as a preservative. There has been increasing concern about parabens due to the high level of human dermatological exposure to this ingredient mainly through daily applications of some cosmetic/personal care products. This study revealed that this ingredient was present in all the systems investigated and is a reflection of its extensive use. The results suggest that nature-based systems were more effective in paraben removal. For instance, the occurrence at the influents of both systems was 100% while this ingredient was below the detection limit at the effluents points of the nature-based system and 33.33% at the Conventional treatment works. Although effluent concentrations at the Conventional works 2 ngL^{-1} – 3 ngL^{-1} were low compared to 9 ngL^{-1} -31 ngL^{-1} at the influent points, it is also important to note that the Nature-based system was highly efficient in the removal of this ingredient with 2 ngL^{-1} to 1593 ngL^{-1} found in the influent without any detected concentration at the effluents points of the Nature-based systems. Having in mind the scale and scope of this study, the occurrence of isobutyl parabens was only 33% in the influent with a wide range of concentrations in the Nature-based system and very low concentrations at the Conventional treatment works. However, it was below detectable concentrations at the effluent points. This does not rule out that some might have been discharged through effluent into the environment but the concentrations would have been negligible.

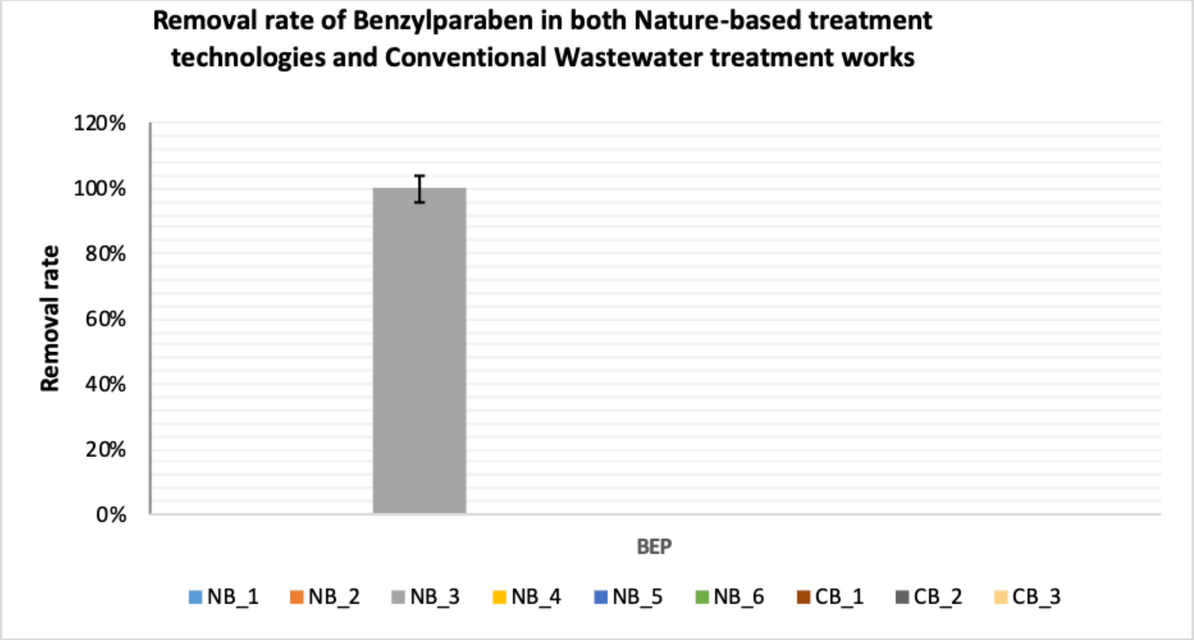


Figure 4h: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of BEP

Figure 4h illustrates the comparison of the removal rates of benzyl paraben in selected conventional and nature-based wastewater treatment plants. Benzylparabens is one of the emerging pollutants that are typically found in the wastewater. This is also used as a preservative in cosmetics. It has been reported that the chain length of the paraben determines the permeability coefficient on the human skin in the order methylparaben > ethylparaben > propylparaben > butylparaben > benzyl paraben (Pozzo and Pastori, 1996). From the nine sites investigated, benzyl paraben was detected at only one of the sites with concentrations ranging from 2 ngL^{-1} - 3 ngL^{-1}.

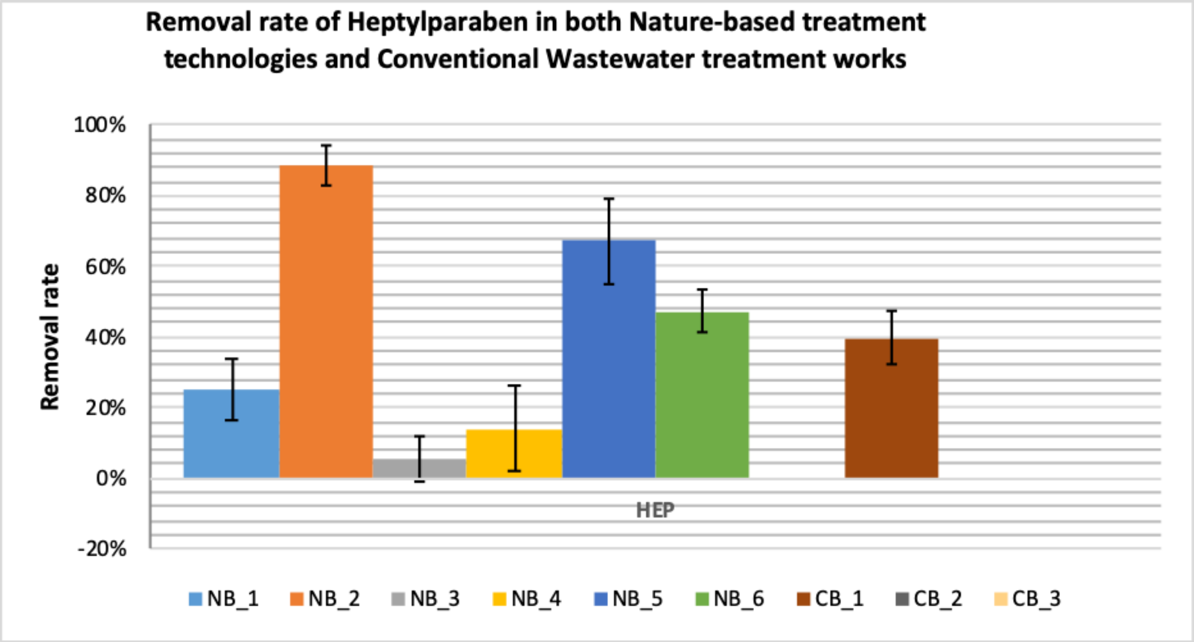


Figure 4i: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of HEP

Figure 4i shows the performance of selected WWTPs in the removal of Heptylparaben (HEP). HEP (E209) and other parabens including Propylparaben (E216), Butylparaben is commonly found in food products (Boberg *et al.*, 2018). Some of the typical foods that contain these parabens as preservatives include processed fish, beer, soft drinks, sauces, desserts, jams, processed vegetables, pickles, flavouring syrups and frozen dairy products. Generally, most of the preserved food items contain parabens (Boberg et al., 2018; Carbajo et al., 2014). The above preservative, Heptylparaben was detected in all the nature-based systems and 33.33% of the conventional systems. However, the removal of Heptylparaben was very low compared to other preservatives. From Figure 4i above, it is evident that the removal in the NB systems 3 and 4 was very poor while it was only in NB2 that we recorded a better performance of the system.

The concentrations of this compound at the influent points of Nature-based systems ranged from 3 ngL^{-1} - 332 ngL^{-1} and 2 ngL^{-1} – 1008 ngL^{-1} at the effluent points, respectively. This compound was also found in the effluent points of all the Nature-based systems which depict the fact that the removal of this compound was very poor across all the systems. However, the concentrations for one of the conventional systems where it was detected in the influents ranged from 3 ngL^{-1} – 9 ngL^{-1} and 3 ngL^{-1} – 6 ngL^{-1} in the effluent. In summary, this is one of the exceptional cases amongst the parabens studied. Future studies should be undertaken to critically evaluate the processes that may have adversely affected the removal of this compound by WWTPs.

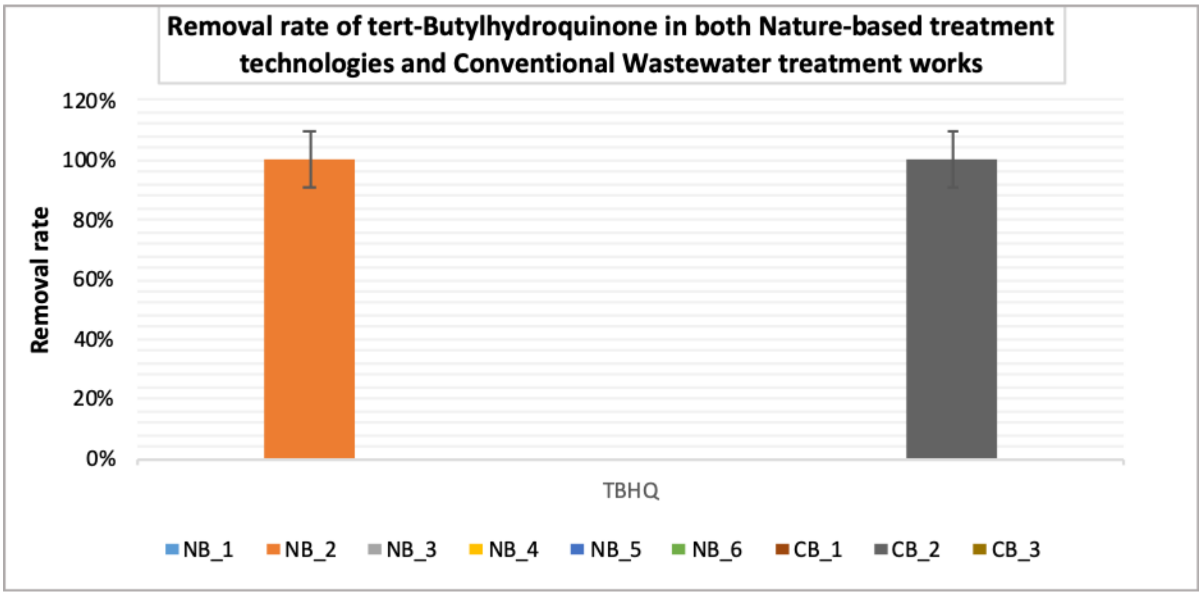


Figure 4j: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of TBHQ

In figure 4j above, TBHQ concentration was very low in most of the systems and was only detected in the Nature-Based 2 and Conventional treatment works 2. However, the removal rate was 100% in both systems where it was detected. This water-soluble compounds are common in food preservation and can also be formed from body metabolism of BHA. Figures 5a-d shows the removal performance of selected conventional and nature-based systems for antioxidants commonly found in household wastewater.

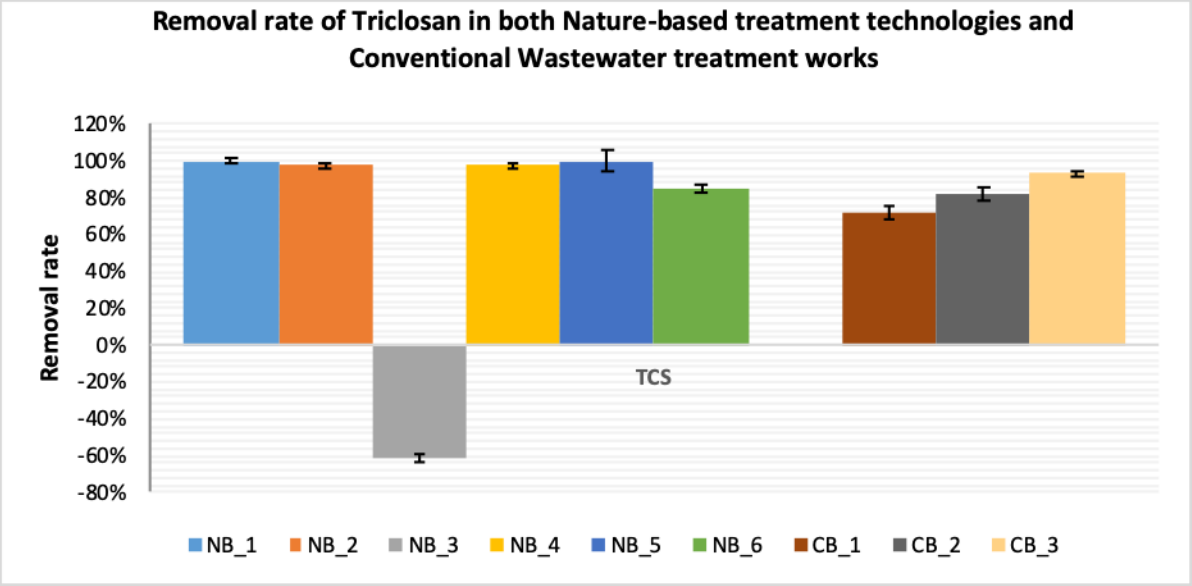


Figure 5a: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of TCS

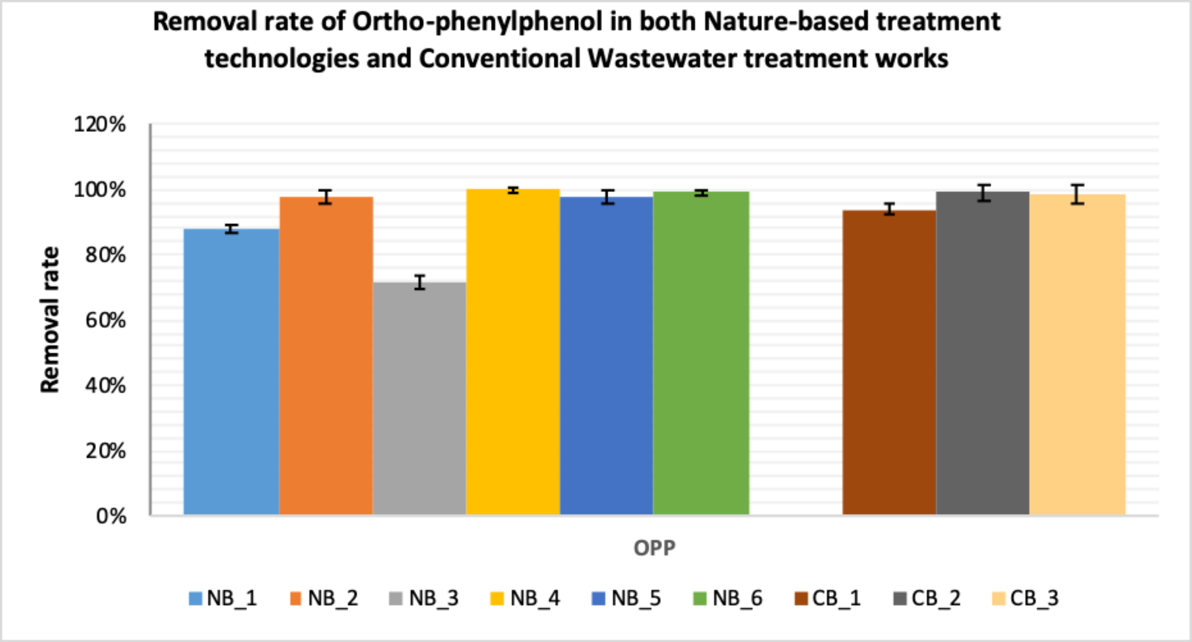


Figure 5b: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of OPP

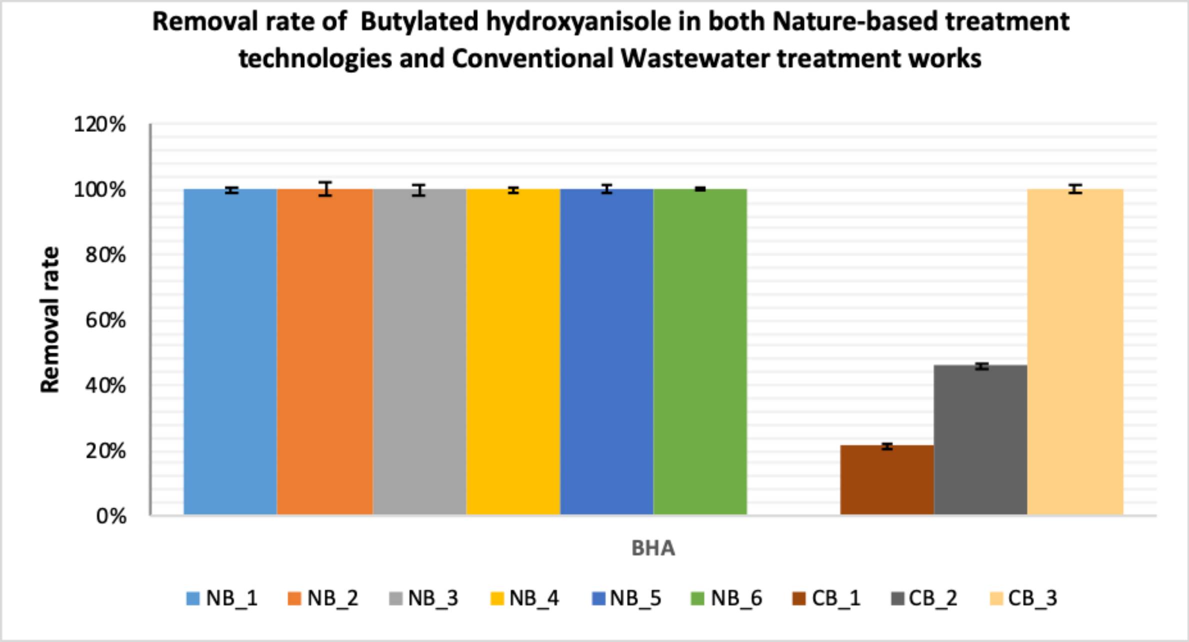


Figure 5c: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of BHA

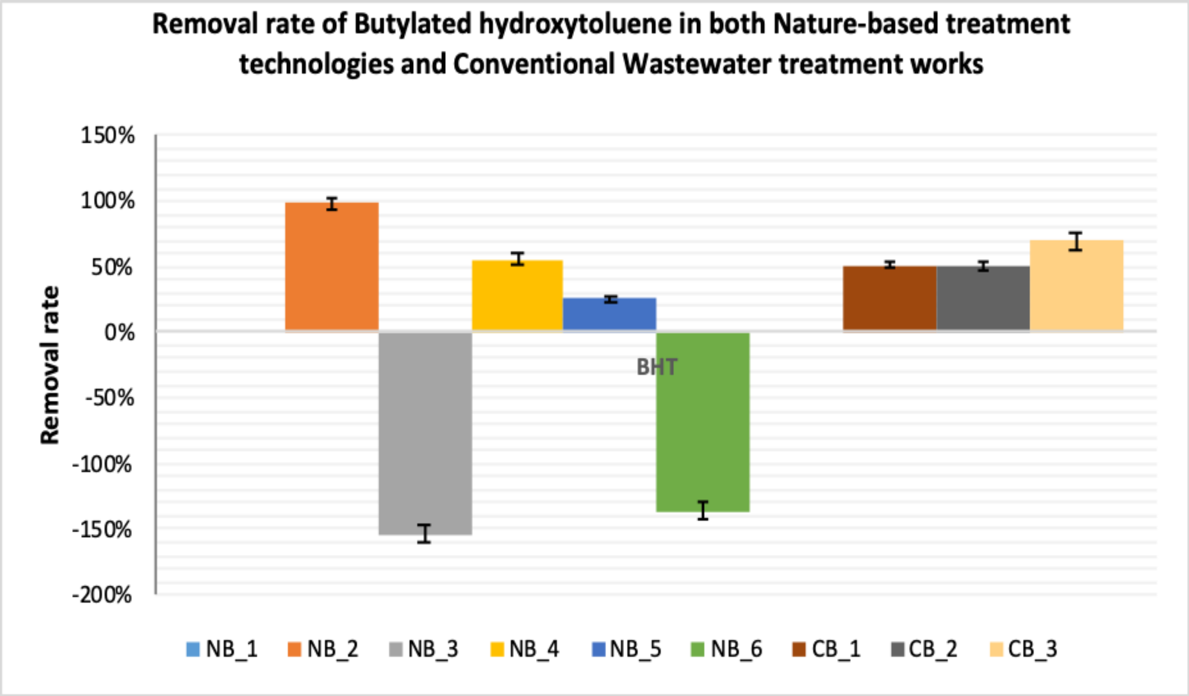


Figure 5d: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of BHT

Figures 6(a-h) illustrate the removal performances of selected WWTP systems for PCPs present in household effluents.

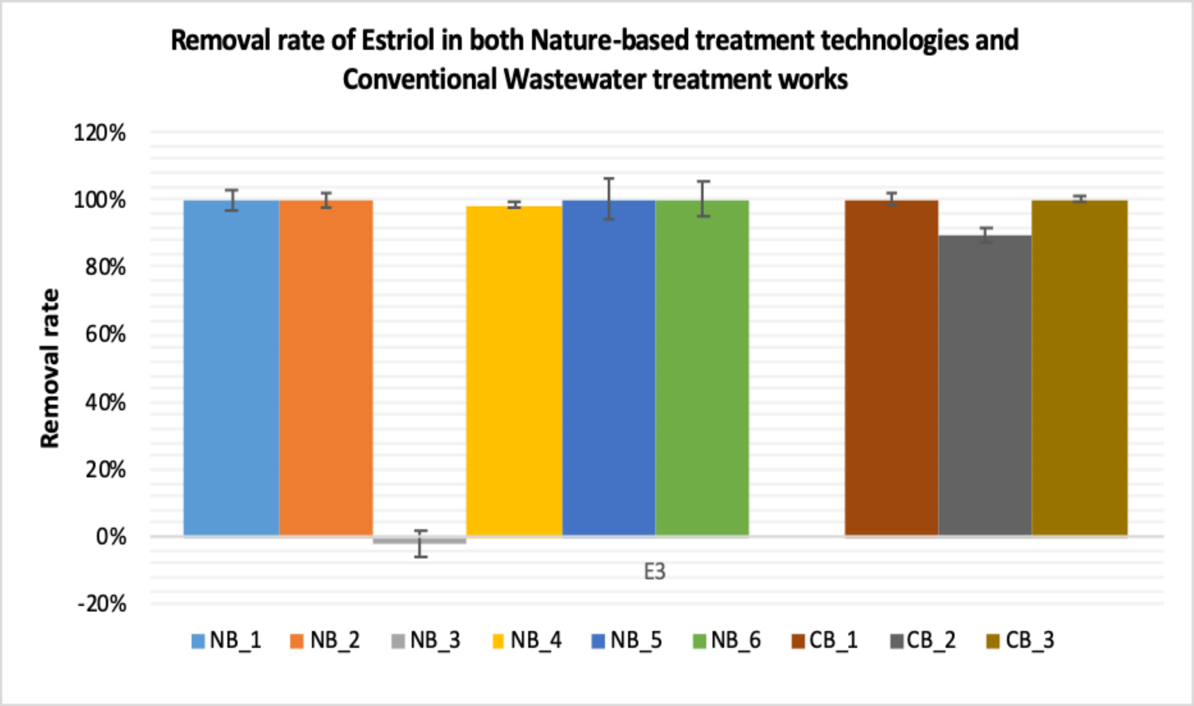


Figure 6a: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of E3

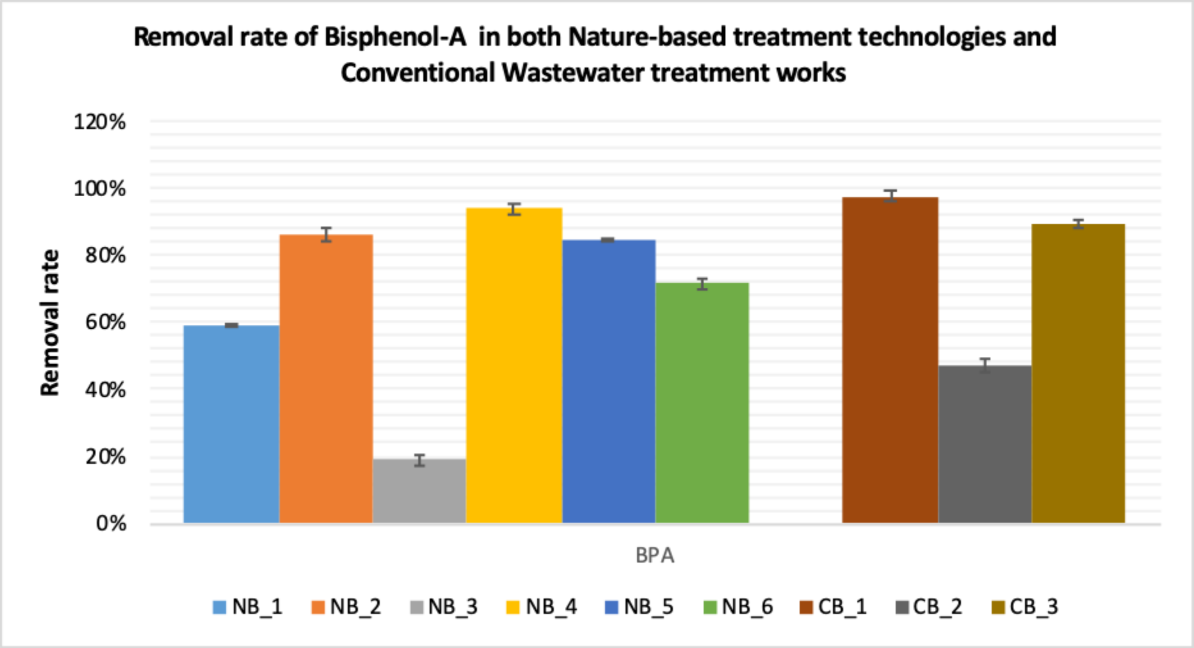


Figure 6b: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of BPA

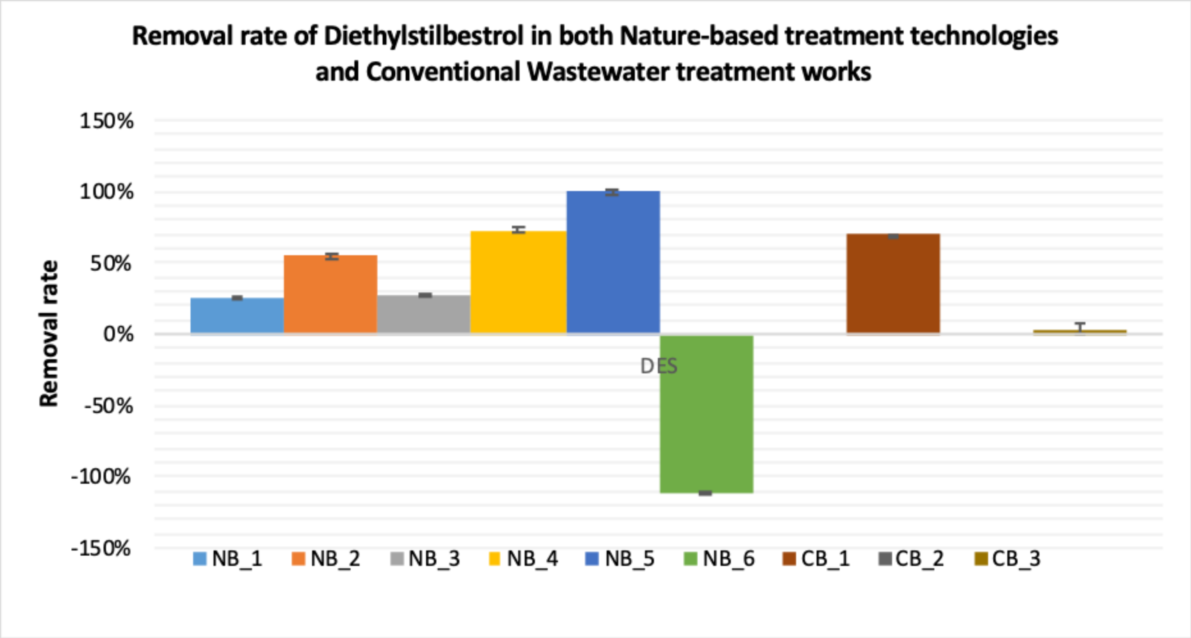


Figure 6c: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of DES

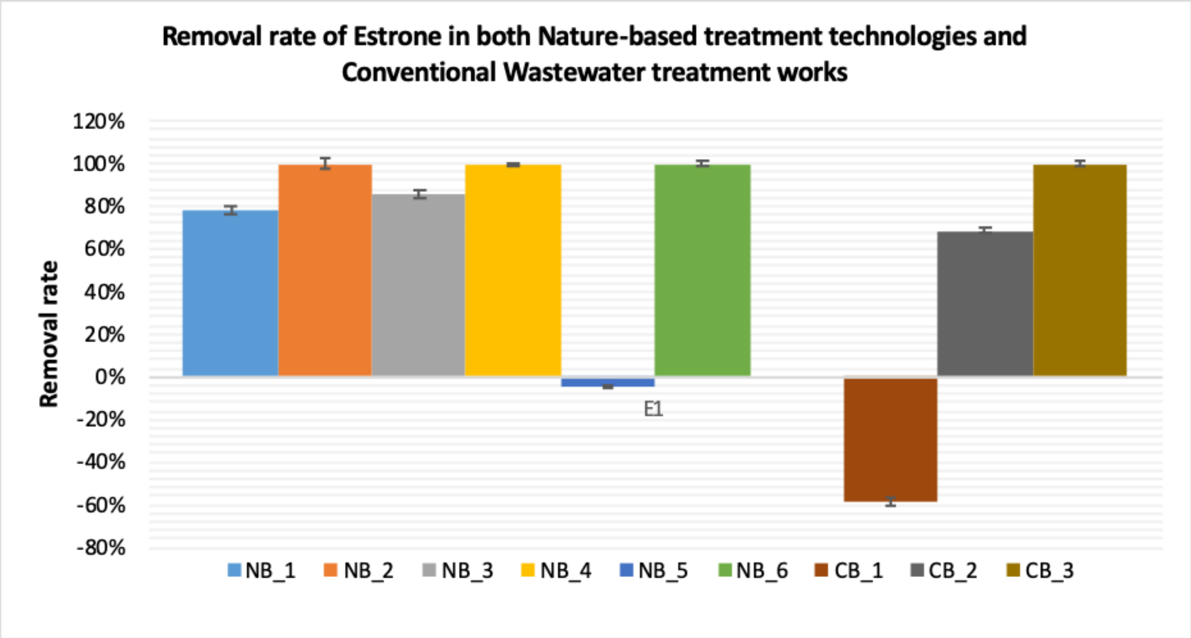


Figure 6d: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of E1

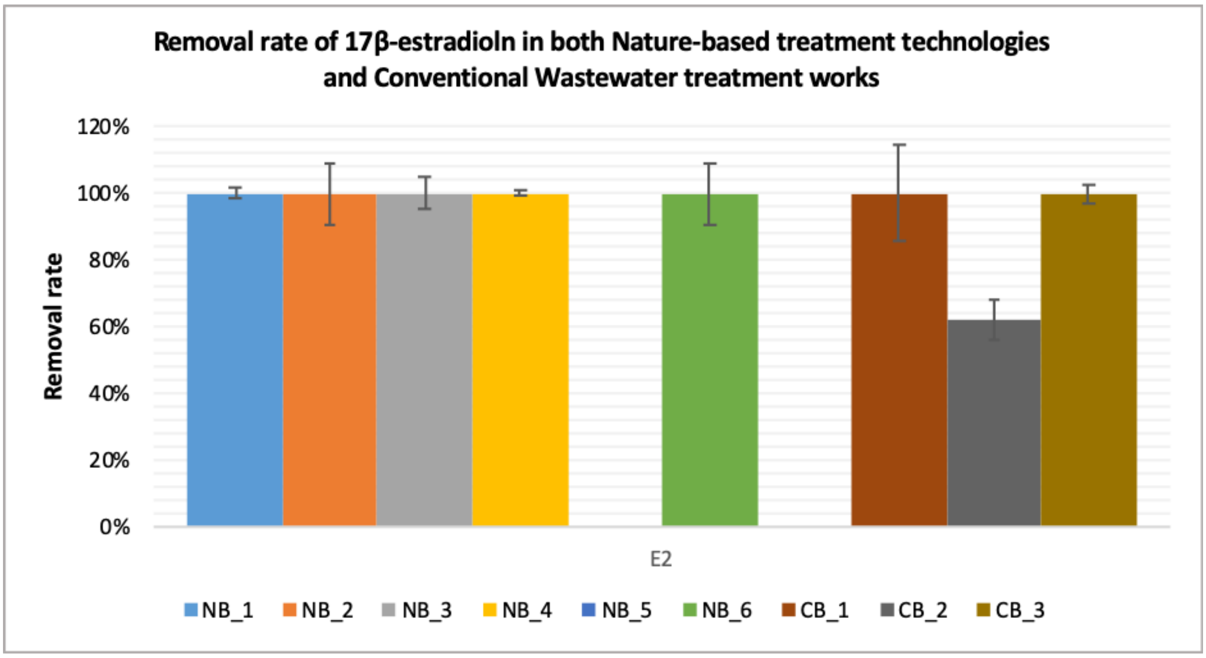


Figure 6e: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of E2

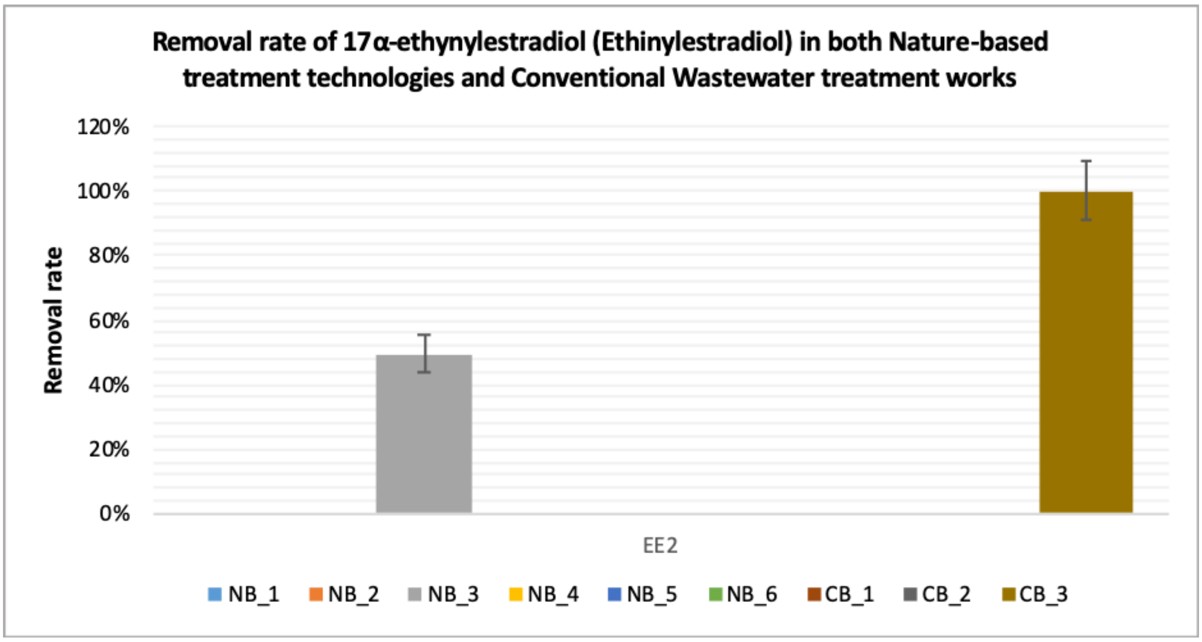


Figure 6f: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of EE2

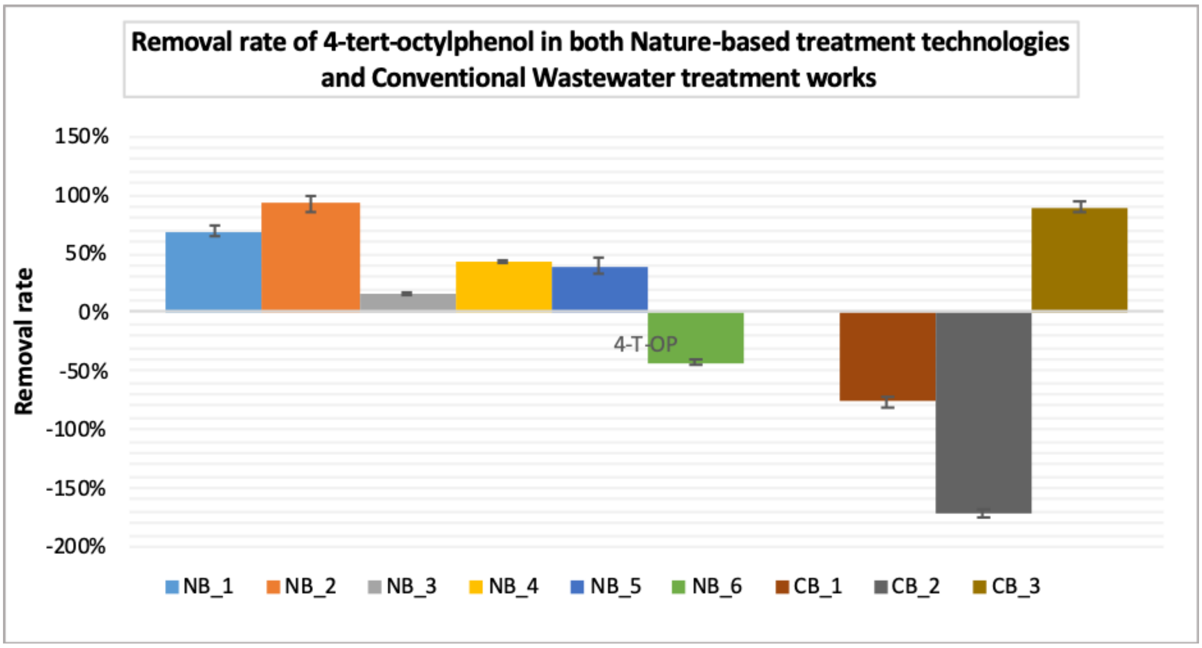


Figure 6g: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of 4-T-OP

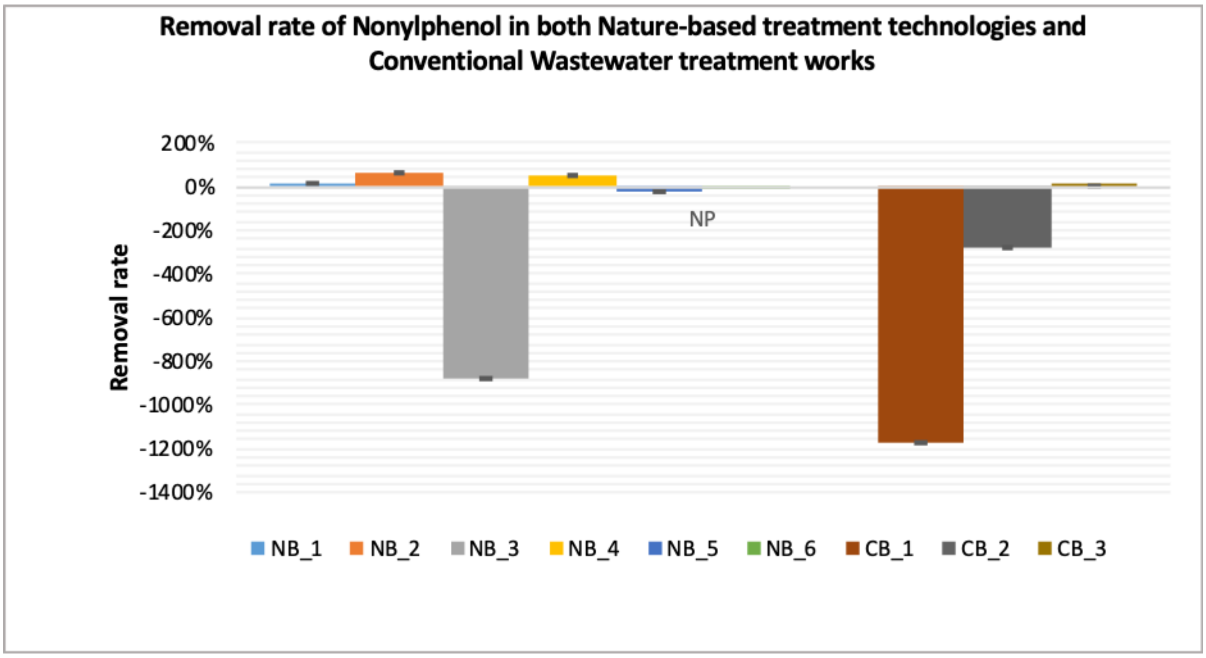


Figure 6h: Performance analyses of six Nature-based (NB) treatments technologies and three Conventional (CB) wastewater treatment works for the removal of NP

It was observed across the compounds investigated that the removal efficiency of each of the treatment plants cannot easily be generalized. Although some compounds were found at concentrations below detection limits and as such, it was difficult to describe their behaviour in both systems. Across figures 5 and 6, both conventional and nature-based systems depict good and comparable removal rates except for a few Antioxidants and Endocrine disruptive compounds. These exceptions are in the removal of TCS, BHT, DES and 4-T-OP in the nature-based systems wherein some of the sites the removal rates with similar behaviour in the Conventional system having removal rates with E1, and 4-T-OP. The removal efficiency of Nonylphenol (NP) was found to be comparable in both system but with slight performance improvement in the nature-based system compared to the conventional system. However, the average removal rates of TCS in the natured based technologies was 70% compared to 83% in the conventional, while BHT was below 57%, DES was between 28% and 37%, 36% to 52% in 4-T-OP, and up to 77% in E1. It shows that average removal efficiency of TCS and DES are comparable contrarily to BHT, E1 and 4-T-OP. These results informed that treatment technology selection and design could be better planned on a chemical by chemical basis as their performances vary from chemical to chemical. It means that design planning should be more focused on the types of chemicals that are to be treated.

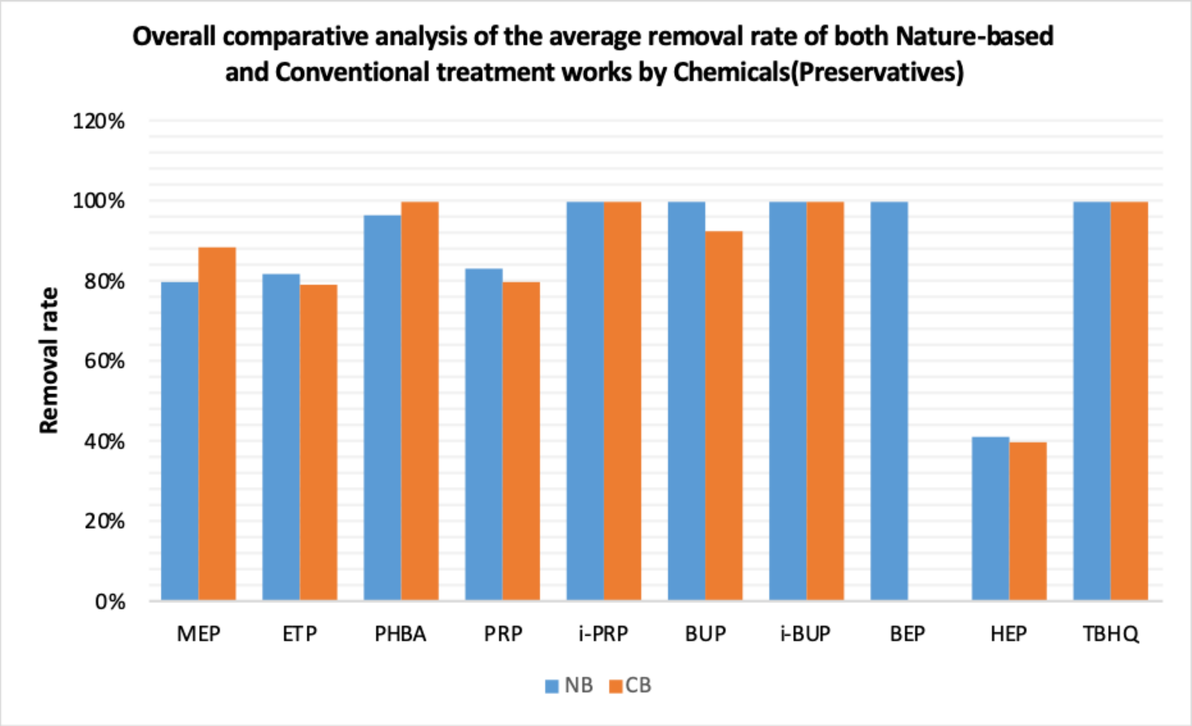


Figure 7a: Comparative analyses of the Nature-based treatments technologies and the Conventional wastewater treatment works for the removal of Preservatives

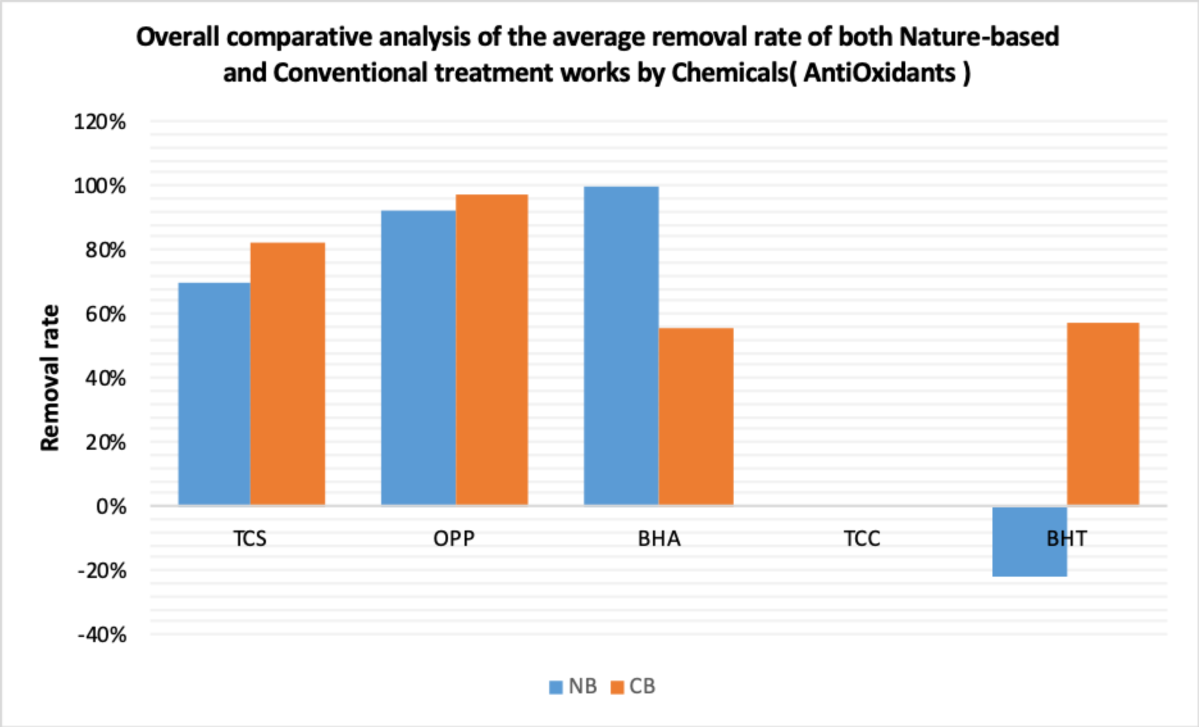


Figure 7b: Comparative analyses of the Nature-based treatments technologies and the Conventional wastewater treatment works for the removal of Antioxidants

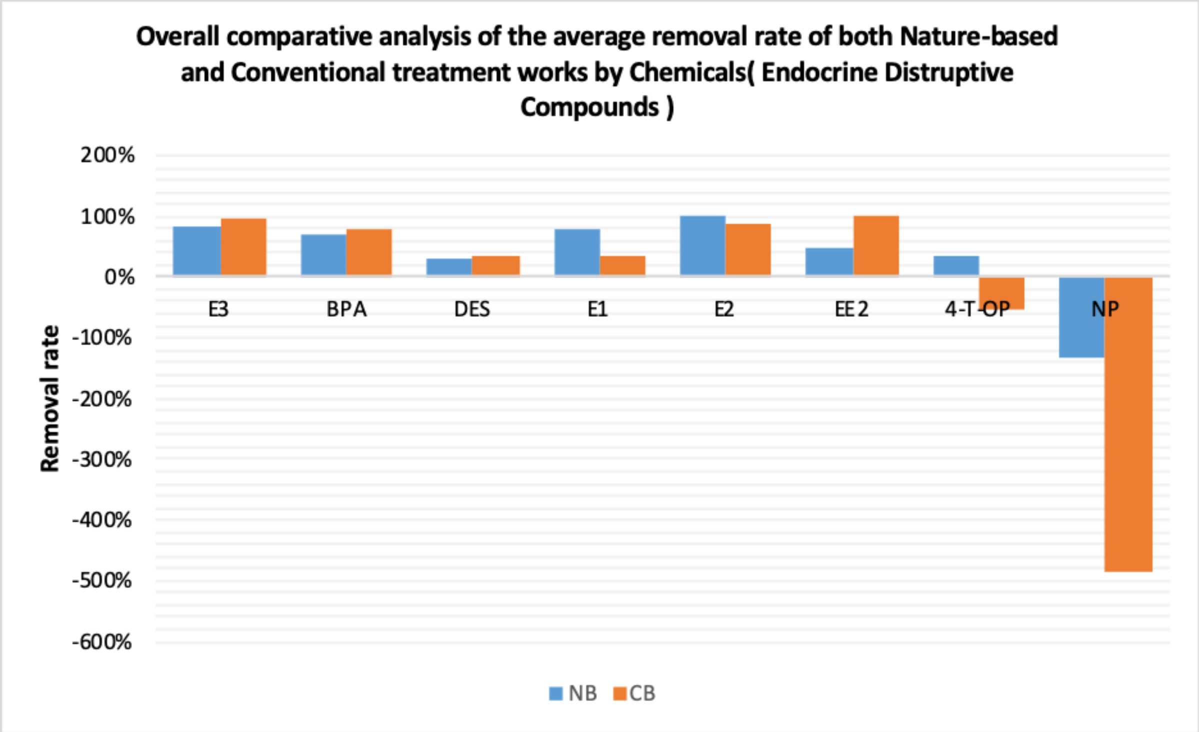


Figure 7c: Comparative analyses of the Nature-based treatments technologies and the Conventional wastewater treatment works for the removal of Endocrine Disruptive Compounds

Considering the data in Figures 7(a-c) above which show average removal rates across both the nature-based and the Conventional systems, it can be concluded that the performance of both systems is chemical-dependent. A statistical analysis of MEP, BUP, ETP, PRP, TCS, and i-BUP confirmed that log K_{oc} has a weak negative correlation of -0.38 with the Nature-based system and extremely weak 0.08 positive correlation with Conventional treatment system while the two-treatment systems have extremely weak negative correlation -0.14 with nature-based system and positive correlation 0.03 with the conventional treatment works. The behaviour of Nonylphenol was exceptional amongst the different treatment works. Calculated removal rates suggest that both systems show low removal rates for Nonylphenol, but the performance of nature-based technologies appeared to be better than the conventional wastewater treatment systems.

Conclusion

This study has been able to evaluate the performance of both Conventional and nature-based wastewater treatment technologies for the removal of Pharmaceuticals and Personal Care product ingredients (PPCPs) from wastewater. Results obtained suggested that the removal efficiencies were more chemical-dependent than technology dependent. However, the age of the studied conventional treatment plants was not considered in this study. It is acknowledged that the selection of suitable technology is dependent on many factors including the system set-up costs, population served, nature of the wastewater such as industrial or domestic, and the prevailing chemicals. Previous reviews and studies have shown that plants and micro-organisms facilitate the removal and recycling of nutrients and metals irrespective of whether they are in water or sediments (Oehmen *et al.*, 2007; Jia and Yuan, 2016). It is also acknowledged that nature-based treatment technology enhances eutrophication as many of the minerals such as nitrogen are used up by the plants that are used in the treatment process. High prevalence of most of the PCPs in both systems with comparable removal rates but except for some antioxidants; Triclosan, Butylated hydroxytoluene which shows negative or very low removal rate less than 1% in 2 out of 6 nature-based technologies were recorded.

The removal efficiency of Endocrine Disruptive Compounds in the nature-based system also recorded some negative removal rates in Estrone, 4-tert-octylphenol, Diethylstilboestrol, and Nonylphenol in 3 out of 6 nature-based systems. However, the removal efficiencies for some of the conventional treatment works had some exceptions with negative removal rates in Estrone, Nonylphenol and 4-tert-octyl phenol in 2 of the 3 works respectively. In summary, nature-based wastewater treatment systems are suggested as a viable alternative to conventional treatment systems. NBWTS are relevant to local communities with low populations, developing economies with limited resources or issues with electricity supply, as these systems do not require the use of power for their operation, unlike conventional systems. Finally, this study revealed that as well as other influential factors present in both wastewater treatment technologies, the efficiency of the systems are largely dependent on the chemical composition of the treated effluents. A further study to investigate the presence of the parent forms of those compounds with negative removals at the influent channels would be imperative to fully establish their removal efficiency and compare both technologies accordingly.

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Supplementary Information

Supplementary Table 2: Standard Calibration Curve parameters for the PCPs (Y = MX + C)

Compounds	calibration function (weighted 1/x)	R^2
MEP	Y = (3 x 10^{-5})X – 3.1383	0.9991
ETP	Y = (3.278 x 10^{1})X – 103.48	0.2962
PHBA	Y = (6 x 10^{-5})X – 2.0039	0.9996
PRP	Y = (3 x 10^{-5})X – 3.6164	0.9987
i- PRP	Y = (6 x 10^{-5})X – 3.2654	0.9990
E3	Y = (1.1 x 10^{-3})X + 0.5688	0.9998
BUP	Y = (2 x 10^{-5})X – 0.0246	0.9961
BEP	Y = (2 x 10^{-5})X – 2.4352	0.9995
i- BUP	Y = (2 x 10^{-5})X – 3.8990	0.9995
TBHQ	Y = (2.5x 10^{-3})X – 74.262	0.9907
TCS	Y = (9.8 x 10^{-3})X + 0.476	0.9950
BPA	Y = (2 x 10^{-4})X – 0.5607	0.9987
DES	Y = (2 x 10^{-4})X + 5.2697	0.9965
E1	Y = (2 x 10^{-4})X + 3.6040	0.9965
E2	Y = (1.8 x 10^{-3})X + 1.7326	0.9974
EE2	Y = (9 x 10^{-4})X + 3.7123	0.9973
HEP	Y = (2 x 10^{-5})X – 0.7506	0.9958
OPP	Y = (5 x 10^{-4})X – 2.7486	0.9994
BHA	Y = (2 x 10^{-5})X + 5.1726	0.9967
TCC	Y = (2 x 10^{-5})X – 25.139	0.8181
4-T-OP	Y = (5 x 10^{-4})X – 3.1080	0.9991
NP	Y = (3 x 10^{-4})X – 22.374	0.9975
BHT	Y = (2 x 10^{-5})X – 18.695	0.9992

4. Paper Two: Effective removal of selected active pharmaceutical ingredients in wastewater by conventional and 'nature-based' treatment technologies

Effective removal of selected active pharmaceutical ingredients in wastewater by conventional and 'nature-based' treatment technologies

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Abstract

The economic sustainability of Conventional treatment plants in an area with high seasonal population turnover or low populations is a major concern due to recurrent costs such as operating costs which often does not justify the investment costs. It has become important to consider alternative treatment system for domestic and industrial wastewater to effectively reduce the release of contaminants into river basins. Fates of 23 active pharmaceutical ingredients were investigated with 8 being detected across 6 nature-based treatment works and 3 within conventional wastewater treatment works. The removal efficiencies of the detected chemicals were investigated across the nine sites and most importantly to determine if the nature-based removal technologies would have similar removal efficiencies to the conventional treatment works. Diffusive Gradients in Thin films (DGT) passive samplers using XAD-Amberlite 18 as the binding gel was used for this study. Across the 3 conventional treatment works, Clarithromycin, CLM effluent concentrations were higher than influent concentrations which suggest that CLM may have entered the treatment system in a conjugate form rather than the compounds that we investigated and this has been shown by Sui et al., (2015) to be amongst some of the PPCPs with negative removals in the secondary treatment system.

In contrast the removal rates for Sulfamethoxazole, SMX ranged from low < 1 % to 43 % and up to 93 % in the case of CFX. Chemical Oxygen demand COD is a measure of all the oxidizable chemicals in the wastewater while Biological Oxygen demand BOD is important in water quality measurement as it indicates the amount of oxygen needed in mg/L by microorganisms and bacteria in the system to fully oxidize the available organic material. The higher the BOD: COD ratio, the better the removal rate (Etchepare & van der Hoek, 2015). However, the poor removal rate in Conventional treatment plant - Olo was expected due to the BOD/COD ratio of 0.45. Removal rates in nature-based technologies appeared to be influenced by influent loads; treatment works to design and the physicochemical properties of the compounds. In the nature-based system, the removal rate of CLM was detected to be higher in the multi-staged pre-treatment system while treatments work with single bed show low removal rates. Except for nature-based Jes treatment technology having removal rates below 1% for SPD, SMX, and CLM, while most of the compounds across nature-based technologies were removed compared to the conventional system. Nature-based treatment technologies can be considered a suitable replacement for conventional treatment works concerning the removal of selected pharmaceutical substances. However, consideration must be given to the required land size for the construction, economic consideration for the population to be served and the wastewater source. The removal rates that are below 1% could be because those compounds entered the treatment works in their conjugate forms while biological processed made them change forms to SPD, SMX and CLM while in some instances, the chemical of interest could be metabolites of the parent compounds which were not measured at the influent channel. Further study on a full scan of the available PPCPs would give more insight and understanding into the sources of these compounds that are prevalent at the effluent rather than concluding the efficiency of the treatment systems or processes.

Introduction

The widespread manifestation of the antibiotic resistance gene has been partly driven by inappropriate use and disposal of antibiotics into surface waters. Human consumption is a major contributing factor to the increasing prevalence of antibiotics in the environment (Watkinson *et al.,* 2007; Zheng *et al.*, 2011) via personal consumption or veterinary prescriptions. Inappropriate consumption of antibiotics has been linked to increased mortality rates as a result of bacteria having now evolved to withstand the effects of antibiotics when taken by the patients. The increase in antibiotic-resistant pathogens (Hopkins & Muller-Pebody, 2015) has been reported by hospitals (Fischbach & Walsh, 2009; Ling et al., 2015) and researchers. Some reports on antibiotic resistance have suggested that new categories of antibiotics should be developed to combat some bacterial infections. Klein et al., (2018) concluded in a study across 76 countries between 2000 to 2015 that, there was increase of 65% (21.1–34.8 billion DDDs) antibiotics defined daily doses (DDDs) while daily consumption rate was found to have increased by 39% which is 11.3–15.7 DDDs per 1,000 inhabitants per day. Antibiotics are also widely used by farmers (Watkinson et al., 2007; Zhou et al., 2011) in intensive food production, beyond treating infections in their livestock but to similarly boost their productivity.

Researchers have developed a range of antibiotics for the prevention and treatment of animal and plant infections, human disease and for promoting and enhancing livestock' growth. The development of antibiotics has exceeded other groups of drugs for human health improvements since the discovery of penicillin in 1928 (Cabello, 2006; Martinez et al., 2002). However, with these developments in mind, the annual mortality rate has been estimated at 700,000 annually as a result of drug-resistant infections, while the forecast for 2050 stands at about 10 million annually. Economically, 100 trillion USD of economic output are at risk due to antimicrobial resistance (O'Neill, 2016). Anthropogenic activities are the major contributors to pharmaceutical ingredients in the environment (Chen, 2016; Zhang et al., 2012). It is, however, important to reiterate that excessive use of antibiotics should be discouraged and controlled, but there will always be an on-going load in wastewater that would need to be treated. Beyond links with the spread of antimicrobial resistance, some of these contaminants have been shown to exhibit detrimental effects on aquatic wildlife such as algae (Akcha et al., 2010; Wilson, Smith, Denoyelles, & Larive, 2003).

Major classes of Antibiotic

Antibiotics are a wide-ranging chemical class derived from natural sources with therapeutic agents which can be divided into seven major categories(Barker, 1998). These classes as stated thus reflect their usefulness in agriculture and human consumption.

β-lactams containing beta-lactam ring in the molecular structure such as penicillin, cephalosporins, monobactams, carbapenems, and amoxicillin. This class of antibiotics are used to treat many bacterial infections to include urinary tract, skin, throat infections, tonsillitis etc. As a result of chemical instability of the β-lactam ring, this class of antibiotics are quickly susceptible to hydrolysis, while penicillin is easily eliminated with an elimination half-life of about 1.4 hours. Due to the short half-life of Penicillin, it is infrequently present in the aquatic environment as well as amoxicillin with a half-life of approximately 62 minutes (Barker, 1998; Hirsch, Ternes, Haberer, & Kratz, 1999). Even though comprehensive consumption rate of antibiotics was not available at the time of this study, records confirmed that penicillin and macrolides have the highest prescription rate of 23% before the year 2000 (Vaccheri, Castelvetri, Esaka, Del Favero, & Montanaro, 2000) while the prescription of penicillin and their consumptions have increased from 43% to 52% during 2007-2015(Di Martino, Lallo, Kirchmayer, Davoli, & Fusco, 2017)

Tetracyclines have a broad spectrum of antibiotics usefulness in the treatment of many infections most predominantly in the developing Nations. Over 60% is excreted in Urine with an elimination half-life of about 8-11 hours. This class of antibiotics have complexing properties that enable them to bind easily to calcium and similar ions to form stable complexes with a strong affinity to suspended organic matters and sediments (H. Chen, Jing, Teng, & Wang, 2018; Hirsch et al., 1999; R. Zhang et al., 2013). This includes ingredients like Sumycin, Panmycin, and doxycycline, Vibramycin while they are easily detectable in the freely dissolved state in the sludge having a typical removal rate of 86.4-93.6% (Wang, Ben, Li, Liu, & Qiang, 2018)

Macrolides: Infections that are caused by Gram-positive bacteria, Streptococcus, Staphylococcus, Enterococcus and limited Gram-negative bacteria such as Actinobacillus pleuropneumonia, soft tissue and respiratory tract infections are treated by this class of antibiotics. It is a substitute for patients with penicillin allergy since it has a slightly wider spectrum than common antibiotics and includes erythromycin, roxithromycin, azithromycin, and clarithromycin which are among the key antimicrobial agents used for human treatments (Parmar & Rawat, 2012; Piddock, Ricci, Stanley, & Jones, 2000; World Organisation for Animal Health (OIE), 2016). The study also confirmed that 50-67% of Macrolides are excreted via bile and faeces, while 30 % of Roxithromycin, 10-20 % of Clarithromycin, 6-12 % of Azithromycin and 5-10% erythromycin's unconjugated parent molecule are excreted via urine (McArdell, Molnar, Suter, & Giger, 2003). They are partly removed during wastewater treatment (Giger et al., 2003; Huset et al., 2008; McArdell et al., 2003)

Fluoroquinolones have a strong affinity to sewage sludge during treatment and as such, higher proportions are adsorbed into the sludge but this does not rule out the fact that some of the agents in the group are still found at the treatment water effluents in a dissolved state (N. Li, Liu, Xue, Wang, & Dai, 2017; Lindberg et al., 2006). Removal rate ranges from 82% to 94% through sewage sludge and 75% to 92% for sludge-amended soil (Golet, Strehler, Alder, & Giger, 2002), while biodegradation has been observed to be responsible for 94.5% to 99.9% removal of this group in the wastewater (Guo et al., 2017) Fluoroquinolones are divided into two major sub-categories (Van Der Heijden et al., 2013). The older category includes Ciprofloxacin, Norfloxacin, and Ofloxacin while the newer category comprises Gemifloxacin, levofloxacin, and Moxifloxacin. Many of this newer category are toxic in the body and withdrawn or with restricted use (Warren, 1997; Zhanel et al., 2002) while some are not even recommended for children(Lipsky & Baker, 1999; Schaad, 2005) as they potentially lead to bacterial resistance and cause arthropathy in juvenile animals.

Active pharmaceutical ingredients such as antibiotics are mostly found in aquatic environments after discharge from wastewater treatment after excretion from consumer consumption (de Jesus Gaffney et al., 2015), disposal of unused pharmaceuticals (Gagnon, 2010; Tong, Peake, & Braund, 2011) and localised sources to include landfill leachate, leakage from septic tanks and hospital discharge (Lu, Tang, Chen, & Sakura, 2008; Walsh & Kunapo, 2009). There are other sources such as agricultural waste-storage or non-localised sources such as surface runoff and unsaturated aquifers in the groundwater and land-application of agricultural wastes and human wastes (R. Li, Zhang, Chu, Chen, & Wang, 2018; Van Epps & Blaney, 2016).

Nature-based treatment technology

Nature-based treatment technologies are also known as reed beds which use biological treatment system of wastewater through secondary and/or tertiary stages (Sundaravadivel & Vigneswaran, 2017) to remove organic pollutants from wastewater. Vegetation such as cattails, reeds, and reed canary grass (Stuart, Gooddy, Bloomfield, & Williams, 2011) are essential in the design of Nature-based or Constructed treatment Wetlands systems for the development of viable microorganism populations that are responsible for the biodegradation of the waste. The wastewater flows through a filter system to remove the gravels and biosolids in a defined flow directly into the treatment lakes or basins(Rozkosny, Kriska, Salek, Bodik, & Istenic, 2014). Different types of wastewater are considered during the design process and appropriate treatment systems are available to provide an adequate level of treatment that meets the needs of users. Some of the design may include vertical or horizontal flow system. Typical wastewaters include polluted stormwater runoff, municipal wastewater, industrial and agricultural runoff. Wastewater undergoes treatment while passing through a horizontal or vertical permeable filtration system by a complex combination of physical, biological and chemical processes. These systems can be designed with sedimentation pre-treatment as shown below in Figure 1 or without sedimentation pre-treatment often referred to the French System (Rozkosny et al., 2014)

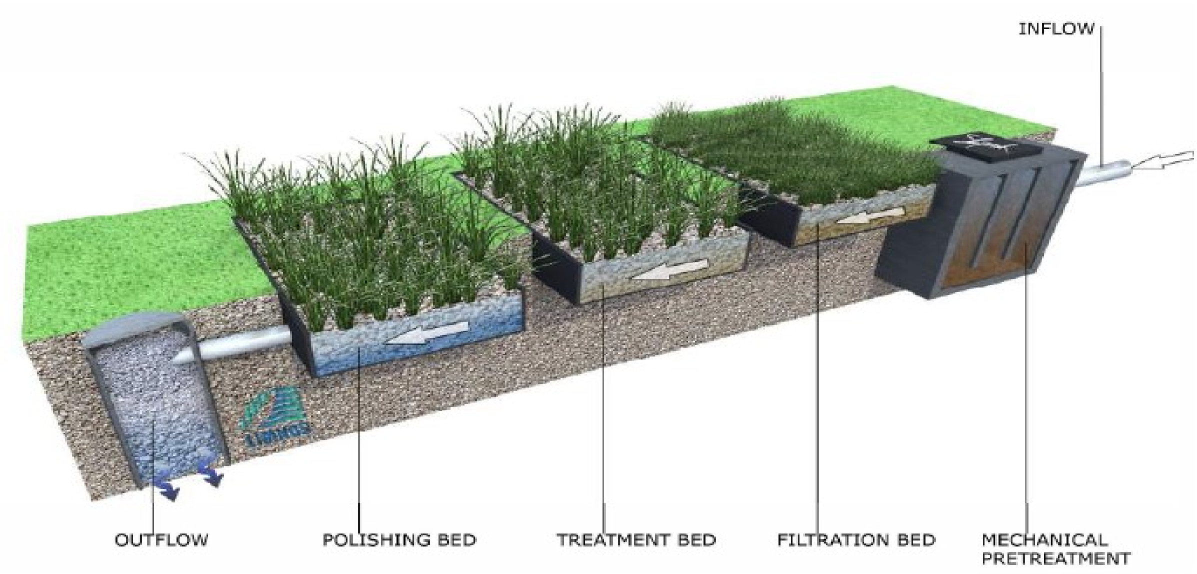

Figure 1. Simple schematic representation of horizontal flow nature-based technology (Rozkosny et al., 2014)

Operations of nature-based treatment technologies are flow controlled with various sections or stages. The studied nature-based technologies have a mix of some of these components; vertical flow reed bed filters VRBF as the first stage with vertical subsurface flow basins SFS-v, horizontal subsurface flow basins SFS-h and free water system FWS. The wastewater flows through the filters to separate the gravels and organic biosolids. There are Horizontal flow HF and Vertical flow (VF) systems in which the VF system has the water flow vertically and the filtration occurs through a 0.2 - 4 mm gravel-sand bed with a filtration height or 0.9 - 1.5m (Nivala et al., 2013). But in the HF systems as shown in Figure 1 above, the flow of wastewater is horizontal through various stages. It is also possible to have a VF treatment technology without mechanical pre-treatment chamber (Reeb & Liey, 2011). The use of wetland macrophytes was initially tried in Germany as far back as 1950 (Vymazal, 2005) and has since then become a promising alternative for the conventional wastewater treatment system (Ávila, Garfí, & García, 2013; Vymazal, 2011)

Nature-based technologies here refer to as NBTs have a range of benefits compared to other treatment methods. Operational costs are very low as the system can function without pumps/electricity whilst construction is very simple. However, the quality of treated water could be improved by the treatment beds design which could be multi-staged or single-stage treatment lakes. NBT affect favourably the climate in its vicinity where plants organically rich water are available to vegetations, increased biodiversity, with huge energy saving as this system does not require energy supply. If properly design, a high treatment performance could be achieved with little maintenance costs. However, it is a very good technology for economical removal of bioavailable organic nutrients, Nitrogen, Phosphorus and Potassium by the macrophytes and this could be seasonally based on climatic conditions. A well-constructed nature-based treatment technology (NBT) could achieve relatively high efficiency of 70% - 83 % COD removal, 48% - 91 % suspended solids removal, 27% - 70 % total nitrogen removal and 26% - 89 % total phosphorus removal efficiency (Álvarez, Ruíz, & Soto, 2008). The above was also corroborated in a different study of nine NBT which was conducted for a consistent period of 3 years by Jenssen et al., (2010) that organic matter removal as biochemical oxygen demand (BOD) was above 80%, total Nitrogen (TN) 32% to 66% and total phosphorus (TP) above 94%. These two studies are in agreement. Studies have confirmed further that the majority of the nitrogen and BODs are removed by pre-treatment filters.

The major concern in the construction of NBT is the availability, of land, the size of which is dependent upon the treatment design, which could involve primary, secondary or tertiary stages while wastewater loads should also be considered in relations to the population that the treatment plant is to serve. Filters in the basic configuration play an important role where high sorption capacity biofilters have shown to be effective in the removal of phosphorus from 95.4

to 99.9% where <1.0 mg P l^{-1} was detected at the effluent water. More also, Ammonia (NH_4) removal rate ranged from 38-80%, total Nitrogen removal ranges from 32 to 66% (Jenssen et al., 2010). The filtration material stands the risk of clogging if the mechanical pre-treatment system is inappropriately designed. Even though there have been some concerns about lack of vegetation growth most especially in the winter seasons in Europe due to unfavourable conditions, consideration should also be given to the plant types.

Suitable vegetation in the NBT plays a vital role in the creation of a viable habitat for microorganisms through the removal of some of the major nutrients by sorption. These aquatic plants include duckweed, green algae (Chlorella Vulgaris) and water hyacinth. The removal efficiencies of the organic nutrients are enhanced by absorption in the form of plant nutrients. These nutrients are used up by the vegetation around the treatment beds. It has been proven that plants can decrease nutrients in tested water leading to improved water quality to a permitted level where such water could be reused for irrigation purposes. A study conducted by Badr El-Din & Abdel-Aziz, (2018) confirmed that Chemical oxygen demand (COD) and biological oxygen demand (BOD5) can be reduced by 43% and 42% respectively in wastewater by duckweeds, 33% and 38% by green algae and 28% and 33% by water hyacinth over a 21 days study. The study confirmed higher N, P, K pollutants removal efficiency of Duckweeds being a promising plant for wastewater treatment. However, Rozkosny et al., (2014) established that NBT can achieve a high performance of COD and BOD treatment of 85% BOD_5, COD 75 % and 30% NH_4^-N while filtration and sedimentation (SS) removal efficiency is 80 %. Removal of phosphorus is by sorption/binding of phosphorus (TP) unto the filter with a removal efficiency of 35 %.

Nature-based technologies are beds composed of the substrate, water column, with water-tolerant plants, and microbes that aid microbial disintegration. This substrate stage could be gravel, sand, or soil containing the growing wetland plants. These treatment technologies use series of treatment beds or reed beds with hydrophyte and macrophyte wetland plants (Bouwman et al., 2013; Pinckney, Paerl, Tester, & Richardson, 2001; Songliu, Hongying, Yingxue, & Jia, 2009). The process is reliant on these plants and the residence time of the wastewater in the treatment ponds. These systems can also be used to store/treat additional wastewater requiring treatment at conventional wastewater treatment plants rather direct discharge into the receiving river or estuaries. The sites selected for the study received daily wastewater loads ranging from 8 to 55,000 m^3/day. Nature-based treatment technology has been described as a successfully sustainable wastewater treatment technology most suited to small to medium-sized communities and for developing countries (Sundaravadivel & Vigneswaran, 2017). Besides their advantages in terms of operational costs, they have also been reported useful for the conservation of some native fauna and flora species, amphibians, invertebrates as well as in improving treated water quality (Brix, 1994).

Aims and Objectives

This study was designed to investigate the removal efficiencies of active pharmaceutical ingredients from wastewater by comparing Nature-based treatment technology with Conventional treatment works. The study also aimed to investigate its suitability as an economic and viable alternative to Conventional treatment works. Removal rates and characteristics of various chemicals in both systems were considered to give insight into the appropriateness of Diffusive Gradients in thin-film passive (DGT) passive sampler as a suitable tool in both deep and shallow sampling medium following up on its time-weighted integrated sampling capability TWA, its portability which supports its suitability for use in any sampling medium irrespective of depth, and its transportability.

Method and Materials

DGT passive Sampler

The first passive water sampler was invented in about 30yrs ago which has since then reduced the operational challenges in water sampling (Kot-Wasik et al., 2007a). Since then, many passive samplers have been developed leading to a reduction in operational limitations faced in the use of active samplers such as power supply, downtime due to system malfunctioning, field calibrations, and security of the active sampling tools (Kot-Wasik *et al.*, 2007). Some of the available passive samplers are Polar Organic Compound Integrative Sampler (POCIS), Membrane Enclosed Sorptive Sampler (MESCO), and Semi-Permeable Membrane Devices (SPMD). Traditional grab sampling was also a popular sampling method which involves taking small volumes of fluids from a medium over some time to build up a representative mixture. This method had been compared with Diffusive Gradient in thin-film (DGT) passive samplers during the feasibility stage of this study and DGT has been found more suitable and sustainable for this study considering the spatiation of the sites costs implication and huge manpower requirements outside the fact that it fails to satisfy the capability of monitoring consistently activities of the chemicals in the sampled medium. Most previous studies have concluded that the reliability of grab sampling method could be ambiguous viz;

1. Temporal variation in chemical signals is significant which is catered for by time-integrated sampling such as DGT, POCIS etc.
2. It is not flow proportional considering the sample volume to the overall flow.etc.
3. The ‘spot check’ method only capture the pollutants around the sampling horizon in the water column at the instant the samples are taken.
4. Also, samples are to be taken over a period to have representative data averaged over time due to changing parameters at the sampled sites.
5. Storage of such samples over a long period may affect the outcome of the analysis/ chemical concentrations due to biodegrading.
6. Inability to identify the pollutant sources as this would require very large samples over spatial distances and time.

DGT was originally designed to investigate labile metals and inorganic chemicals in water and soils (Chen, 2013) but has since then been used for the quantification of organic compounds such antibiotics, personal care products ingredients (X. Gong, Li, Wu, Wang, & Sun, 2018; Xie et al., 2018) and bioavailability of nutrients such as phosphorus in water and sediments(Y. Gong et al., 2015). Mengistu, et al., (2012) concluded their study in Johannesburg that sources of pollutions can be monitored over a long period using DGT than the grab sampling as a result of its Time-Weighted Average (TWA) ability as well as pollutants source apportionment. This device accumulates pollutants over the sampling period using Fick’s first law of diffusion and as well as providing a Time-Weighted Average concentration of chemicals without any need for field calibrations, unlike the active sampling tools. This sampling toolkit has been extensively used in sampling inorganic compounds while there is a paucity of data relating to Organic chemicals and Endocrine Disruptive compounds.

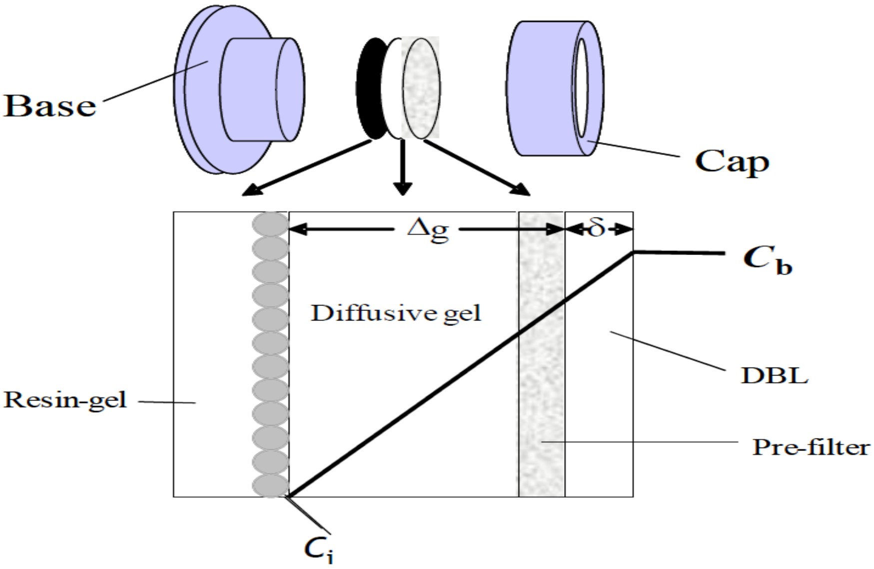

Figure 2. Schematic representation of DGT components: DBL diffusive boundary layer; C_i concentration at the diffusive gel-resin boundary; C_b as the concentration in the aqueous medium (Source: Chen 2013)

Preparation of DGT

Standard DGT preparation method was followed as contained in the method section of this study. However, the diffusive layer uses agarose gel while the binding gel used for this study was Amberlite XAD-18 with particle size 63um-150um. Other reagents and components of the o-DGT remain unchanged. DGTs were prepared in the Lancaster University laboratory and all in a single batch of binding gel and diffusive gels.

Sampling Preparation

The DGT samplers were made up of Amberlites XAD-18 binding gels to absorb antibiotics. These binding gels were prepared to a thickness of 0.56mm using 0.35mm spacers while they are placed between agarose diffusive gel of 0.80mm and the GHP 0.45um membrane filter. The preparation information for all the various components is contained in chapter 2 of this study.

Chemicals and Reagents

Gel solution was obtained from DGT Research Ltd, Skelmorlie, Bay Horse Rd, Quernmore, Lancaster. LA2 0QJ, UK.). This Gel solution has a storage lifespan of at least 3 months in a refrigerator at (4ºC). Conversely, ammonium persulphate solution was prepared daily by dissolving 0.1g of dry ammonium persulphate in 1g of water. N,N,N'N'-Tetramethylethylenediamine (TEMED), 99% was purchased from Sigma-Aldrich (UK). XAD-18 Amberlite was purchased from Dow Chemical Company and made to (65µm-150µm bead sizes). The Organic solvents used for this project work are HPLC grade Acetonitrile and Methanol that were purchased from Fisher Scientific (UK). Other components of the DGT samplers, Diffusive gel was made from Agarose powder that was purchased from Bio-Rad Laboratories (UK), GH Polypro 0.45um 25mm Hydrophilic Polypropylene membrane filter was purchased from PALL Incorporation in Michigan, and the 0.22um syringe filter. The MQ water was made in Lancaster laboratory while the assembly and preparation of various gels were also made in the Lancaster University Laboratory.

Sampling and sampling techniques

This study was carried out in Florence and its environs in Italy. Study sites selection was based on the availability of various types of the treatment system, which is particularly challenging due to travel distance for nature-based treatment technologies that are under consideration for viability as alternative treatment system to conventional sewage treatment plants. The systems ranged from simple multi-staged processes to tertiary systems depending on the source of waste and the influent loads viz population equivalents that the system has been designed to serve. Sampling was carried out over 7 days to investigate time-integrated removal rates using matched influent-effluent samples at each work. Sampling took place across six nature-based wastewater treatment technologies and three Conventional WWTPs. Three conventional treatment works were in urban/industrial catchments with influent loads ranging from 26,173 m^3/day to 34,524 m^3/day. Daily COD load ranges from 6,229 kg COD/day to 7,774 kg COD/day and BOD loads of 2,058 kg BOD/day to 4,050 kg BOD/day. The six constructed wetlands were categorised into two groups with daily loadings ranging from 7.5 m^3/day to 525 m^3/day while the larger two sites had wastewater loads of 19,500 m^3/day and 55,000 m^3/day respectively.

Deployment and Removal

Fifty-Four o-DGT samplers were deployed across six nature-based treatment systems and three conventional sewage for seven days. The average temperature at the time of deployment was 18±3°C. At the time of deployment at the nature-based treatment technology, influent water average pH were 6.8±0.1 and 6.9±0.2 at the effluent while the average pH at the time of removal of the DGT were 6.9±0.0.1 and 7.1±0.2 respectively. However, the average pH at the conventional treatment works influent water was 7.1±0.4 and 7.3±0.5 in the effluent at the time of deployment while 7±0.2 and 7.2±0.2 were recorded at the time of removals respectively. The DGT samplers were deployed using mesh bags as the deployment device for this study across the entire system.

DGT Extraction Process

Each of the Amberlite XAD-18 resins gels was removed from the DGT moulding and placed in a 15ml vials. The samples were spiked with 50µl of a mixed internal standard containing SMX-d4, CAF-13C3, OFX-d3, and ETM-13C2 in an equal proportion. Samples were extracted with 5ml of Methanol in an ultrasonic bath for 30mins. The methanol was decanted into another vial and the process repeated but without adding internal standard. A further 2ml MeOH was used to rinse the vials and the aggregated 12ml extract was reduced to dryness under a stream of Nitrogen gas at 40°C. The extract was reconstituted in 1ml Acetonitrile. A 200µl aliquot was taken and exchanged into 20/80 ACN/MQ and then filtered using 0.22um syringe filter into amber vials for instrumental analysis.

Analysis of Pharmaceuticals ingredients

Quantitative analysis was undertaken using a Shimadzu Triple Quadrupole Mass Spectrometer HPLC LCMS-8040. The mobile phases were A, 0.2% formic acid in MQ water, while B was Acetonitrile 20%. The gradient program was as follows: 0–1 min, 10% B, this was then increased to 80% B within 13 min, and then increased to 100% of B in 5 min, which was held for 4.5 min, after that decrease to the initial condition of 15% B within 1 min. Finally, 7 mins of post-run were set between each sample injection to ensure the re-equilibration of the column. The injection volume was 10µl while the column and the tray temperatures were set at 25 °C. A 10µl aliquot of the extract was injected onto an XBridge^R C18 column (Waters Corporation., 3.5µm, 2.1mm. 100mm length) fitted with a guard column (Waters Corp., XBridge^{R} BEH C18, 3.5µm, 2.1mm, 5mm length).

Table 3 Reagent Information

Name	Abbreviation	Purity	Supplier
Acetonitrile	ACN	HPLC	Fisher Scientific (UK)
Agarose	AG	Bio-analysis	Bio-Rad Laboratories (UK)
Ammonium Formate	(AF)	analytical	Fisher Scientific (UK)
Ammonium persulfate	APS	≥ 99%, analytical	Sigma-Aldrich (UK)
Gel solution	-	-	DGT Research Ltd (UK)
Milli-Q water	MQ water	(> 18.2 MΩ cm-1	Waters Corporation (UK)
Methanol	MeOH	HPLC	Fisher Scientific (UK)
N,N,N′,N′-Tetramethylethylenediamine	TEMED	≥ 99%, analytical	Sigma-Aldrich (UK)
Sodium chloride	NaCl	≥ 99%, analytical	Sigma-Aldrich (UK)
XAD-18 Amberlite	XAD-18	-	Dow Chemical Company

Quantification

Internal calibration curves for nine concentrations (1, 2.5, 5, 10, 25, 50 100, 250, and 500ng/ml) were prepared for quantification of target analytes in each of the samples. Standard calibration curves showed good linearity with a correlation coefficient of 0.999 while 0.5 ng/ml visual detection limit was adopted for this study.

Quality Assurance

As part of the experimental design and implementation, field blanks, and laboratory blanks were analysed. Samples of the DGT samplers were taken to the field and returned to the laboratory without being deployed. Blanks were processed with the other deployed samples to check if there have been contaminants in the process of transporting the samples. Empty vials were spiked with the internal standards and the organic solvents that were used for the extraction. The extracts were subjected to the same process as the real samples to correct for any instrumental or laboratory contaminations.

Results and Discussion

Concentrations of antibiotics in influents of Wastewater treatment works

The concentrations of the accumulated compounds were quantified as ng/ml which is equivalent to ng/sampler. Concentrations were converted to bulk water concentration using equation 1 below; To accurately determine the bulk concentration C_b (ngL^{-1}), diffusion coefficient D_e of the compounds under investigation at the field temperature is required and this may be calculated based on the measured concentration at $25^{O}C$ in the laboratory with 0.88mm thickness diffusive agarose gel layer. (C.-E. Chen, Zhang, & Jones, 2012; C. Chen, 2013).

$$\mathbf{C_b} = \frac{\mathbf{M(\Delta g+\delta)}}{\mathbf{D_e At}} \qquad (1)$$

$$\boldsymbol{Log\ D_{e(t)}} = \frac{\mathbf{1.37023}(\boldsymbol{t}-\mathbf{25})+\mathbf{8.36}\times\mathbf{10^{-4}}(\boldsymbol{t}-\mathbf{25})^{\mathbf{2}}}{\mathbf{109}+\boldsymbol{t}} + \boldsymbol{log}\,\frac{\boldsymbol{D_{e25}}(\mathbf{273}+\boldsymbol{t})}{\mathbf{298}} \qquad (2)$$

Temperature is represented as t at the deployment site, De is the Diffusion coefficient at 25°C and De (t) is the calculated diffusion coefficient at the temperature t.

The following compounds were all investigated; Sulfapyridine (SPD), Lincomycin (LIM), Trimethoprim (TMP), Norfloxacin (NFX), Ofloxacin (OFX), Ciprofloxacin (CFX), Amoxicillin (AMX), Sulfamethoxazole (SMX), Clarithromycin (CLM), Erythromycin-H2O (ETM-H2O), Roxithromycin (ROM), Erythromycin (ETM). However, Sulfapyridine (SPD), Lincomycin (LIM), Trimethoprim (TMP), Norfloxacin (NFX), Ofloxacin (OFX), Ciprofloxacin (CFX), Sulfamethoxazole (SMX) and Clarithromycin (CLM) were within a detected and reportable limit. Other compounds were not detected or detected below a significant concentration that is reportable. Not all compounds were detected at these sites, and the tables below show the concentration of the detected antibiotics while others were either absent at the time of study or below the detection limit. The result confirmed that most of the compounds detected were the compounds with low hydrophobicity (low $\log K_{ow}$ of -1.03 to 0.91) except for CLM with $\log K_{ow}$ of 3.16 while other compounds with $\log K_{ow}$ above 1 were not detected within a reportable limit. The water solubility of CLM is 0.34mg/L at 25°C. A summary of the concentration data for the detected antibiotics from both nature-based technology and conventional wastewater treatment works are shown in Table 1 below.

Table 1. Concentrations of antibiotics in influents and effluents of treatment works

Chemical of Interest	Influents		Effleunts	
	Nature-Based Treatment Works Range (ngL^{-1})	**Conventional Treatment Works** Range ngL^{-1})	**Nature-Based Treatment Works** Range (ngL^{-1})	**Conventional Treatment Works** Range (ngL^{-1})
Sulfapyridine (SPD)	7-12000	7-79	7-1300	5-85
Lincomycin (LIM)	11-17	6-16	7-14	6-11
Trimethoprim (TMP)	9-45	9-75	5-9	5-24
Norfloxacin (NFX)	7-17	10-11	4-16	nd
Ofloxacin (OFX)	17-880	14-960	15-220	95-410
Ciprofloxacin (CFX)	12-410	12-460	12-113	30-55
Sulfamethoxazole (SMX)	8-69	8-81	8-42	4-46
Clarithromycin (CLM)	11-38	11-17	4-157	15-85

Chemical uptakes from both the influents and effluents channels at all the investigated channels were used to obtain removal rates. This was calculated using the equation below.

$$\frac{\text{Influent mass uptake - Effluent mass uptake}}{\text{Influent mass uptake}} \quad \text{x} \quad 100 \qquad (3)$$

Table 2: Removal rates of antibiotics at the three conventional treatment plants investigated with the removal rates

Conventional Treatment Plant - Olo

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Influents	-	13.00	-	-	-	-	-	11.00
Effluents	-	6.20	4.60	-	95.00	55.00	4.50	15.00
Rem_rate	0%	52%	0%	0%	0%	0%	0%	-36%

Conventional Treatment Plant - Pon

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Influents	78	6	62	11	650	240	47	-
Effluents	85	-	-	-	410	45	42	85
Rem_rate	-9%	100%	100%	100%	37%	81%	11%	0%

Conventional Treatment Plant - Gav

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Influents	22	16	75	-	960	460	81	-
Effluents	5	-	24	-	230	30	46	17
Rem_rate	75%	100%	68%	0%	76%	93%	43%	0%

Calculated removal efficiencies

There is an observable variation in antibiotics within each type of works, as well as between the different treatment technologies. The variations are discussed below on treatment works by works basis both in the Conventional system and the Nature-based technologies

Conventional Treatment Plant -Olo, the occurrence of SPD, and NFX were below the detection limit in both the influent and effluent channels while the removal rate of LIM was 52%. However, TMP, OFX, CFX, and SMX were measured in the effluent while their occurrence at the influent was below the detection limit. CLM was found to have a negative removal rate which is <1% at the effluent channel indicating that the concentration at the effluent was greater than the influent. The possible cause of this could be attributed to the influx of these compounds in the preceding period which has not been degraded or discharged, influx before deployment time as well as conjugates of some other metabolites. However, this pattern has also been reported by other researchers (Ni & Zeng, 2015) and this study was not designed to investigate a wider spectrum of the compounds through which the parent compounds might have been detected at the influents channels. It is conceivable that residence time at various systems could also be considered as one of the factors. It implies that the system was probably not in a steady-state due to variations in the wastewater influx over the period.

For the **Conventional Treatment Plant-Pon**, the removal rates for LIM, TMP & NFX were 100%, OFX was 37%, HFX 81% and 11% removal rate for SMX. SPD had a removal rate of <1% indicating poor removal rate with a concentration of 78 ngL^{-1} at the influent and 85 ngL^{-1} at the effluent. This is an indication that the system was not suitable for the removal of LIM. It may also be presumed that the concentration of LIM at the effluent might have been controlled by the conjugate of this compound which was not measured or investigated by this study. However, CLM was not detected in the influent but had a concentration of 85.2 ngL^{-1} in the effluent sample.

Data from the Conventional Treatment Plant – Gav suggests removal rates ranging from 43% for SMX to 100% for LIM. NFX was not detected whilst CLM 17 ngL^{-1} was measured at the effluent without any measurable signal at the influent. This was an observable pattern across the three works which could be better explained various unknown activities that might have happened in the previous week coupled with wastewater residence time in the treatment works. There is however a consistent behaviour of CLM across the three treatments works. However, its detection at Olo treatment works influent was lower than the concentration at the effluent. On the other hand, Pon and Gav were very efficient in the removal of SPD, LIM, TMP, OFX, and CFX while NFX was not detected at all at the Gav treatment works. However, removal of SMX across the plants ranged from 0 to 43% which shows that all the plants were not as effective in the removal of this compound as for CLM. Gav had the highest daily influents loads of 34524m^3/day with BOD/COD ratio of 0.52 compared to Olo with BOD/COD ratio of 0.45. One could infer that the BOD: COD ratio has a direct relationship with the removal efficiency at these works. Zaher & Hammam, (2014) confirmed that for a typical wastewater treatment system, BOD/COD ratio of 0.3 to 0.6 is common for untreated municipal wastewater. However, in circumstances where the untreated wastewater BOD/COD ratio is 0.5 or greater, such wastewater is considered treatable biologically but in situations where the ratios are below 0.3, such wastewater is considered toxic.

None of the Conventional treatment plants had been designed for the removal of all antibiotics most especially the CLM across the three and OFX, CFX in the case of It could therefore be inferred that the treatment of wastewater could be influenced by various factors that include the design of the treatment plants (e.g. residence time) and other factors like solubility of antibiotics, BOD/COD ratio etc (W. Li, Shi, Gao, Liu, & Cai, 2013; H. Zhang, Liu, Feng, & Yang, 2013; X. Zhang et al., 2017)

Nature-based /Constructed Wetland WWTPs

There's a range of constructed wetland technologies ranging from single secondary stage to multi-stage system (Songliu et al., 2009; Sundaravadivel & Vigneswaran, 2017) while the flows could take any of the forms vertical subsurface flow (VSF), free water surface flow (FWS) or horizontal subsurface flow (HSF) (L. Li, Li, Biswas, Nian, & Jiang, 2008). All these are very important in the removal efficiencies of the Constructed Wetland. This is a system where the influent water passes through the coarse solid removal stage through to the sedimentation tank before the filtration system and into the Free Water System where the filtered water resides. These wetland plants supply the nutrients and oxygen that are required for the microbial growth in the substrate. However, the microbial population is responsible for the treatment of the system by either aerobic or anaerobic disintegration. The filtration stage could be horizontal or vertical or a combination of both depending on the design of the system. There are two basic types of Constructed Wetland or nature-based treatment technologies; There is free water surface (FWS) which has shallow beds with aquatic vegetations where contaminated water are treated by the exposed plant. However, the other type is known as subsurface flow (SF) wetland where the free water surface is not exposed to the atmosphere. In SF, the water level is kept below the top of the permeable treatment subsurface that supports the growth of vegetation while the quality of treatment is improved by the matrix of aerobic and anaerobic zones.

Table 3: Removal rate for Pharmaceuticals using Nature-Based treatment technologies.

Nature-based treatment plant - Cas

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Inf	115.00	6.60	14.00	-	-	-	-	12.00
Eff	-	14.00	-		-	-	-	-
Rem_rate	100%	-112%	100%	na	na	na	na	100%

Nature-based treatment plant - Dic

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Inf	660.00	-	44.00	7.00	880.00	410.00	69.00	-
Eff	326.00	12.00	-	3.70	17.00	-	42.00	4.70
Rem_rate	51%	0%	100%	47%	98%	100%	39%	0%

Nature-based treatment plant - Mar

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Inf	-	-	7.40	-	710.00	-	-	-
Eff	-	-	-	-	-	-	-	23.00
Rem_rate	na	na	100%	na	100%	na	na	0%

Nature-based treatment plant - Mos

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Inf	11,600.00	17.00	-	-	880.00	-	-	-
Eff	1,295.00	12.00	-	-	-	-	-	31.00
Rem_rate	89%	29%	na	na	100%	na	na	0%

Nature-based treatment plant - Gor

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Inf	-	-	-	-	210.00	49.00	-	17.50
Eff	-	-	-	-	-	-	-	-
Rem_rate	na	na	na	na	100%	100%	na	100%

Nature-based treatment plant - Jes

	SPD	LIM	TMP	NFX	OFX	CFX	SMX	CLM
Inf	17.00	-	45.00	-	400.00	190.00	20.00	38.00
Eff	32.00	-	5.00	-	220.00	110.00	27.00	160.00
Rem_rate	-88%	na	89%	na	45%	42%	-35%	-321%

Data from the nature-based treatment plant -Cas shows that the treatment system had 100% removal rates for SPD, TMP, and CLM. However, NFX, OFX, CFX, and SMX were below the detection limits in both influent and effluent. Conversely, LIM had a negative removal rate of <1% which is not in agreement with other NBT in this study. It is important to note that this treatment works only receives an average of 75 m^3/day influent water and so may not be directly comparable with other NBT in this study. This treatment technology covers a land area of 2,014 m^2 and was designed as a replacement for an activated sludge system which was no longer adequate to meet the demand of the community. The system lacks a sedimentation stage but is composed of gridded equalization tanks with RBF (Reed Bed Filter) + VF (Vertical Flow) + FWS (Free Water System), at the vertical flow reed bed filter, the accumulated solids are removed after 15-20 yrs for agricultural uses as fertilizer. The second stage has two vertical-subsurface flow systems which promote the evapotranspiration process of the aquatic plants while the effluents water is used to recharge the groundwater through infiltration and then reused for irrigation purposes. The stage design system could help to subject the compounds showing negative removals into further treatments which may in turn reduce their prevalence at the effluent.

Data from the **Nature-based treatment plant – Dic** suggested high removal efficiencies for TMP, CFX, and OFX. However, CLM and LIM were detected in the effluent but not in the influent because the concentrations were too low.

Invariably, this is a complex situation as wastewater residence time and dilution rate play important roles in the calculated removal rates of these compounds. It is however assumed that the system is in a steady state of supply. There is a widespread consensus that dilution reduces the concentration of hydrophilic compounds in the wastewater(Le Corre et al., 2012) while biological degradation of pollutants or conjugates of some antibiotics could also be a responsible fact for dominance of antibiotics ingredient at the effluent with low concentrations at the influents (Joss et al., 2006). Calculated removal rates of SPD, NFX, and SMX ranged from 40% - 50%. However, due to half-life degradability of the compounds, hydraulic retention times also play an important role in wastewater treatment(Gros, Petrović, Ginebreda, & Barceló, 2010). LIM half-life is 3.99 ± 0.25 hours(Sharma, Dumka, Singla, Kaur, & Singh, 2019), SPD half-life is 5 to 10 hours(Challis, Carlson, Friesen, Hanson, & Wong, 2013; Taggart, McDermott, & Roberts, 1992), while NFX has an estimated half-life of 3–4 hour(Stein, 1987)

This study suggests that **Nature-based treatment plant – Mar system** was 100% efficient for the removal of TMP and OFX. However, CLM was found in the effluent but was below the detection limit at the influent. Other compounds SPD, LIM, NFX, CFX, and SMX were below the detection limit in both the influent and the effluent.

Similarly, only OFX, CFX, and CLM were detected at **Nature-based treatment plant** – **Gor** with 100% removal efficiency. This plant treats 55, 296 m^3/day of combined sewer overflow for 2017 population equivalent. Even though the water solubility of CLM is low 0.34mg/L, biodegradation and sorption could have been responsible for its nondetectable concentration at the effluent in addition to the fewer population.

Nature-based treatment plant – **Mos** is a small urban wastewater pre-treatment plant with one stage (1bed) treatment system with very low daily influents load of 16 m^3/day designed to cater for the residents of the area with 60 population equivalents. Only a few compounds were detected at this treatment plant while TMP, NFX, CFX, SMX were not detected at all. However, the removal rate for SPD was 89% removal rate, for LIM it was 29%, for OFX a 100% removal rate, whilst CLM follows the previous pattern with 31 ngL^{-1} measured in the effluent water without any detection at the influent wastewater. This system shows that the most prominent compound is SPD with 11,500 ngL^{-1} in the influent and 1,300 ngL^{-1} in the effluent with $logK_{ow}$ 0.35 while the system is in a steady state of wastewater influx. Although this ingredient has been banned since 1990 and could only be found in those that have used it at one point or the other.

For the Nature-based treatment plant – **Jes** LIM and NFX were not detected in either influents or effluents while the removal rates for other compounds range from <1% CLM to 88 % TMP. These 2 staged treatment plants is an urban wastewater tertiary pre-treatment site with a 2-stage system and a load of 19,465 m^3/day serving a 60,000-population equivalent. Mores stages would be suitable to improve removal rates. This hybrid system consists of 2000m^3 sedimentation pond with 1 hectare horizontal submerge flow system SFS-h and 5-hectare free water surface system. However, accumulate sludge in the sedimentation pond are periodically pumped into a wetland. Clarithromycin adsorbs to suspended solids and sewage sludge while its pKa 8.99 indicates that it will exist in the cation forms between pH 5 and 9. DGT measures freely dissolved ions while low solubility 0.33 mg/L of CLM with its high sorption to suspended solids, large population being served with high daily influent loads inform the reason for its poor removal efficiency by the treatment system.

Chemical Concentrations in the Nature-Based Treatment Technologies

Contaminant concentrations in NBT are reduced in the treatment system through processes such as volatilization, ion exchange, chemical reaction, adsorption, and biodegradation. Biological activity and volatilization are influenced by ambient temperature. At colder temperatures, the upper bed layer insulates the microbial community in the free water system (FWS) and this effect is lower in the subsurface flow system (SFS). At a lower temperature, enzymes activity is decreases and protein macromolecules are disrupted (Strambini & Gonnelli, 2007). The removal rate of **Sulfapyridine**, SPD ranged from 51%-100% in any system where it was detectable except for Nature-based treatment plant-Jes where it was <1% in this study. The treatment plant-Mos is a rural treatment plant with only 1 bed serving a population of about 60. The daily influx was very low and the dilution was also very low.

Lincomycin is a polar antibiotic with a high water-solubility (927 mg/L at 25 °C) and is widely used in the pig and poultry industry. It was detected at a higher concentration in the effluent of Nature-based treatment plants Cas and Dic leading to a removal rate of <1% and 0% (12 ngL^{-1}) at the effluent respectively whilst the removal rate at treatment plant Mos was 29%. The treatment plant was designed to replace an activated sludge system that was no longer considered adequate for the community. However, the design of the plant also played an important role in this process. At the preliminary stage, vertical flow reed bed filters (RBF), the biosolids accumulate on this surface and these solids are removed after every 15-20years and used as organic fertilizers. However, the second stage has two vertical subsurface flow basins (VF) which provide habitats for the wetland plants. It can be deduced that the design of this system is a significant factor in the treatment of Lincomycin while the design of treatment plant Dic is also a multistaged system SFS-h + SFS-v + SFS-h + FWS. Overall, a multi-stage treatment system helps in the treatment process where extra stages could be useful during excess wastewater influx as well as in achieving better overall performance through a longer residence time.

Trimethoprim minimum solubility in a study was 0.28 g/L at pH 3.22, 25 degrees C and could be as high as 1 g/L at 24 ºC (Dahlan, Mcdonald, & Sunderland, 1987). Nature-based systems selected for this study were found to be effective in the removal of this compound. Studies have shown that TMP removal by sorption to activated sludge might be very low (Göbel, Thomsen, McArdell, Joss, & Giger, 2005), while another study suggested that it was found in the same concentration in the raw sewage as in the effluent water (Lindberg et al., 2006). However, a trimethoprim removal rate of 74 ± 14% has been recorded using a type of sand filter (Göbel, McArdell, Joss, Siegrist, & Giger, 2007) which suggest that they were removed in a crystalline form. This study corroborated our observations across the NBTs that were investigated.

Norfloxacin was detected at low concentrations in the treatment plant Dic with an average daily wastewater influx of 525 m^3/day. This antibiotic has a strong absorption which increases rapidly between the pH <5 or pH> 10. Although the removal rate of this slightly water-soluble compound stood at 47%. NBT-Dic is a secondary treatment plant that serves 3500 people. The four-stage system, comprising horizontal subsurface flow system (SFS-h) linking vertical subsurface flow system (SFS-v), horizontal subsurface flow system(SFS-h) and supplying into free water surface (FWS) covering a land area of 6080 m^2 was designed to create a high biodiversity area for 16 Tuscany's autoctone or native species of vegetations(Person & Typology, 2003). This design would have been very effective in the removal of Norfloxacin by sorption but its low solubility in water has an impact on its detection as DGT only measures dissolved compound and the undissolved part of the compound could have been absorbed into the organic matter. Organic matters or sewage sludge was not examined in this study which would have given us a clearer insight into the chemical partitioning.

Ofloxacin is a water-soluble antibiotic. It was detected in all the treatment plants except Cas. This treatment plant covers a landmass of 2014m^2 with daily influent loads of 75 m3 per day, having strong fluctuations in the number of people that it serves from a few dozens in the winter to up to 1000 during the summer. This system is therefore subjected to huge variations in the concentrations of compounds detectable in the wastewater. This explains why many of the investigated compounds to include Ciprofloxacin, Sulfamethoxazole and Norfloxacin were not found in this plant.

Ciprofloxacin This compound has a low $LogK_{ow}$ 0.4 which shows that the compound is highly insoluble in water. It is 30,000 mg/L at 20 °C in water. Compounds with high $LogK_{ow}$ increases with hydrophobicity while a low $LogK_{ow}$ is hydrophilic. The effective removal of this compound was across all the treatment plants where it was present apart from treatment plant -Jes that has 42% removal rate which suggests that its removal could have been predominantly by sorption. Studies have shown that Ciprofloxacin could have as high as 2.27 - 2.42mg kg^{-1} dry weight biosolid concentrations (Petrie *et al.*, 2014) which suggests that its removal must have been influenced by the presence of organic matters. It serves a population of 60,000 and the treatment plant has been designed to also receive stormwater and as such, the plant receives an average of 19,465 m^3/day wastewater. The 60,000m^2 treatment plant has a typology Sedimentation basin + SFS-h + FWS and has been observed not to be as efficient as other plants in the treatment of most of the compounds. However, Ciprofloxacin is widely used to treat clinical bacteria infections to include cancer.

Sulfamethoxazole is removed by adsorption into the organic matters with a water solubility of 610 mg/L (at 37 °C) (Ryan *et al.*, 2011; Rioja *et al.*, 2014). This corroborates the investigation outcomes that DGT only measure dissolved compounds in the water. This compound was detected only in the treatment plant -Dic and Jes with the removal rates ranging from 0% - 39%. The removal of this compound in Dic must have been favoured by the multi-stage design of the treatment plant while the sedimentation design of the Jes plant could have been responsible for the poor removal rate. However, degradation of 5-methyl-isobutyl-3-carboxamide gives rise to Sulfamethoxazole which could lead to increased concentrations in the effluent.

Clarithromycin. Studies have confirmed that 25% of Clarithromycin is excreted in an unchanged form (Calamari, Zuccato, Castiglioni, Bagnati, & Fanelli, 2003; Göbel et al., 2005; Rioja et al., 2014). The concentrations of Clarithromycin in the investigated treatment sites are similar to those reported by McArdell et al., (2003) as well as data reported by other studies (Golet, Alder, & Giger, 2002; Yamashita et al., 2006). This study confirmed that in all the treatment plants with urban wastewater sources, the concentration of Clarithromycin at the effluent is higher than at the influent which reflects poor removal by sorption or degradation.

However, treatment plants Gor and Cas had a 100% removal rate. Treatment plant Jes has a removal rate of <1% while it serves a very large population of 60,000 with daily influent loads 19,465 m^3/day.

Conclusion

The occurrence, concentration, and removal efficiencies of 23 pharmaceuticals at 3 Conventional WWTPs and 6 Nature-based treatment systems around Florence, Italy were investigated using DGT passive samplers. A total of 8 antibiotics were routinely measured with concentrations ranging from 6 ngL^{-1} – 960 ngL^{-1} in the influents and 4.5 ngL^{-1} – 410 ngL^{-1} in the effluents of the conventional WWTPs, while some concentrations were below the detection limits of the method. Antibiotic concentrations ranged from 6.6 ngL^{-1} to 11,600 ngL^{-1} in the influents and 3.7 ngL^{-1} - 1,295 ngL^{-1} in the effluents of the nature-based plants. The study confirmed that removal rates were influenced by factors including NBT designs which include multi-stage/single bed, partitioning behaviour, and the pH of the wastewater. Some of the antibiotics ingredients were detected at higher concentrations in the effluent than in the influent which could have occurred as a result of the degradation of the conjugated forms in the course of the treatment process where the system has become a source of the chemical via desorption. This study has not investigated the contributions made by the degradation of parent chemicals in the treatment process as well as other biochemical reactions that could have increased the concentrations of chemicals of interest. It means that in many of the instances, the chemicals that are reported at the effluents might not have been the signals that were picked at the influents which imply that even though the treatment plant may have good removal efficiency, but it is often difficult to be determined based on the effluent concentrations. The contributions of the parent or conjugate forms could be further investigated in future studies using by conducting a full chemical spectrum scanning rather than targeted compounds as investigated by this study.

Some of the exceptional removal rates were recorded in the conventional treatment works, ranging from <1% (CLM(Conventional treatment-Olo)) to 100% while a removal rate rates of <1 % (CLM(Nature Based-JES)) to 100% was reported in the nature-based system. This is in line with the outcome of other studies earlier conducted by other researchers which suggests that the increased concentrations at the effluent channels could have been as a result of the compound of interest being produced in the process as a metabolite of parents compounds that were not detected at the influent channels(Ni & Zeng, 2015). This does not suggest anything about treatment works performance but an indication that some biochemical activities might be happening in the system. It implies that a full chemical spectrum scan of the parents' forms and some other metabolites in the group would be ideal to investigate most especially these compounds with negative removals. Some individual antibiotics ingredients could pose a low risk to human health and as such, toxicology analysis of these ingredients would also be useful. The study therefore concluded that both removal systems and efficiencies are chemical dependent which corroborates the findings of Vymazal & Kröpfelová, (2009) that constructed treatment wetlands to be very effective in the removal of suspended and organic solids. It can be concluded that Nature-based treatment technologies are effective in the removal of organic compounds, suspended solids which collaborate the long-term survey conducted by Vymazal and Kröpfelová, (2009). It can also be suggested as a good alternative to the Conventional treatment system most especially in the localities where the population is very low and could not justify the investment in the conventional system. It means that land availability, daily influent loads, population, and types of wastewater have to be considered in deciding the suitable nature-based removal technologies design.

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5. Paper Three: Long term monitoring of removal efficiency of selected pollutants during Wastewater Treatment

Long term monitoring of removal efficiency of selected pollutants during Wastewater Treatment

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Abstract

This study evaluated seasonal variations in personal care products (PCPs) ingredients detected in a weekly influents and effluents samples from a wastewater treatment plants (WWTP) in Lancashire, Northwest of England for 52 weeks. An additional 4-week study was conducted to investigate their fate and behaviour in the activated sludge treatment process. Flow data for the works during the period ranged from 169,529 m^3/week to 589,608 m^3/week. 23 compounds were examined, out of which 21 were quantified in both influents and effluents of the wastewater treatment plant. There was a 100% occurrence rate for MEP at 23 ngL^{-1} to 2,300 ngL^{-1} in the influent, ETP 5 ngL^{-1} to 6,900 ngL^{-1} and BUP at 1 ngL^{-1} to 370 ngL^{-1} per sample. A further study confirmed a reduction in the chemical concentrations from influents to effluents except for TBHQ, OPP, 4-T-OP and E3. This study discusses the role of sorption to sewage sludge and biodegradation as possible removal mechanisms in this system. The concentration varies over the year, but it is inconclusive to say that the variation was affected by seasonal changes as there were no clear seasonal boundaries observable. However, it can be suggested that the sources were heterogeneity in nature. The removal rate of the chemicals over the further 28 days study showed only very minimal variability with a very good removal performance of the treatment works. Extract of ETP, PHBA, PRP, BPA and MEP shows an average of 57% to 92% removal rate. Besides, DGT has been demonstrated to be a suitable long-term monitoring tool for PCP ingredients like some other passive samplers such as POCIS.

Introduction

Seasonal variations in contaminant levels in the environment can be linked to variations in contaminants concentrations at source due to anthropogenic activities (Gómez, Herrera, Solé, García-Calvo, & Fernández-Alba, 2012), contaminant mobility (Keen, Knapp, Hall, & Graham, 2018) and water flow interdependent elements (Smeenk, 2012; Loos et al., 2013). Therefore, it is reasonable to suggest the fact that migration and dilution of contaminants changes with the season (Macdonald *et al.*, 2005; Bexfield & Jurgens, 2014). Water is the common agent of transportation for contaminants in the environment and is an important component of the ecosystem (Barnett & Feasey, 2018), and the need for non-contaminated water cannot be overemphasized (Fuerhacker, 2009). Also, the European Commission set up the Water Framework Directive (WFD) and its daughter directives to govern the members' states on sustainable use of freshwater and preservation of water basins (Fuerhacker, 2009; Brack et al., 2017). Domestic and industrial wastewater treatment has become necessary to preserve the quality of water basins, make the water reusable (Becerra-Castro et al., 2015; Cho, Luong, Lee, Oh, & Lee, 2011) and prevent reintroduction of contaminants and pathogens into the estuaries and river basins. The use of activated sludge treatment process of wastewater due to its heterogeneity in terms of the sources and presence of pathogens has been found effective (Carbajo et al., 2014). It is therefore important to understand the seasonal chemical variations and trends in the influx of wastewater into the treatment works to enable regulators to predict the impact of some of the unremoved chemicals that are reintroduced into the environments via wastewater discharge (Clarke & Cummins, 2015; Grobelak et al., 2017; Xie et al., 2016) and via sludge application to agricultural land (Pal, Gin, Lin, & Reinhard, 2010; Vidal-Dorsch et al., 2012).

Treated water is commonly reintroduced into river systems, as well as being used for irrigation purposes in some areas (Pedrero, Kalavrouziotis, Alarcón, Koukoulakis, & Asano, 2010; Becerra-Castro et al., 2015). There are however situations where there is excess stormwater, the overflow can be directly discharged into the river without subjecting it into full screening and treatment process (Cantwell, Katz, Sullivan, Borci, & Chen, 2016; Phillips et al., 2012), although the environmental impact of this practice is difficult to assess due to lack of accurate records and data on the ecotoxicology of the discharged water. Effluent water has been reportedly used for irrigation (Al-Nakshabandi *et al.*, 1997; Toze, 2006; Fereres & Soriano, 2007), and can be reused for other purposes only when bacteria and other microorganisms or pathogens have been eliminated or greatly reduced (Cho et al., 2011). However, reclamation and reuse of wastewater are some of the ways of overcoming water scarcity (Hudman, 1999). There have been concerns for increasing levels of pollution due to agricultural use of wastewater treatment end products (Thorburn, Wilkinson, & Silburn, 2013) such as sewage sludge, effluent water and as such, a holistic understanding of seasonal fluctuations in contaminant reintroduction into the environment would be crucial to alleviate some of the challenges (Macdonald *et al.*, 2000; Fleeger, Carman & Nisbet, 2003; Bolong *et al.*, 2009).

A large body of research exists on the release of organic contaminants into aquatic environments that can lead to increase toxicity (Fleeger et al., 2003) which could endanger aquatic organisms (Kennedy, 2011; Kühnert, Vogs, Altenburger, & Küster, 2013). Contaminant-induced alteration in oxygen and nutrients dynamics may modify ecosystem function (Atkinson, Julian, & Vaughn, 2014) which could inadvertently prompt ecological alterations where sensitive species may be adversely affected. Other studies have investigated the concentrations of personal care products ingredients such as parabens. The significant presence of propylparaben and butylparaben were revealed in the river studied while there have been concerns about human health implications following the occurrence of several trace PPCPs in drinking water (Carmona et al., 2014; Juliano and Magrini, 2017). Carmona et al., (2014) observed a decreasing trend in the PCP's concentration in the order wastewater>surface water>drinking water, while the high concentrations in surface water in Turia River of 7µgL-1 of propylparaben has been linked to the discharge of both treated and untreated wastewater into the river. High concentrations of PCPs were also detected in both mineral and tap waters from Turia river basin, Spain. This raises several concerns about human health consequence to exposure and bioaccumulation due to consumption.

Sampling river water for European Commission Wastewater Framework Directive WFD compliance is usually achieved using grab samples but this has its drawbacks which include the inability to carry out flow-weighted sampling, costs, need for huge resources and many more. A passive sampling method is considered as a viable alternative for this study. This study (in line with other previous studies) investigates the performance of o-DGT (Organic-Diffusive Gradient in Thin-Films) passive sampler for Water Framework Directives compliance monitoring. It was proposed in the European Union in Directive 2000/60/EU that all members states should achieve ‘good status’ for all their freshwater, transitional, and coastal water basins by 2015. This directive has been revised in Directive 2008/105/EC, Directive 2013/39/EU, and with the most recent one in Decision (EU) 2015/495. These have led to an increasing number of priority substances on the list, while also creating a Watch-list of priority chemicals. These priority substances are to undergo monthly surveillance, investigative and operational monitoring. Beyond the huge requirements of manpower using active and grab sampling techniques, it requires a lot of time and financial resources to effectively coordinate long-term monitoring of water basins. The Commission has made it open for each member state to decide on their tools while the commission is more result-oriented (Allan et al., 2006; Liefferink, Wiering, & Uitenboogaart, 2011). To sustainably implement and make informed decisions, sampling techniques have to be very cost-effective and must be able to detect contaminants at levels below the prescribed Environmental Quality Standards (EQSs). Passive sampling with DGT has been described an ideal tool for regulatory monitoring which meets several WFD requirements with special design to address some of the concerns such as temporal and spatial resolution, as well as bioavailability of contaminants (Gonzalez et al., 2005). DGT also provides time-weighted average (TWA) contaminants concentrations in water (Allan et al., 2007), can screen for pollutants (i.e. present/absent), and can be used to identify pollutant sources, as well as merging the assessment of pollution loads across national boundaries (Vrana et al., 2014).

With consideration of increasing levels of pollution in our river basins, a consistent and easy to operate approach to pollutant monitoring would be useful to understand and suggest suitable proactive measures to keep the quality of water basins under control. This study is built on the fact that we need water for human existence, dignity and food production (World Health Organization, 2013). We are however aware that water is a medium for ecotoxicology where groundwater, some drinking water’s, surface waters, seawaters and sewage treatment plant (STP) effluents have been reported on various occasions to contain some levels of contaminants (Fent, Weston, & Caminada, 2006). It is therefore important that we develop a systematic, simple, and cost-effective approach of monitoring environmental contaminants.

Aims and Objectives

The study has been designed to use a passive sample to investigate the monitoring of organic contaminant concentrations over a year which will encompass all the seasons of the year. Sequel to increasing trend and acceptance of passive sampler in environmental pollutants monitoring, there are increasing need for monitoring data from various researchers. These data can be used to measure a seasonal variation in the organic pollutants in both WWTP influent and effluent to determine removal rates, and also to investigate the reliability of various passive samplers. Accurate knowledge of removal rates and their variability is essential in determining both chemical emissions and risks. This study was also designed to assess the suitability of o-DGT for long-term monitoring of active ingredients in the wastewater across the seasons. Information about removal efficiency would also be obtained by analysing the contaminants data with the flow data giving an indicative idea of wastewater loads periodically as well as the removal rate. This would provide us with an idea of the seasonal performance of the treatment works over the investigated period as well as that of the sampling toolkit.

A parallel study was also conducted for four weeks to examine the partitioning of contaminants in the treatment system, which involved sampling the activated sewage sludge alongside the influent and effluent channels of the treatment works.

Chemicals of Interest

The study was centred on monitoring the presence of preservatives, antioxidants and Endocrine disruptive compounds in the wastewater treatment. There were 23 chemicals in these categories out of which 21 were reported while 2 of the chemicals were present below detections limits and as such were not reported: Methylparaben (MEP), Ethylparaben (ETP), 4-Hydroxybenzoic acid (PHBA), Propylparaben (PRP), Isopropylparaben (i-PRP), Butylparaben (BUP), Isobutylparaben (i-BUP), Benzylparaben (BEP), Heptylparaben (HEP), Triclosan (TCS), Ortho-phenylphenol (OPP), Butylated hydroxyanisole (BHA), Triclocarban (TCC), Butylated hydroxytoluene (BHT), Estriol (E3), Bisphenol-A (BPA), Diethylstilboestrol (DES), Estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (Ethinylestradiol) (EE2), 4-tert-octylphenol (4-t-OP), Nonylphenol (NP), tert-butylhydroquinone (TBHQ).

Nonylphenol (NP) and Butylated hydroxytoluene (BHT) were not detected and so are not included in the discussion. Considering the range of the chemicals that were detected, a few selected compounds that were continuously present have been analysed in more detail to provide insight into the removal patterns over 52 weeks. These include Methylparaben (MEP), Bisphenol-A (BPA), 4-Hydroxybenzoic acid (PHBA), Propylparaben (PRP) and Ethylparaben (ETP).

Methodology

Sampling Procedure: Deployment and Retrieval

Sampling campaign commenced in June 2017 and concluded in June 2018. For 1 year in a cycle of 7 days, o-DGTs passive samplers were deployed in triplicates at the influent and effluent channels of the treatment works in a mesh bag enclosed in an aluminium cage as shown in Figure 1 below. o-DGT samplers were prepared in the lab on deployment day, while the deployment for the new week and retrieval for the previous week are carried out simultaneously. Upon removal of the metallic cage, the rags and organic matters surrounding the cage were cleansed. Figure 1 below shows some of the issues encountered there on the field, most especially in the area of coagulated organic matters around the cage, and particularly at the influent channel, while the effluent channel was cleaner being at the final effluent of the treatment system. Recycled water in the works was used to wash the cage thoroughly before a new mesh bag is inserted with a new set of o-DGT for the new week while the retrieved mesh bag is removed from the cage. The retrieved mesh bags containing 3 o-DGT each for influent and effluent channels were stored in a separate polythene bag and safely transported to Lancaster University laboratory for further processing. The surface of the samplers was rinsed with Milli-Q water before the commencement of o-DGT extraction processes.

Nitrile gloves were used while handling the DGT housing to prevent contamination and these were changed at every location to prevent cross-contamination. Freshly prepared DGT were always removed from polyethene bags and arranged into mesh bags before being placed inside the aluminium cage and then immersed into the influent channel or effluent channels as quickly as possible within few minutes to keep them moist. On the day of removal, DGT surface areas were rinsed with Milli Q water to remove any grit or organic matter from the moulding and stored in polythene bags while being kept moist and to minimise exposure to air as was practically possible. Samples were immediately extracted upon arriving in the lab which was usually less than 2 hours from the time of retrieving them from the deployment site. Meanwhile, samplers were deployed in triplicates to assess sampling variability.

1 Influent cage during the deployment stage

2 Influent cage in-situ

3 Influent cage at the removal day

4 Effluent cage pre and post sampling

Figure 1: Deployment Device at the influent and Effluent of treatment works

An additional study was undertaken to understand chemical partitioning into sewage sludge, effluent water and removal by the activated sludge treatment process. This included an in-situ deployment of o-DGT into the influent, activated sludge and effluent of the wastewater treatment plant for 28 days. DGT samplers were removed in triplicates on the 7th, 14th, 21st and 28th days respectively giving rise to 36 samples. The o-DGT samplers were enclosed in the mesh bags every week and deployed using the aluminium cage as a result of the high flow rate and high content of organic matter at the influents. However, this deployment process had to be kept constant in the sewage sludge and effluent channels for consistency. New sets of DGT were deployed upon the retrieval of the previously deployed samples within 10 minutes. Retrieved o-DGT samplers were stored in pre-labelled separate zip lock bags, and securely transported to the laboratory and the entire process of retrieval, deployment and transportation to the laboratory for extract was less than 2 hours on each of the sampling days.

DGT: Analyte extraction phase

Hydrophilic Lipophilic Balance, HLB Resin gels were removed from the DGT holder following the DGT extraction procedure. The cap, filter paper and diffusive gel were disposed of while HLB binding gels were placed in clean, pre-baked 15 ml amber vials that have been pre-labelled for each of the sample locations with names. Resin gels were spiked with 50µl 1 ppm mixed internal standard (IS) of 23 compounds while 5 ml of acetonitrile was added to each of the bottles before ultrasonication for 30 minutes. The solvent was decanted to a new 15ml vial while the process was repeated for another but without IS to enhance analytes recovery. The analyte rich solvents in were also decanted into the respective 15 ml vial while the vial containing the resin was rinsed with 2ml of solvent and decanted into the vials containing the 10ml organic solvent to make 12ml per sample. The extract was concentrated under a gentle flow N_2 gas until the extracts were nearly dry. This was reconstructed by spiking the extracts in each of the vials with 1 ml of acetonitrile, ACN and filtered with a 0.2 µm PTFE syringe filter into 2 ml amber GC V-shaped vials. Vials were stored in the freezer for further processing for LCMS analysis.

LC-MS/MS analysis

0.2 ml of each sample was transferred to a V-shaped 2 ml vial and diluted with MQQ water at a ratio 20/80. The target compounds were separated by a Waters XBridge^R C18 column (Waters Corporation., 3.5µm, 2.1mm. 100mm length) fitted with a guard column (Waters Corp., XBridge^R BEH C18, 3.5µm, 2.1mm, 5mm length) on a Shimadzu LCMS-8040. Before LC/MS analysis. The LC-MS/MS setup and methodology for mass analysis was set up thus; Eluent A1 was Milli Q water, A2 was 5 Mm NH_4OH in 500 ml MQ water, B1 was ACN while B2 was 5 Mm NH_4OH in ACN. Eluents A1, B1 was de-gassed in an ultrasonic bath for 30mins. Compounds quantification carried out using Multiple Reaction Monitoring (MRM). Peaks were manually integrated for most of the detected compounds while compounds with the smoothest peaks i.e. minimal shoulders, were further analysed. The limits of quantification (LOQ) was 0.5ng.

Results and Discussion

The commonly used active sampling techniques (autosamplers) can provide individual samples or time-integrated samples but are expensive and time-consuming to operate compared to passive sampling system which integrates dissolved pollutant concentration over a designed period (Kot, Zabiegała, & Namieśnik, 2000). However, conventional grab or spot sampling approach is convenient but does not give a representative indication of the sampling medium over a period. o-DGTs has been adopted for this study based on their ability to address most of the concerns with the other sampling methods. The samplers were deployed in the influent and effluent channels of the treatment works for 52 weeks. The samplers were changed weekly in triplicate in each of the channels apart from the sampling week 27 and 43 which coincided with holiday periods. The flow data for the period under investigation was also obtained from the site operators to provide an idea of the daily influx of wastewater in m^3/day which was processed to weeks/months to correspond to other data. Flow data confirmed the highest and lowest flows through the works between 589,608 m^3/week and 169,529 m^3/week respectively.

Out of 23 chemicals initially investigated, only 21 were quantified due to concentrations being consistently below detection limits. There was a small spike in the flow data between week 22 with 292,640m^3/w, 589,608m^3/w in week 23 to 366,032m^3/w in week 25 but without any significant impact on the chemical concentrations. This also reinforces the fact that even though exposure of DGT to large water volumes is important in determining uptake concentrations, other factors such as chemical sources, the solubility of chemicals and dilution are important. For example, if the source of the chemicals is constant during the period with high water influx into the treatment works, there is a tendency for a high dilution which would lead to a low concentration of various ingredients per cubic meter of water. Figure 2 depicts that average daily rainfall was consistently high during this period up to January 2018 with little variation in the flow data. The temperature was observably low between November 2017 to March 2018 as well as average monthly precipitation except for January 2018. Please see Table 1 and Figure 2 for the graphical representation. However, Figure 3A shows the amount of chemical present in the influent between June 2017, week 1 and December 2017 week 26 were higher on an average compared to January – June 2018. Although, concentrations of individual chemicals varied over the period.

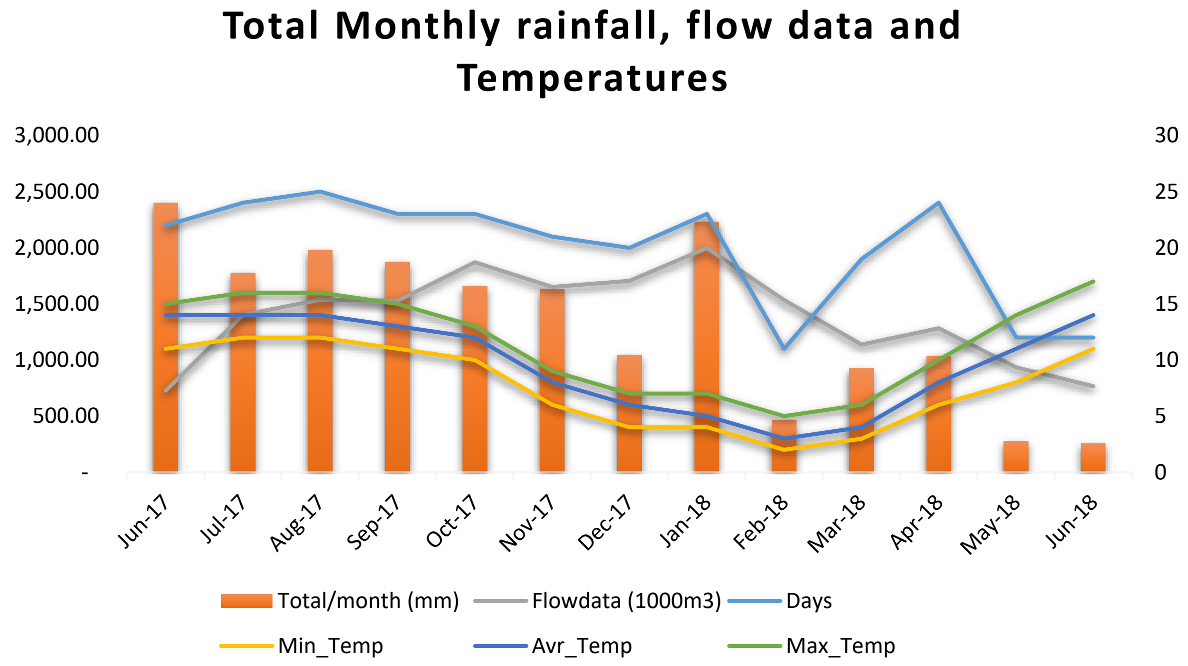

Figure 2: A schematic representation of monthly Flow data(m^3/month), rainfall data(mm), and temperature(^{0}C)

Figure 2 shows a higher rainfall in the first half of the study with an average rainfall of 110 mm in June 2017 compared to 12.5 mm in June 2018. With a few exceptions, it can be observed that rainfall varies with the flow data while the temperature also shows similar patterns. It can be inferred from the study that flowrate in some instances was inversely proportional to the o-DGT uptake. Observably, the mass of chemical uptake in the second half of the investigation shows a direct relationship between rainfall and chemical availability in response to the available chemicals in the wastewater influent. This suggests that uptake of chemical is flow-dependent provided the supply of PCP ingredients into the process is steady while some exceptions do occur due to other factors such as pH, the concentration of organic matter, temperature and many more.

Table 1: Average rainfall in Lancaster and LWTTP flow data between the June 2017- June 2018 (WorldWeatherOnline.com, 2019)

	Average rainfall (mm)			**Monthly Flow data**		**Temperature**		
Date	**Days**	**Daily Avr(mm)**	**Total/ Mth (mm)**	**m³**	**'1000m³**	**Min**	**Avr**	**Max**
Jun-17	22	109	2398	727296	727	11	14	15
Jul-17	24	74	1776	1403936	1404	12	14	16
Aug-17	25	79	1976	1535456	1536	12	14	16
Sep-17	23	82	1873	1529192	1529	11	13	15
Oct-17	23	72	1661	1870160	1870	10	12	13
Nov-17	21	78	1628	1650888	1651	6	8	9
Dec-17	20	52	1040	1704888	1705	4	6	7
Jan-18	23	97	2232	1998355	1998	4	5	7
Feb-18	11	42	465	1535041	1535	2	3	5
Mar-18	19	48.60	923	1138350	1138	3	4	6
Apr-18	24	43.18	1036	1282357	1282	6	8	10
May-18	12	23.30	280	935834	936	8	11	14
Jun-18	12	21.28	255	766262	766	11	14	17

* From 20th – 30th June 2017 to month-end

In situ Deployment at the Influent

Influent channel concentration data are presented in Figure 3A. The highest detectable concentrations of the 21 reported chemicals in the treatment works was 13,000 ngL^{-1} out of 290,000 m^3 wastewater influx in the week 14 where 4,900 ngL^{-1} was detected at the effluent channel of the treatment works in September 2017. Samplers were deployed at the influent in triplicate and retrieved weekly for the entire period of 52 weeks except for week 27 for Christmas plant shutdown and week 43 for the Easter break, respectively. The average wastewater loads range from 169,529 m^3/week in week 51 to 589,600 m^3/week in the week 23rd. The flow rate does not have a direct relationship with the total DGT uptake which confirmed the fact that DGT only measures the dissolved PCP ingredients which the undissolved components are not accounted for by the DGT uptake data.

Chemical List

Out of 23 PCP ingredients that were investigated, only 21 were reportable as the other ingredients Nonylphenol, (NP) and Butylated hydroxytoluene (BHT) were not detected in a reportable concentration. The 21 quantified compounds are Methylparaben (MEP), Ethylparaben (ETP), 4-Hydroxybenzoic acid (PHBA), Propylparaben (PRP), Isopropylparaben (i-PRP), Estriol (E3), Butylparaben (BUP), Benzylparaben (BEP), Isobutylparaben (i-BUP), Tertiary butylhydroquinone (TBHQ), Triclosan (TCS), Bisphenol A (BPA), Diethylstilboestrol (DES), Estrone (E1), 17β-estradiol (E2), α-Ethinylestradiol (EE2), Heptylparaben (HEP), Ortho-phenylphenol (OPP), Butylated hydroxyanisole (BHA), Triclocarban (TCC), 4-tert-octylphenol (4-T-OP). There was a weekly consistent occurrence of some chemicals at the influent channel which includes MEP at 23 ngL^{-1} per sample to 2,300 ngL^{-1}, ETP 5 ngL^{-1} to 6,900 ngL^{-1} , BUP 1 ngL^{-1} to 370 ngL^{-1} . However, the rest of the investigated compounds were not detected every week of the year. Figure 3A depicts uptake of the PCP ingredients over 52 weeks starting from June 26th.

There was an observable high uptake of Triclocarban, TCC in the week 18 and 19 at the influent channels as well as high uptake of BPA in the week 14. Week 18 and 19 had a high flow data of 400000-500000 cubic meter. Considering the high influent loads, even though the as of 2016, studies confirmed that the use of Triclocarban has reduced to 40% while it was banned by FDA in 2017(Fransisca & Dianursanti, 2019) with other 17 antibacterial compounds, the high influx must have been based on industrial discharge. Laboratory studies have shown that removal of Triclocarban, as well as Triclosan compounds by degradation, could take a few weeks with a half-life of 108 days and 18 days respectively (Ying, Yu, & Kookana, 2007). It is important to note that these compounds even though many have been banned, but this study was conducted around the time when many manufacturers of personal care product and other users of these would be disposing of their unwanted stockpile.

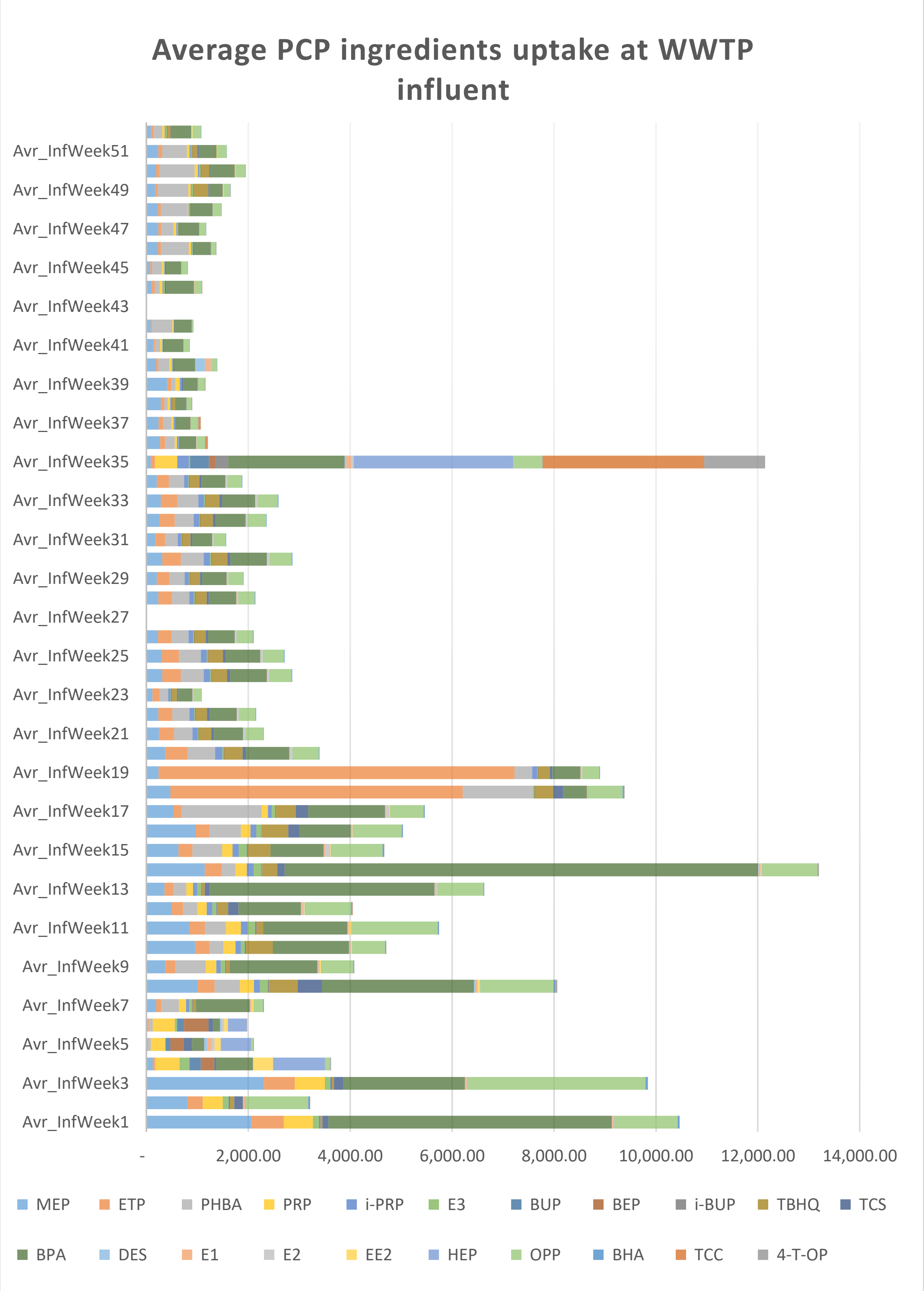

Figure 3A: Average PCP ingredients uptake over 52 weeks in the influent channel of treatment works in the Northwest of England.

In situ Deployment at the Effluent

The effluent channel is cleaner to a wide range of water quality parameters than the influent with the effluent water being released directly into the estuary. All the PCP ingredients that were detected at the influent were detected at the effluent but a very substantially reduced concentration. There were weeks when some of the ingredients were not detected while Ortho-phenylphenol was the only PCP ingredient that was consistently detected in all the weeks of the year. This was detected at 7 ngL^{-1} to 292 ngL^{-1} over the sampling period. This did not occur across all the weeks in the influent but a maximum concentration of 3,500 ngL^{-1} per sampler was detected over the 52weeks. This is commercially used a preservative in the food industry (St. John et al., 2001) with a high $\log K_{ow}$ of 3.28 suggesting that its removal is mostly by sorption to sewage sludge.

Statistical distributions with kurtosis of -1.2 for PHBA, 1.9 for i-PRP, and 0.58 for TCS confirmed that others outside kurtosis range of ±3.00 had some weeks of outliers or spikes in the DGT concentrations. This demonstrates chemical influx fluctuations confirming that the system was not at a steady-state level over the 52 weeks. This study corroborates other studies such as Jelic et al., (2011) which have found some Personal Care Products ingredients at the effluents of wastewater treatment works. It therefore can be used to demonstrate the removal efficiency of the treatment works. However, the overall removal process could be summed up as losses of a parent compound because of different chemical and physical transformation, sorption to solid matter and biodegradation. The behaviour of PCP ingredients in the treatment works vary with their properties as the hydrophobic compounds with high $\text{Log}K_{ow}$ tends to be removed by sorption while low $\text{Log}K_{ow}$ or the Hydrophilic compounds tend to biodegrade or be removed by other processes. Figure 3B below shows PCP uptake at the effluent channel over the 52weeks.

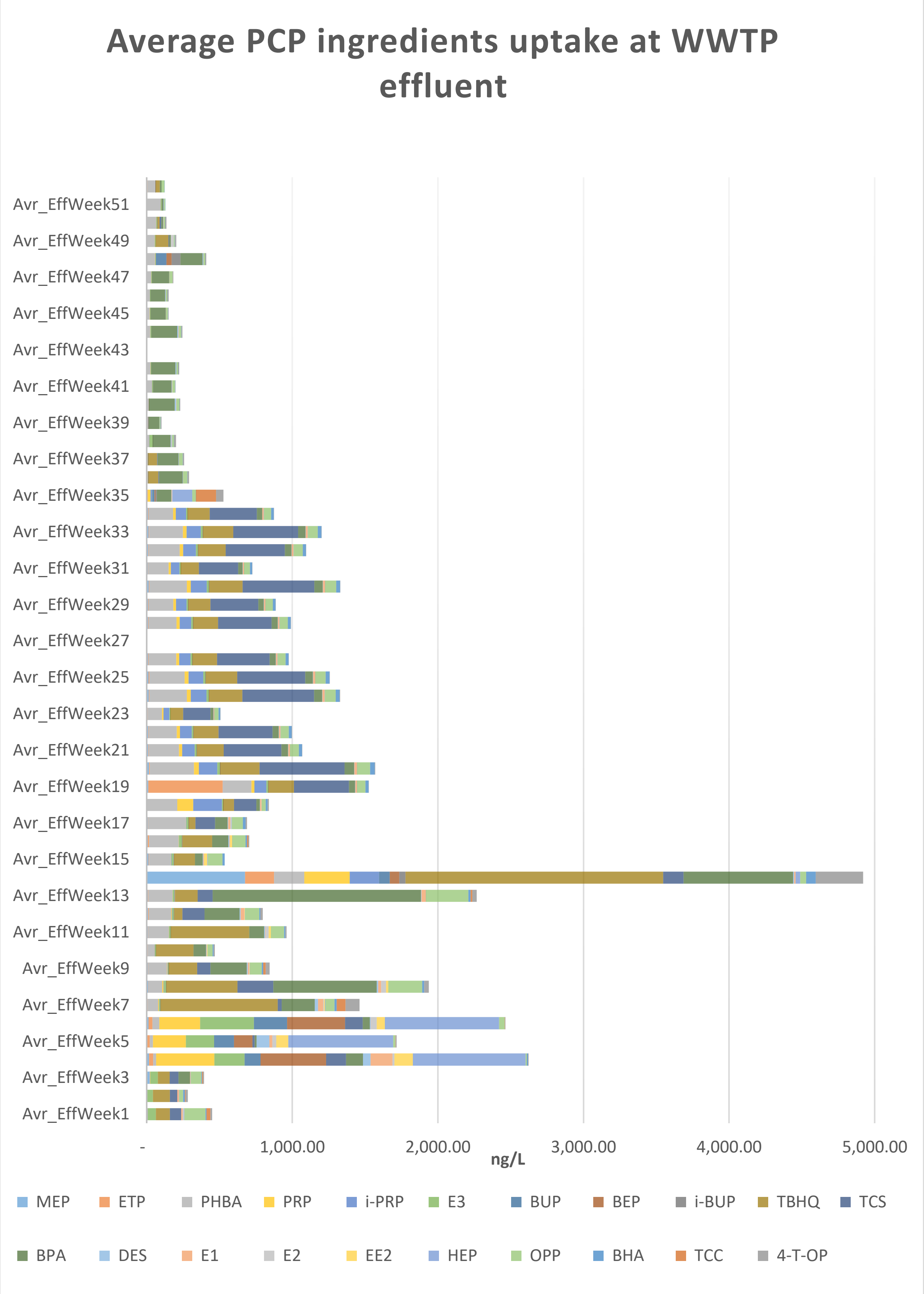

Figure 3B: Average PCP ingredients uptake over 52 weeks in the effluent channel of treatment works in the Northwest of England.

Calculated Removal Rates

Removal rates during this period were observed to vary considerably for many of the compounds most especially HEP and 4-T-0P having a removal rate of -5,100% and -2,800% respectively. These were as a result of an average effluent concentration of 0.61 ngL^{-1} and 1.70 ngL^{-1} to influent concentrations of 32 ngL^{-1} to 343 ngL^{-1} respectively. For other compounds removal rates for MEP ranged 41% - 95%, ETP had an average removal rate of 85% with the effluent concentration of <1% to 511 ngL^{-1} , PHBA has the average removal rate of 63% where the highest removal rate of 98% was observed on some days. PRP and i-PRP have a maximum removal rate of 100% with an average of 87% and 66% respectively across the entire year. E3 has a maximum removal rate of 100.0% with an average removal of 39% across the year. The average removal rate was as a result of the effluent concentration of 370ngL^{-1} as against the influent concentration of 44% in week 6, likewise week 38 with an influent concentration of 5ngL^{-1} and effluent concentration of 23ngL^{-1} . BUP had an average removal rate of 100% except for week 48 where the influent and effluent concentrations were 1ngL^{-1} and 72ngL^{-1} respectively. Similar patterns were recorded except for OPP, BPA, and MEP where the effluent concentrations were lower than the influent concentrations with removal rate ranging from 41% to 100%. The concentrations at the effluent channels in some of the weeks were observed higher than in the influents. Total rainfall for September 2017 was 1,873 mm as recorded over 23 days with an average daily precipitation of 81.45 mm. However, the flow data for the month was 1, 529,192 m^3/month while the following October 2017 had a higher flow of 1,870,160 m^3/month but with less daily rainfall precipitation of 72.21mm/day over 23 days.

There was an indication that some of the wastewaters that were received in the treatment work during week 14, the last week in September 2017, might not have been subjected to a full treatment due to system breakdown or excess loads of wastewater during the month probably overstretched storage and treatment capacity of the system. In this circumstance, the works could have discharged some of the water after the initial screening into the estuary to ensure that they maintain their operational limit or capacity. Please see supplementary table 1 showing removal rate statistics.

There is an observable relationship between the flow data and the amount of rainfall during the investigation period which does not follow a similar sequence with chemical concentrations. Data presented in Figure 2 suggests that chemical concentrations at any point in time are dependent on many factors. Some months such as weeks 22 to 34 where there are high wastewater loads, the detected chemical aggregates are not as high as those detected within week 1 and 19. Azzouz & Ballesteros, (2013) reported a variation in the removal efficiency of Pharmaceuticals and Personal care products where higher concentrations are observed in the autumn and winter compared to summer and spring. This confirmed usage patterns which vary with seasons of personal care products ingredients. It can however be seen that there is a downward trend in the reported concentration towards the last 2 seasons of the study which observably has a direct relationship with daily loads and total rainfall.

Some of the parameters affecting the concentration and treatment conditions such as their sorption onto organic matter, organic matter loads, pH, temperature, covary (Cirja, Ivashechkin, Schäffer, & Corvini, 2008). While most of the detected chemicals behaved differently, the above factors including their Physico-chemical characteristics may have to be considered to gain a full insight into their behaviour over the four seasons of the year.

Chemicals concentrations in week 35, 13th – 20th February 2018 do not follow the patterns in the second half of the deployment season giving rise to 12,142 ngL^{-1} chemical uptake per sampler during the period. This high concentration was as a result of few compounds such as BPA 2,280 ngL^{-1} , HEP 3,129 ngL^{-1} , TCC 3,172 ngL^{-1} , and 4-O-TP 1,195 ngL^{-1} . These constitute an anomaly compared with the previous week and the sudden influx may have been as a result of some additional sources in the weeks preceding the week 35th. There has been a huge influx of 447,773 m^3 wastewater in the preceding week while a reduction of about 160,000 m^3/week from that volume has been recorded in the following week. However, the average removal rate for week 35 was 95.7% for the reported chemicals. There was also a sudden reduction in rainfall from an average of 97.04mm/day over 23 days to 42.25mm/day over 11 days in February while the temperature was at its lowest point of 2 degrees. The influent wastewater influx was 1,535,041 m3/month with total rainfall of 464 mm/month giving an indication that the wastewater must have been discharged from storage somewhere which could be responsible for the high concentration of these ingredients.

There is also an observable outlier in week 14 which had a total flow rate of 834,992 m3 in the preceding 2 weeks with an average rainfall of 1,873.35 mm for 23days. It is envisaged that operations at the treatment works must have experienced high flow conditions resulting in short residence time and poor treatment levels since similar outlier was observed at the effluent for the same period. But conversely, due to process residence time, it is suggested that this could have also resulted from huge wastewater influx experienced in the previous 2 weeks. Although, Tert-Butylhydroquinone TBHQ which is practically insoluble in water with a solubility of 1 mg/ml was predominant in the effluent during the week 14 with a high concentration of BPA at the influent in the same week. These two compounds are fairly insoluble in water. However, DGT measures the dissolved concentrations and having such a spike shows that the influx of this compounds must have been precarious while many of the compounds might have been removed by other forms of removal in the system.

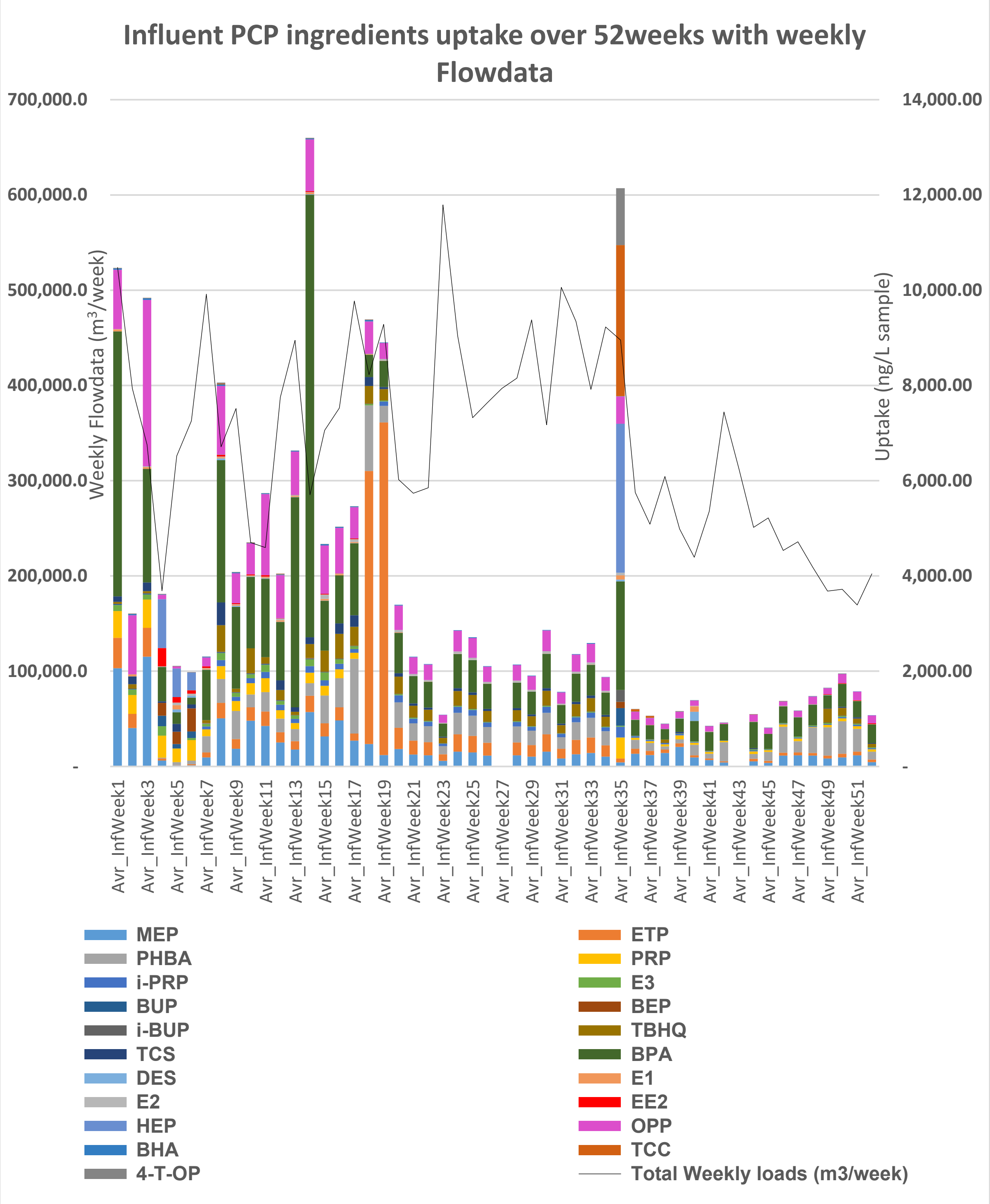

Figure 4A: Average chemical concentrations for 52 weeks in the effluent channel of treatment works in the Northwest of England to the flow data.

The following selected compounds shall be reviewed to investigate the relationship that exists between the removal rates and the logK$_{ow}$ which have been selected based on their LogK$_{ow}$. Methylparaben (MEP) has a LogK$_{ow}$ of 2.00, Bisphenol-A (BPA) with LogK$_{ow}$ 3.64, 4-Hydroxybenzoic acid (PHBA) LogK$_{ow}$ 1.39, Propylparaben (PRP) LogK$_{ow}$ 2.98 and

Ethylparaben (ETP) LogK_{ow} 2.49. Amongst the removal mechanism is sorption of compounds into sludge which is used by the farmer for land nourishment.

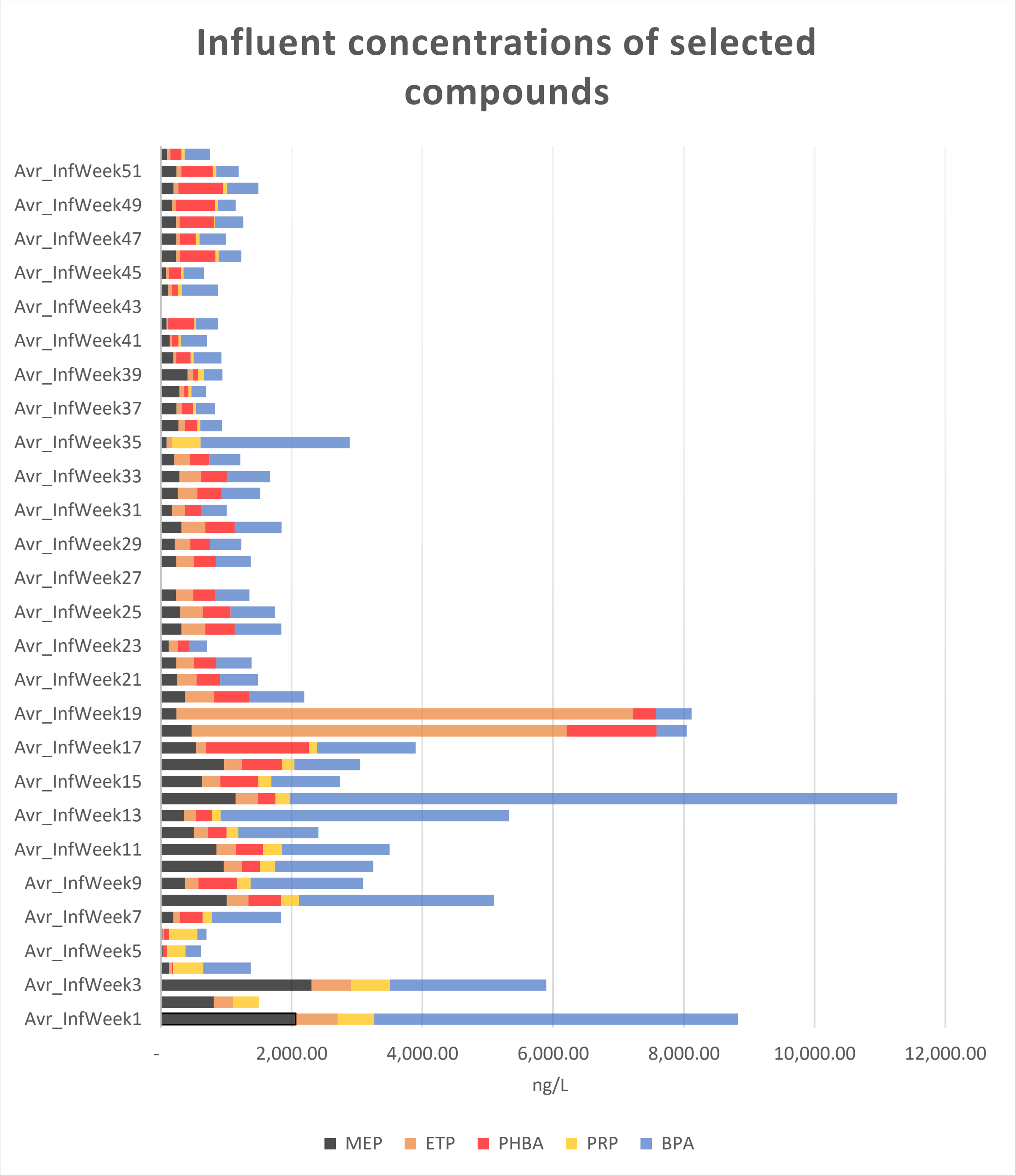

Figure 4b: Chemical concentrations per sample of selected compounds at the influent channel of wastewater treatment plants

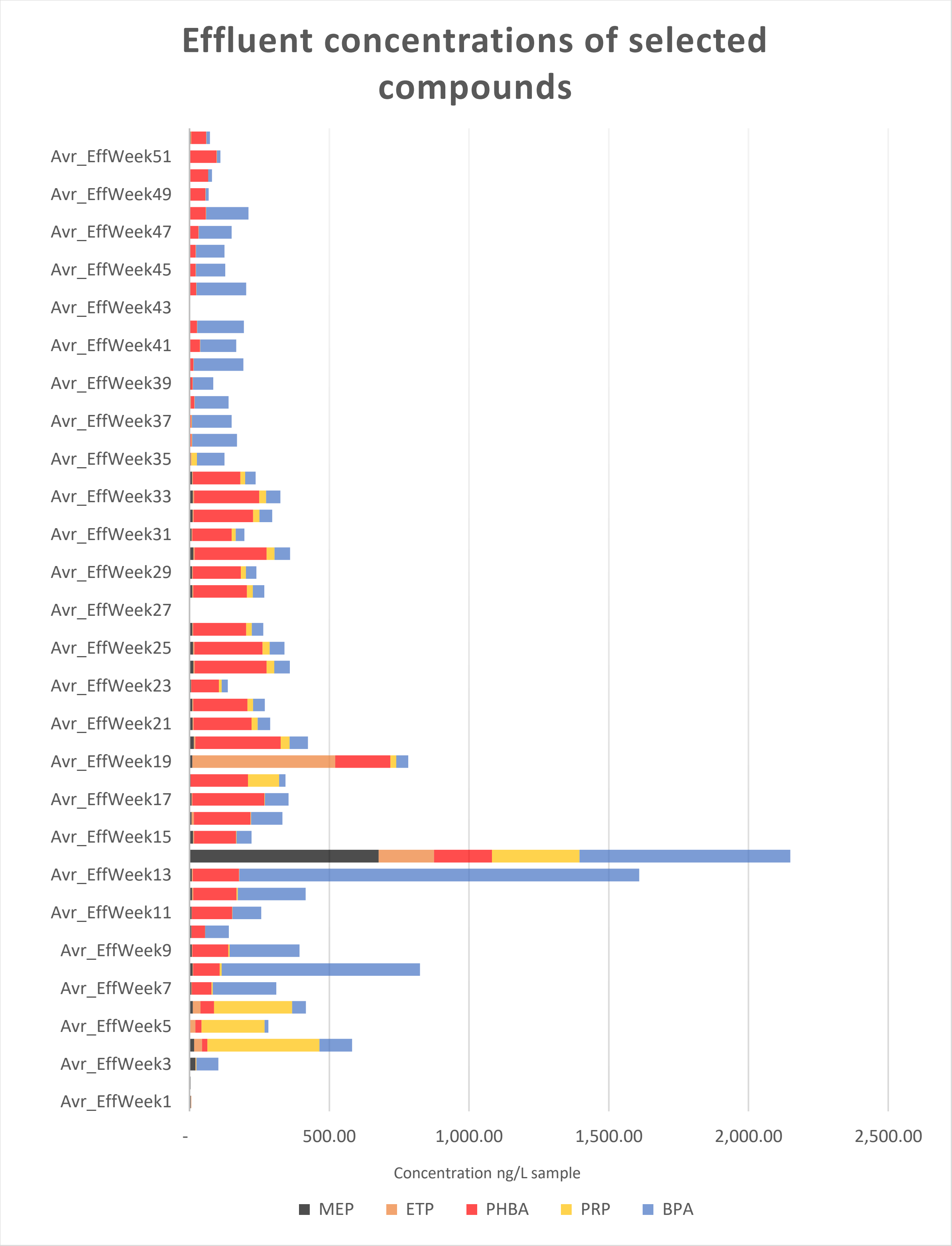

Figure 4c: Chemical concentrations per sample of selected compounds at the effluent channel of wastewater treatment plants

Following up on the result, Figures 4(A-C) has been drawn up to show the potential relationship between the removal rate and LogK_{ow}. The selected compounds are observed to be mostly detected across the year. Comparatively, BPA has the highest logK_{ow} 3.64 with removal rate ranging from 97 % to 100% while PHBA with the logK_{ow} 1.39 had removal rate ranged from 75% to 100%. Other compounds such as Methylparaben (MEP) with LogK_{ow} of 2.00 had a removal rate of 82% to 100%, Propylparaben (PRP) LogK_{ow} 2.98 having removal rate of 65% to 99% and Ethylparaben (ETP) with LogK_{ow} 2.49 ranged from 16% to 100%. PHBA occurs almost across all the weeks showing that the compound has been detected extensively at the effluent. High logK_{ow} suggests that removal rate across these compounds is likely to have resulted from sorption rather than degradation. Amongst the removal mechanism is sorption of compounds into sludge which is subsequently processed into agricultural fertiliser.

Table 2: Comparative table of Removal Rates, LogK_{ow} and Water solubility at 25 °C

Compounds	**Removal rate**	**LogK_{ow}**	**W_s(mg/L) at 25 °C**
Bisphenol-A (BPA)	97% to 100%	3.64	120
Propylparaben (PRP)	65% to 99%	2.98	500
Ethylparaben (ETP)	16% to 100%	2.49	885
Methylparaben (MEP)	82% to 100%	2.00	2,500
4-Hydroxybenzoic acid (PHBA)	75% to 100%	1.39	5,000

The above data suggested that water solubility and Logk_{ow} covary, which is perhaps not surprising. Meanwhile, compounds that are less soluble in water tend to be removed by sorption to a greater degree (Anderson & Hansen, 1955; Galil & Wolf, 2001), while the soluble compounds are more available to be removed by degradation and other chemical processes.

In situ Deployment at the Influent, Sewage sludge and Effluent

This section of the study was to investigate the likelihood of any relationship between chemical concentrations at the influent, activated sludge and effluent for 4 weeks. This part of the study should provide insights into the relative importance of different treatment stages with the WWTW.

Previous data in this study was presented as an amount of chemical per ml, which is equivalent to the accumulated mass per sample. The DGT equation can be used to accurately determine the bulk concentration C_b of analytes in the sampled medium from the accumulated mass in this case. Determining the bulk concentration C_b requires the diffusion coefficient D_e of all the compounds at the various sampling temperatures for all the sampled locations. DGT concentrations have been determined at 25^{O}C in the laboratory using 0.88mm thickness diffusive agarose gel layer and the D_e at various temperature was therefore calculated using equation 2 (C.-E. Chen, Zhang, & Jones, 2012; C. Chen, 2013). Average bulk concentrations for each of the samples were calculated from the D_e at the average temperature in the field at the time of deployment and removal of the samplers.

$$\mathbf{C_b = \frac{M(\Delta g+\delta)}{D_e A t}} \qquad (1)$$

$$\boldsymbol{Log\ D_{e(t)} = \frac{1.37023(t-25)+8.36\times10^{-4}(t-25)^2}{109+t} + log\frac{D_{e25}(273+t)}{298}} \qquad (2)$$

Where t stands for the temperature at the deployment site, D_e *is the Diffusion coefficient at* $25^{o}C$ *and* $D_{e\,(t)}$ *is the calculated diffusion coefficient at temperature t.*

The outcome of this study is represented below in figure 5(A-E). The concentrations have been grouped using the influent aggregates into 4529 ngL^{-1} to 6201 ngL^{-1}, 2200 to 3966 ngL^{-1}, 549 ngL^{-1} to 810 ngL^{-1}, 58 ngL^{-1} to 154 ngL^{-1}, 7 ngL^{-1} to 98 ngL^{-1}considering the number of personal care ingredients that were investigated as well as the removal rates. The data has been presented using some charts with groupings of chemicals per chat to provide a clearer illustration of the findings.

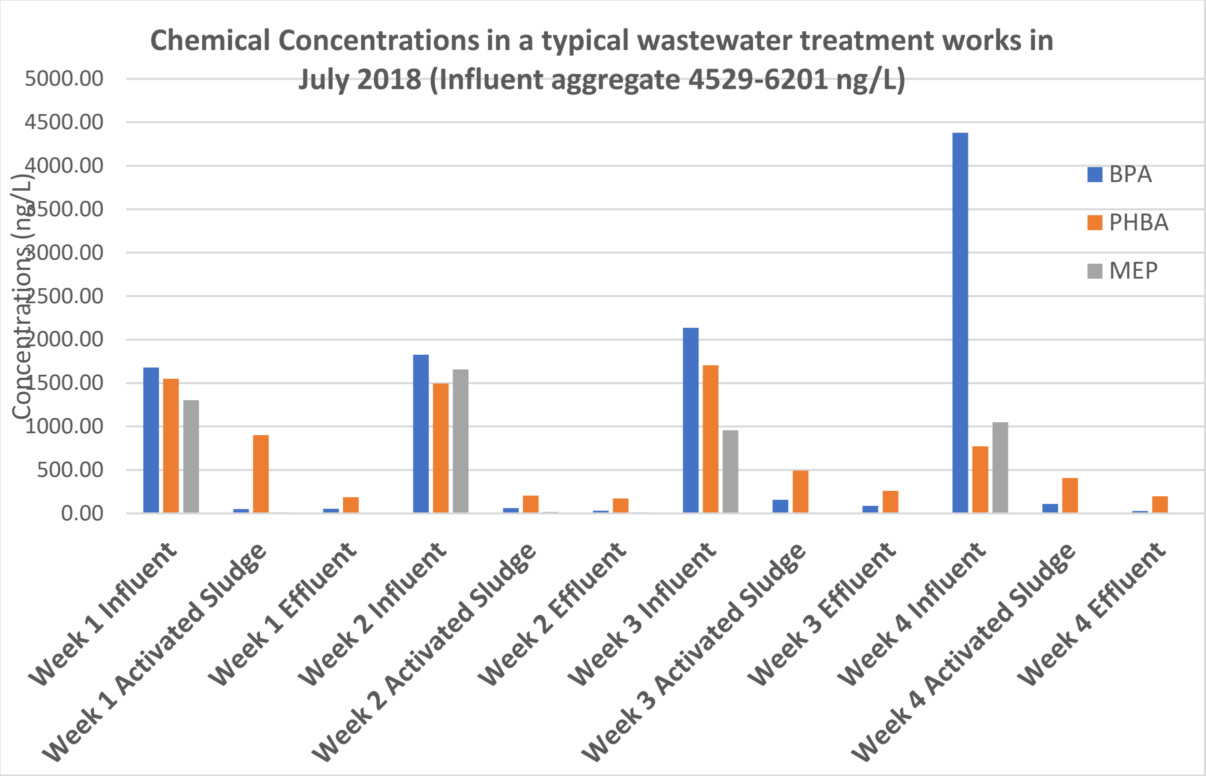

Figure 5A: Chemical concentrations across 4 weeks measured from influents, activated sludge and effluents grouped chemicals concentration

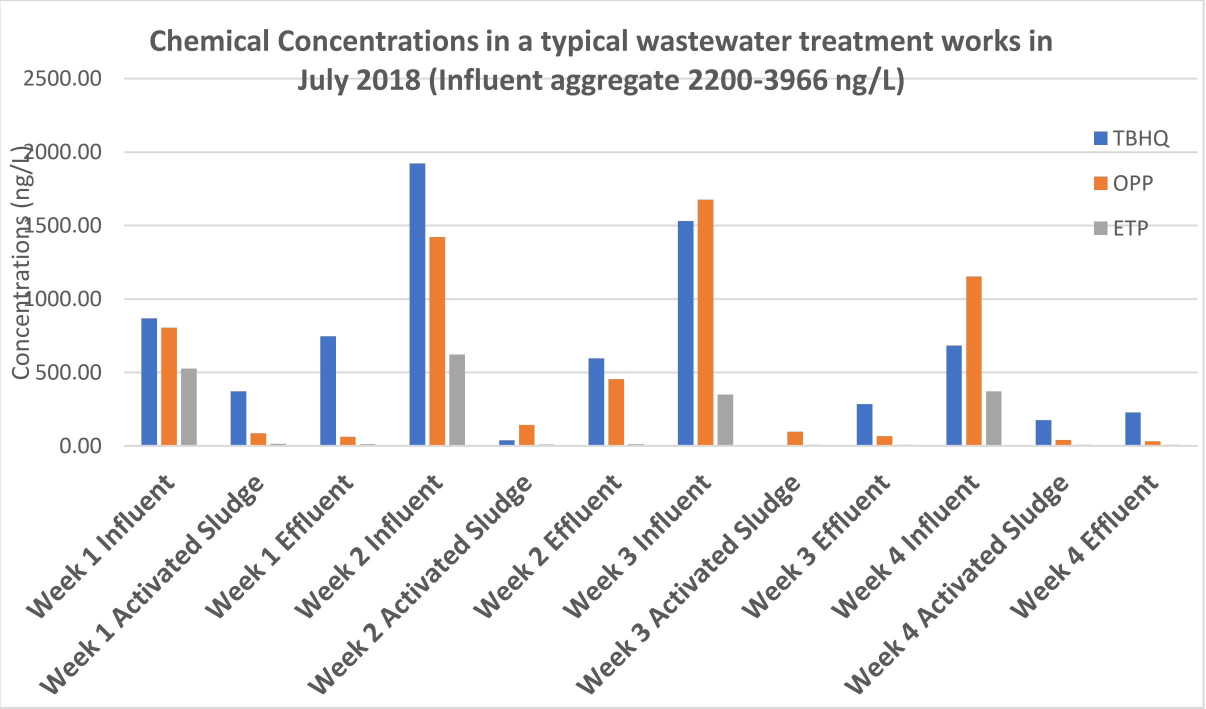

Figure 5B: Chemical concentrations across 4 weeks measured from influents, activated sludge and effluents grouped chemicals concentration

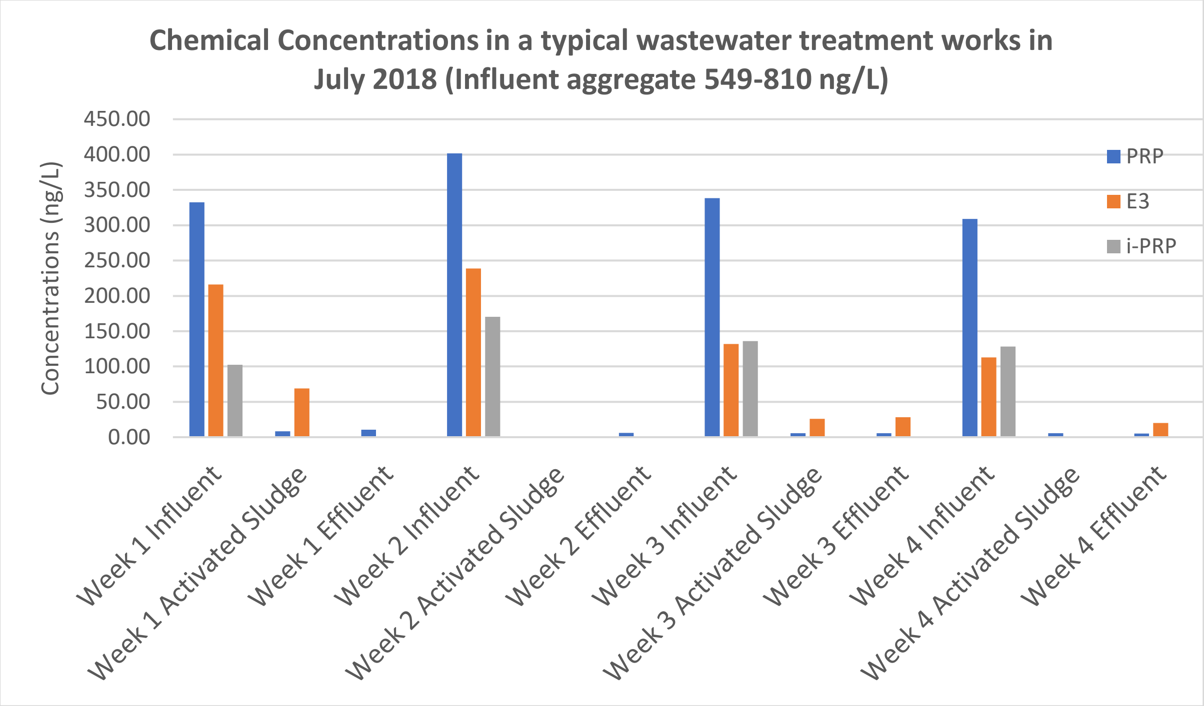

Figure 5c: Chemical concentrations across 4 weeks measured from influents, activated sludge and effluents grouped chemicals concentration

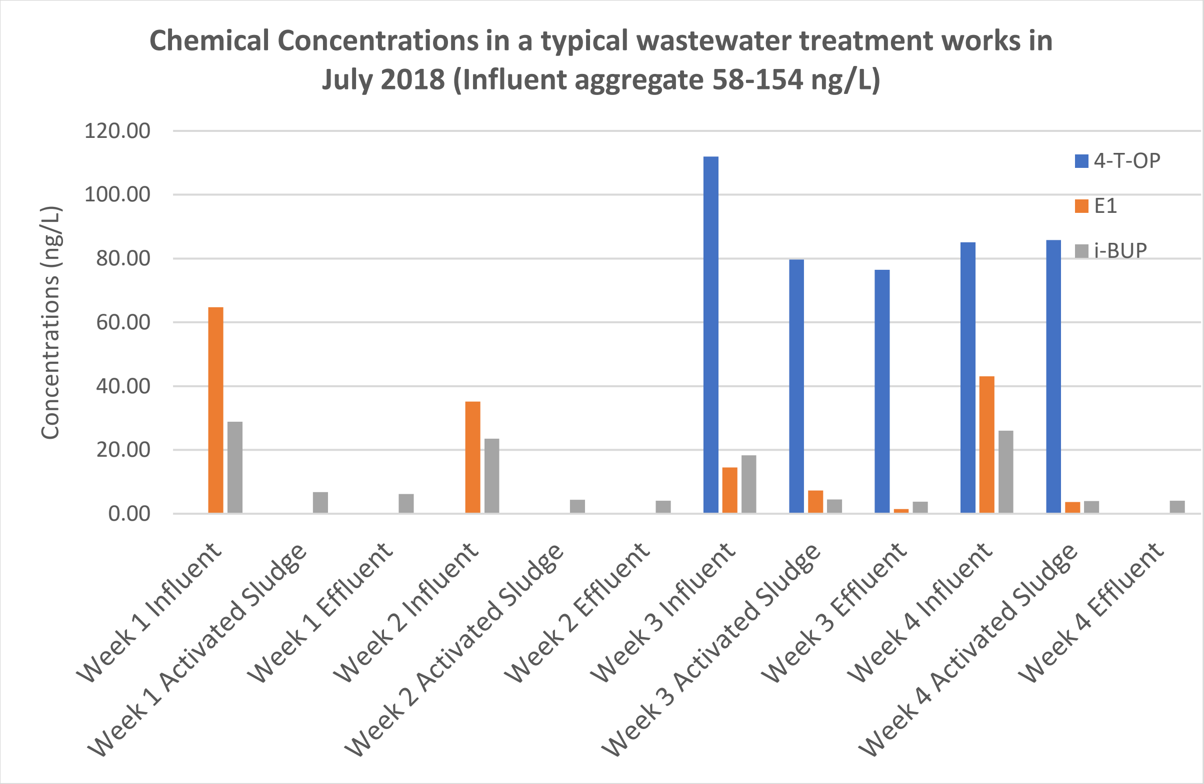

Figure 5d: Chemical concentrations across 4 weeks measured from influents, activated sludge and effluents grouped chemicals concentration

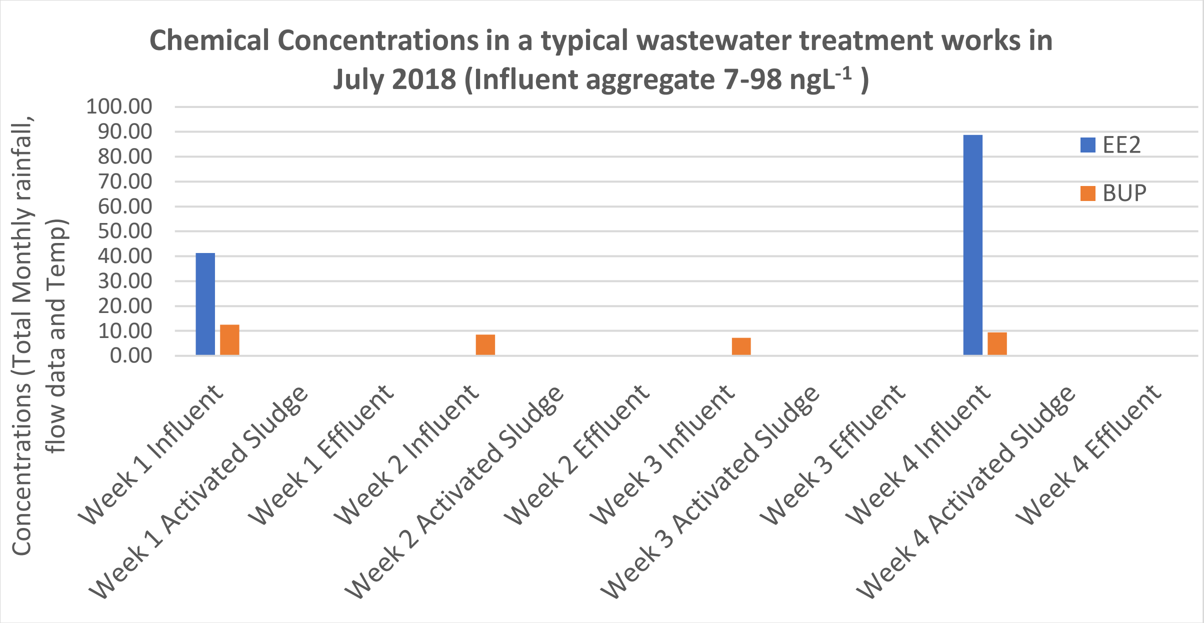

Figure 5e: Chemical concentrations across 4 weeks measured from influents, activated sludge and effluents grouped chemicals concentration

Some of the investigated compounds including BEP, HEP, E2 and TCC were below the detection limits and as such were not reported. Figure 5E shows that chemical concentrations diminish as the wastewater progresses through the treatment process except for TBHQ, OPP, 4-T-OP and E3. The two major processes for pharmaceuticals and PCPs removal is sorption to sludge and biodegradation (Nakada et al., 2007; Onesios, Yu, & Bouwer, 2009; J. Wang & Wang, 2016). Depletion of the chemical compounds from influents through to effluents of wastewater confirms that the Activated Sludge treatment plant is effective in the removal of PCP ingredients. Although there is always an exception to every system which will be discussed further. Sewage sludge is subjected to further treatment to reduce the adherent pathogens before disposal. Disposal through its use for agricultural purposes is becoming increasingly popular amongst farmers (and is a key disposal route for the UK), which now raises questions about the future impact of some of these contaminants on the environment and human food chain via accumulation by root tubers and via the water cycle as some are leached into groundwater. Some contaminants can be transported to surface waters via runoff. Any bioactive metabolites produced because of degradation are reintroduced to the aquatic environment via numerous routes as complex mixtures but primarily through both untreated and treated sewage. Aquatic organisms are often faced with the pollution which affects their lifecycle resulting from continuous exposure. However, there is a possibility of continual accumulation of some of these ingredients over time in the biota since some of these pollutants are persistent (Daughton, Christian G., 1999). Studies have revealed that removal rate of Steroid oestrogens, (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinylestradiol (EE2) in activated sludge treatment systems demonstrated a better removal efficiency of up to 100% compared to 75% in the trickling filter treatment system (Ting & Praveena, 2017).

Removal of steroids like estrogens in the primary treatment unit occurs via sorption onto primary sludge, and then onto micro-flocs which then undergo biodegradation in the secondary treatment unit. Even though the treatment works has been efficient in the removal of steroid estrogens, it was however found not to be efficient in removing TBHQ, OPP, and 4-T-OP. Table 3 suggests that lower concentrations were recorded in the influent compared to the effluent concentrations. This could be because of many factors to include measurement uncertainties resulting from biofouling encapsulating the sampling device. Processing plant downtime was not considered as well as other factors which often dictates the residence time for the wastewater in the treatment works. Removal rates for personal care products ingredients at the wastewater works are evaluated by a mass balance between the influent and effluent concentrations. These compounds had removal rates ranging from 14% TBHQ in week 1 to 100% E3 in the same week.

Table 3: Removal rates of some selected compounds with low concentrations in the sewage sludge but higher concentration at the effluent channels

	TBHQ	**OPP**	**E3**	**4-T-OP**
Week 1	14%	92%	100%	0%
Week 2	69%	68%	100%	0%
Week 3	81%	96%	79%	32%
Week 4	67%	97%	82%	99%

TBHQ: Tertiary butylhydroquinone is a water-soluble synthetic aromatic organic compound. It is used as a preservative in foods production and acts as an antioxidant (Sun & Ho, 2005; Dolatabadi & Kashanian, 2010). **4-T-OP: 4-tert-octylphenol** has been reported to occasionally exceed 1μg/g (dw) in municipal sludge while the detection occurrence varied widely both in sludge and sediments (Remberger, Kaj, Palm, Sternbeck, & Brorström-lundén, 2003). This agrees with the outcome of our experiment where the detected concentration was below 1μg/g (dw) with a varied concentration across the weeks.

OPP: Ortho-phenylphenol is a compound widely used as a fungicide in the fruit packing industry and disinfectant formulations. Experiments have shown that biodegradation of its label compound in the activated sludge was very rapid where 50% of the experimental concentration biodegraded in 24 hrs compared to 1 week in the river water (Gonsior, Bailey, Rhinehart, & Spence, 1984). Following up on the biodegradation of **E3: Estriol** in activated sludge, its low concentration could have been attributable to the length of time in which the samplers were deployed. It can therefore be suggested that these compounds were not prominent in the activated sludge or not present in activated sludge as compared to the effluent water due to biodegradation, and strong affinity to organic matter.

DGT has been considered for long-term monitoring of organic pollutants due to its properties to include TWA chemical accumulations, low costs of production and operations. This sampling tool only samples dissolved phase compounds while the insoluble phase is removed by sorption in the activated sludge. The 28 days deployment at Influent, activated sludge and the effluent highlighted the partitioning of sewage chemicals and the removal potentials which can be interlinked with the 52 weeks sampling campaign. There is high variability in the removal rate of some compounds over the 52 weeks such as HEP having an influent concentration of 0.61 ngL^{-1} to effluent concentration of 31.78 ngL^{-1} and 4-T-0P with influent concentration of 1.86 ngL^{-1} to effluent concentration of 94.81 ngL^{-1}. There was less variability in the study undertaken over the 28 days from 14% to 100%. This tells us that the season of the year could influence the removal performance of the treatment works through the hydraulic surge in wastewater influx, heterogeneity of sources of compounds over time, variation in flow rate, climatic influence on degradation and variation in sampling tool batches over the period. It is important to note that chemical properties also play an important role in their fate and behaviour in the wastewater treatment system. However, various chemicals exhibit different removal rates and as such, discussing the suitability of o-DGT for long-term monitoring would have to be viewed in line with the chemicals of interest. It can therefore be suggested that o-DGT is a durable sampling tool and could be used for long-term monitoring of contaminants in the treatment works while consideration is given to the selection of deployment devices based on the water flow velocity in the various environment.

Conclusion

This study has investigated the use of DGT to monitor environmental organic pollutants over 52 weeks further to increasing concerns about the bioaccumulation of emerging organic contaminants in the environment because of anthropogenic activities. Utilization of personal care products such as cosmetics, as well as preservatives, are practically difficult to control due to their important and variety of uses. However, there are regulations in place to deal with safe use, registration, evaluation, authorization and restrictions of chemicals (REACH) which has been in force since 2007 (Andrew & Wright-Williams, 2018) and the EU cosmetic directive (Varvaresou et al., 2009). Many products contain most of these wide-ranged active ingredients with EU market value of €9,993million annually in 2007 being 15.7% of the total market value of Personal care products in the EU 27 nations (Rossi, Antonia, & Hoffman, 2007). Having considered some of these factors leading to their enrichment in the environment as well as WFD requirements, o-DGT was selected for a long-term monitoring tool of the compounds over 12 months in a treatment works within the North West of England. 21 out of 23 compounds investigated were reported in this study. Accurately determining removal rates is a key factor to be considered in environmental risk assessment of organic contaminants. It is however impossible to accurately measure some of the compound's removals due to their forms which were different from the targeted metabolites investigated leading to a higher concentration at the effluent channels than the influents. This corroborated the outcomes of some other studies where high ecotoxicological concentrations of PCPs were detected from the effluent water some few distance away from the discharge point (Blair, Crago, Hedman, & Klaper, 2013). It was established from the study that chemical present in the influent can be partitioned into sludge (primary or secondary) or be removed by chemical/biological degradation while the unremoved or untreated chemical is discharged through the effluent water into rivers or estuaries(Margot, Rossi, Barry, & Holliger, 2015). But stricter legislation and continuous monitoring would be useful to combat reintroduction of organic contaminants into the environment through the agricultural application of sludge cakes and treated water in irrigation. The selected compounds for the follow-up study involving the activated sludge also showed similar removal result to the long-term monitoring data.

Studies in the treatment work using o-DGT are best conducted using the sampling device such as aluminium cages to avoid the samplers being damaged by organic matter in the wastewater and to also reduce the possibility of the samplers being buried in the organic matter. This study shows some variations in the chemical concentrations over the period confirming heterogeneity of sources of the organic contaminant (Benotti et al., 2009; Xiao, Xie, & Cao, 2015). Overall, o-DGT has proved to be suitable for monitoring the dissolved chemical presence in the wastewater over a long period with some few suggestions. It appears that there is a direct relationship between chemical uptake and other factors such as temperature, rainfall, and wastewater flow data.

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Supplementary Table 1 showing removal rate statistics

	Mean	**Standard Error**	**Median**	**Mode**	**Kurtosis**	**Range**	**Minimum**	**Maximum**	**Conf Level (95.0%)**
MEP	0.92	0.03	0.98	0.96	13.12	1.00	0.00	1.00	0.06
ETP	0.89	0.05	0.99	0.99	14.08	1.77	-0.77	1.00	0.09
PHBA	0.59	0.05	0.55	0.00	0.80	1.80	-0.15	1.65	0.09
PRP	0.57	0.07	0.97	0.00	-1.72	1.43	-0.43	1.00	0.14
i-PRP	0.56	0.06	0.95	0.00	-1.70	1.45	-0.45	1.00	0.13
E3	0.35	0.18	0.74	0.29	27.77	8.49	-7.49	1.00	0.36
BUP	-0.64	1.34	0.86	1.00	50.94	69.70	-68.70	1.00	2.69
BEP	0.04	0.28	0.05	1.00	44.45	14.93	-13.89	1.04	0.57
i-BUP	-0.45	1.21	0.87	0.87	51.53	62.88	-61.88	1.00	2.42
TBHQ	-0.26	0.27	0.25	0.00	27.10	12.81	-11.81	1.00	0.53
TCS	-1.97	0.55	0.00	0.00	0.46	18.17	-7.37	10.79	1.11
BPA	1.10	0.30	0.92	0.92	50.91	16.40	0.00	16.40	0.61
DES	0.09	0.09	0.30	0.30	2.24	2.97	-1.97	1.00	0.18
E1	0.26	0.11	0.63	-0.73	0.78	3.77	-0.75	3.02	0.23
E2	0.53	0.10	1.00	1.00	4.39	3.12	-2.06	1.05	0.20
EE2	0.27	0.07	0.00	0.00	2.87	2.77	-1.77	1.00	0.15
HEP	-1.32	1.00	0.00	0.00	47.26	52.39	-51.39	1.00	2.02
0PP	0.85	0.03	0.84	0.83	13.20	1.97	0.00	1.97	0.07
BHA	0.04	0.11	0.47	-0.92	0.02	3.55	-2.55	1.00	0.23
TCC	-0.21	0.39	0.00	0.00	47.95	20.51	-19.51	1.00	0.77
4-T-0P	-2.00	1.09	-0.03	0.00	29.69	50.97	-49.97	1.00	2.19

6. Paper Four: Fate and Availability of Emerging Contaminants in Sewage Sludge-Amended Soil

Fate and Availability of Emerging Contaminants in Sewage Sludge-Amended Soil

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Abstract

There is a growing concern about the impact of organic contaminants such as PPCPs on the agricultural lands, which increases the dissolved bioavailable contaminants for plant uptake. This study was designed to address this concern and to suggest some likely solutions to the problems. DGT is a passive sampling tool that measured bioavailable chemicals in soil, water and sediments with time-weighted average ability. A study was initially undertaken to understand this concern and it suggested a reduction in MEP, PRP, PHBA, and ETP sorption to DGT from day 14 to 21 due to biodegradation since there was no resupply of these compounds. This led to a further study that examined the sewage slurry for 21 days with and without NaN_3 while same samplers were deployed 24 hours before the retrieval day to examine the available chemicals at the end of each week concluded that a further period more than 21 days might be necessary to further reduced bioavailable PCPs in the sewage cakes amended soils by allowing more time for degradation and desorption. It was revealed that NaN_3 inhibits degradation of PCPs while a 24-hour deployment shows that MEP, ETP, PRP needs longer time more than 14 days for desorption and degradation before land use by the farmers. Sludge slurry prepared in a proportion of 15g of sludge cakes with MQ water in a ratio 3:37 for the latter study confirmed that a high concentration of BPA, PHBA, MEP, NP and BHT out of which 2589 ngL^{-1} was adsorbed to DGT in 7 days while 681 ngL^{-1} was adsorbed on the day 21. However, PHBA increased from 859 ngL^{-1} to 2114 ngL^{-1} over the period while MEP increased from 19 ngL^{-1} to 1497 ngL^{-1} respectively. This study suggests that a minimum of 28 days would be pathogenically saved between the sludge cake application to the farmland and cropping which is suggested to be safer with reduced and low contaminants concentrations.

Introduction

Wastewater treatment plants utilizing the activated wastewater sludge treatment method, have been reported to have up to 85% efficiency for the removal of personal care products (PCPs) (Kasprzyk-Hordern *et al.,* 2009) and pharmaceutical ingredients from wastewater (Grassi *et al.,* 2012; Tran and Gin, 2017). Chemical and biological processes can lead to the degradation or sorption of some of these organic contaminants during the wastewater treatment process (Ifelebuegu & Ezenwa, 2010; Council, 2016), while some are released back into the environment via discharge to surface waters. The contaminant load that sorbs onto sewage sludge solids as a result of their strong affinity to organic matter can subsequently present a threat to agricultural systems via the use of sludge as a fertilizer (Yu and Wu, 2011; Yu *et al.,* 2013). Studies have shown that majority of the sludge applications for agricultural purposes (Petrie *et al.,* 2014) are carried out by the farmers without previous knowledge of the emerging contaminants present in the sludge (Petrie *et al.*, 2014). The absence of adequate studies on the characteristics of sewage sludge used in agriculture has led to poor information on the fate of organic contaminants in soils and their toxicity to terrestrial organisms (Porter, 2002; Stuart *et al.,* 2011; Maier et al., 2014; Petrie et al., 2014).

Constituent chemicals within the PCP group have been generally characterised as 'emerging organic contaminants' (Mailler et al., 2014; Chen, 2016) which are principally intended for human external use (Chen, 2016; Etchepare & van der Hoek, 2015) and are also known as daily household or lifestyle products (Sorensen et al., 2015a). They are found as additives or preservatives in products like fragrances, disinfectants, ultraviolet filters and insect repellents (Petrie et al., 2014; Wang et al., 2014; Sorensen et al., 2015a, 2015b). Once utilised, many of the PCPs are generally released into the wastewater treatment process without undergoing metabolism which leaves a large proportion of their constituents in their original forms within the environment (Carbajo et al., 2014; Arlos *et al.,* 2015; Vanraes et al., 2015). However, a proportion can bioaccumulate in the body after use and can either be excreted in their original form or as metabolites in urine (Barrett, 2005; Tolls et al., 2009). Studies have shown that a significant amount of these compounds are produced and consumed annually (Eriksson, Auffarth, Eilersen, Henze, & Ledin, 2003; Lee, 2010; Sarpila & Räsänen, 2011), with little or no data on production and consumption patterns. Pal *et al*, (2014) other researchers have shown that the number of emerging contaminants being discharged into the environment as a result of anthropogenic activities is increasing which reflects the increasingly wide range of consumer products to include cosmetics and personal care products. The chemical ingredients for the cosmetic formulations is in several thousand, with annual production in thousands of tonnes (Juliano & Magrini, 2017).

Some of the PCP constituents such as parabens are used daily in skincare and beauty products to increase the shelf-life of these products (Crinnion, 2010; Ma et al., 2013; Ocaña-González, Villar-Navarro, Ramos-Payán, Fernández-Torres, & Bello-López, 2015). They are also used as anti-bacterial ingredients in cosmetics even though some constituents have been found to mimic oestrogen (Oishi, 2001; Golden *et al.,* 2005; Liao, Liu and Kannan, 2013) and others have been linked to skin cancer, breast cancer, reproductive problems and hormonal imbalance. Some synthetic antioxidants to include BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), are used in food production and as preservatives in moisturizers and lipsticks (Darbre et al., 2004; Ye, Bishop, Reidy, Needham, & Calafat, 2006). The International Agency for Research for Cancer has classified some of these compounds as carcinogens and mutagens (Soni *et al.*, 2002; Konduracka *et al.,* 2014; Roeder, 2014) such as butylated hydroxytoluene, and diethylstilboestrol. Considering the persistence and uncontrolled use of some of these chemicals, any risks to the environment must be identified and quantified.

It has also been suggested in other studies that temperature and sludge retention time (SRT) do not influence the removal rate of PCPs (Carballa, Omil, Ternes, & Lema, 2007) during the treatment process. The use of sewage sludge by the farmers as a fertilizer (Xu *et al.,* 2009; Yang *et al.*, 2011; Verlicchi and Zambello, 2015), which is currently not regulated for organic contaminants (Jones-Lepp & Stevens, 2007) is subject to application guidelines to ensure that risks from pathogens and heavy metals are minimised. The use of sludge cakes on agricultural farms as fertilizers and soil enhancers is regulated by the amended Sludge use in Agriculture Regulations 1989. Sewage sludge is to be reused for certain crops and vegetation types to avoid ingestion of pathogens which could be harmful to human health such as infested salad crops and vegetables. This EU regulations also dictate that sewage sludge is not spread directly onto grassland, not to be over-applied by the farmers, while the sludge and the soil are to be monitored for a range of metals (Blöc & European Commission, 2005; DEFRA, 2012).

Aims and Objectives

The agricultural land application of sewage sludge cakes is gaining increasing acceptance as the most environmentally and economically favourable disposal option as agricultural fertilizer or soil enhancer. This study was designed to examine the fate, partitioning, and degradation of selected Personal Care Products ingredients in sewage sludge-amended soil matrix. It is expected to give an insight into the reasonable days to be allowed by the farmers between sludge cake application and cropping to reduce crops infestation by the pathogens and as well protect the farmland operators. It is envisioned that the study would contribute to the knowledge bank for policy formation to ensure that the risks of using sewage sludge on agricultural soils are reduced or avoided for organic contaminants. It is also expected to give guidance to all the stakeholders to understand the risks and controls associated with the sludge cakes utilization process.

Description of Site

Lancaster Wastewater Treatment Works (WWTW) is situated in an area of open countryside off Old Aldcliffe Lane, approximately 2.5km south of Lancaster City Centre and 250m to the North-West of Stodday. This site is surrounded by agricultural lands except on the west which has Special Protection Area (SPA), River Lune Site of Special Scientific Interest (SSSI), and Morecambe Bay Special Area of Conservation (SAC). The treated sludge cakes have a water holding capacity of 23.25%. The average annual raw sludge production per year stands at 3,603 tonnes which gives a high economic opportunity available in the area while the average dry solid sludge production at the plants stands at 4.49%. This treatment plant serves over 100,000 people living in Lancaster, Heysham and the waste from Windermere and Kendal in Cumbria

Sewage Sludge

Sewage sludge or biosolids is the semi-solid material produced as a sewage treatment by-product of domestic or industrial wastewater. Sewage or wastewater are received at the influent channel of the treatment works to the primary settling tank. Almost 50% of the suspended organic solid matter or raw sludge settle within an hour before anaerobic processes become active and the sludge is removed from the sedimentation tank to avoid putrescence. Figure 1 below, is a simple general diagrammatic representation of the process leading to sludge treatment.

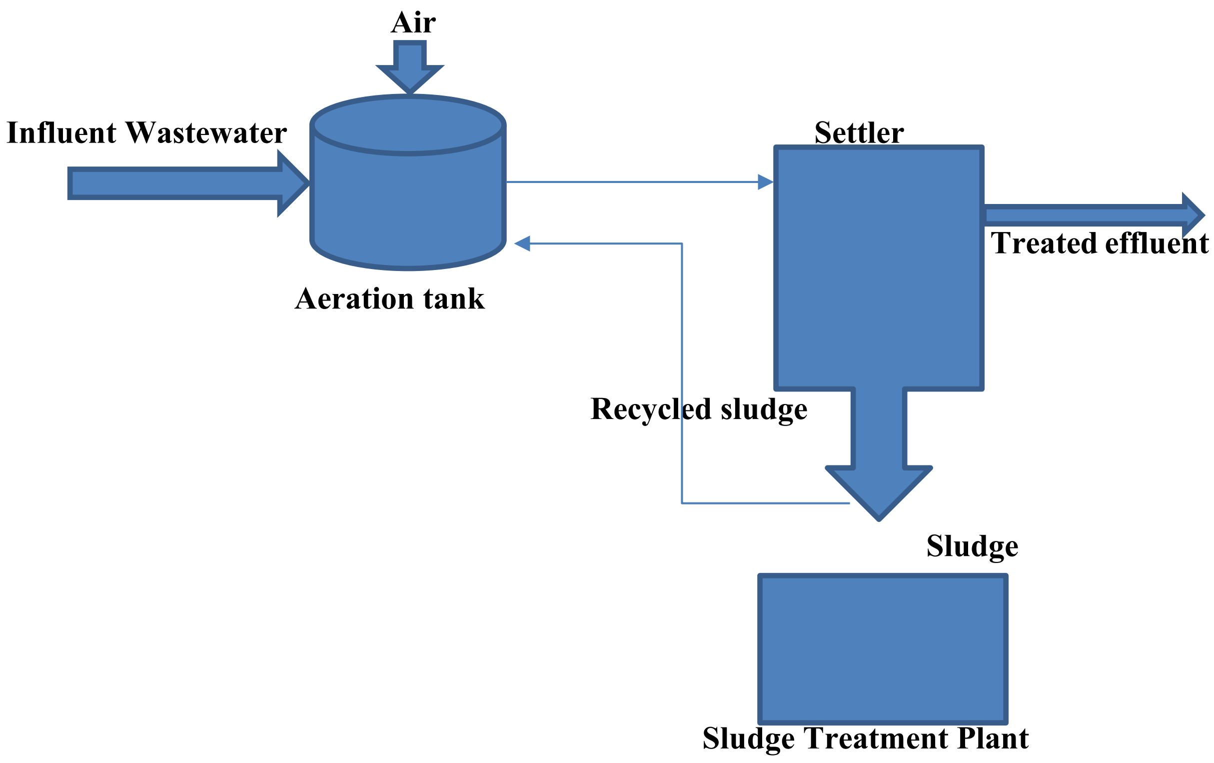

Figure 1: A simple schematic wastewater treatment process

Wastewater Treatment Process

Wastewater contains a complex mixture of chemicals from the use of various personal care products, pharmaceuticals, and endocrine-disrupting substances. These substances represent potential risks to human health and aquatic organisms. Exposure to antibiotics released into surface water environments has contributed to the widespread occurrence of resistance genes (Heath, Kosjek, Cuderman, & Kompare, 2006) which could make most of the medical interventions riskier if various control measures are not enforced. Wastewater can be made reusable by using the appropriate treatment process to remove the contaminants which include physical wastewater treatment, chemical wastewater treatment, biological wastewater treatment and sludge wastewater treatment.

The physical aspects of wastewater treatment include sedimentation, aeration, and filtration. The most used filtration medium is a sand filter, which is used to remove grease and other non-soluble organic materials from the wastewater. However, the biological process which can be categorised into aerobic, anaerobic, and composting are useful in the reduction of organic matter from wastewater. Aerobic processes involve decomposition by aerobic bacterial decomposing organic wastes such as human waste, food waste etc using oxygen while the anaerobic process such as fermentation process waste without the use of oxygen at elevated temperatures. Although this process has proven effective in the removal of organic solids, dissolved nutrients such as phosphorus and nitrogen, may not be removed.

Chemical treatments are also important wastewater treatment approach that involves the use of chemicals to treat and purify the water for secondary use. These methods include the use of chlorine to reduce bacterial load and ozone as an oxidising agent. Neutralization is also important to achieve effluent water at pH 7. Such water can be used for agricultural purposes or further treated for domestic use. The sludge treatment process deals with the treatment and disposal of sewage sludge. Surface waters that receive wastewater effluent discharge have been shown to contain a wide range of PCPs and pharmaceutical contaminants (e.g. Grabicova et al., 2015). However, many partitions to the sludge or biosolids and will be present in the environment due to the agricultural application to enrich the soil nutrients. The commonly used PCP ingredients present in sewage sludge have been investigated which has provided some evidence of toxicity (Carbajo et al., 2014; Etchepare & van der Hoek, 2015) suggesting an element of risk associated with using sewage sludge for agricultural purposes. It is therefore important to understand the scale of associated risks posed to the living organisms in the receiving water bodies as well as herbivores and humans through the consumption of plants and vegetables.

Sewage sludge produced by the wastewater treatment process is a combination of a primary sludge produced by initial sedimentation and a secondary biological sludge produced after the biological process. The resulting sludge contains a wide range of pathogenic bacteria, viruses, and protozoa along with a complex chemical loading. Wastewater treatment plants use a wide range of treatment processes to stabilize the sludge (i.e. to reduce pathogenic content) which include mesophilic anaerobic digestion, thermophilic aerobic digestion, pasteurization, and composting. The resultant product that is intended for agricultural use is usually dewatered to make a sludge cake. The drive for sludge utilization for agriculture purposes is its nutrient content including nitrogen, phosphorus and the presence of organic matter which improves soil structure and water retention. Figure 2 below shows the activated sludge section of treatment works where the sludge undergoes aeration as part of the processes to treat the pathogens.

Figure 2: A section of Wastewater activated sludge plant.

There is a range of disposal and reuse methods for sludges such as incineration and agricultural fertilizers, but these are subject to regulation and risk assessment whilst costs are also a consideration. Disposal at sea was banned in 1998 across the EU which inadvertently increased the quantity that was being incinerated but considering the high level of costs of incineration, agricultural use seems to be the most viable option today and this thereby places the burden upon the utility companies and the farmers to ensure safety and that the pathogens were well treated before applying the sludge. Table 1 suggests agricultural use of sewage sludge as the most economical and viable disposal option, but considerations need to be given to the health hazard (Fytili & Zabaniotou, 2008; Kelessidis & Stasinakis, 2012; Martín, Camacho-Muñoz, Santos, Aparicio, & Alonso, 2012; Werther & Ogada, 1999). There are some restrictions in the UK and France which are stricter than in other countries. Some of these restrictions centre around the number of heavy metals, reductions of pathogens and organic substances, dry soils spread per unit land at every point in time, additional nutrients, Nitrogen and Phosphorus added to the soil, choice of crops, and restriction on the access to the farmland where the sludge has been applied (Bresters *et al.*, 2007). Sewage sludge output was quantified to be 1.4million tonnes dry solid in the year 2010 which is an increment of 0.4million from 1992.

There has been increasing disposal of sewage sludge through reuse for agricultural purposes from 47% in 1992 to 81% in the year 2010. Agricultural use of sludge is cost-efficient over incineration with the added benefit of adding valuable nutrients and organic matter to soils[6]. However, it is important to control the environmental impact over successive applications and impacts on living organisms. Figure 3 provides an idea of stakeholders' positions on this matter where the landowners, retail companies and local organisations are resisting the use of sludge for agriculture purposes. Sewage sludge production, treatment, disposal, and recycling involves some important stakeholders which can be categorised into a farming community, industry, water and waste industry, local authorities, national authorities, and the citizens. These are shown in the chart below;

Table 1: Sludge disposal in the United Kingdom by various routes ((Department for Environment Food and Rural Affairs, 2012)**)**

	1992	**2008**	**2010**	**Cost to consumers**	**Environmental sustainability**
Applying to land/other reuse	47%	87%	81%	medium	high
Incineration	9%	12%	18%	high	medium
landfill	13%	1%	1%	medium	high
Sea/Others	2%	0%	0%	n/a	n/a

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[6] https://www.lancaster.ac.uk/lec/sites/cswm/seal/sewage.html

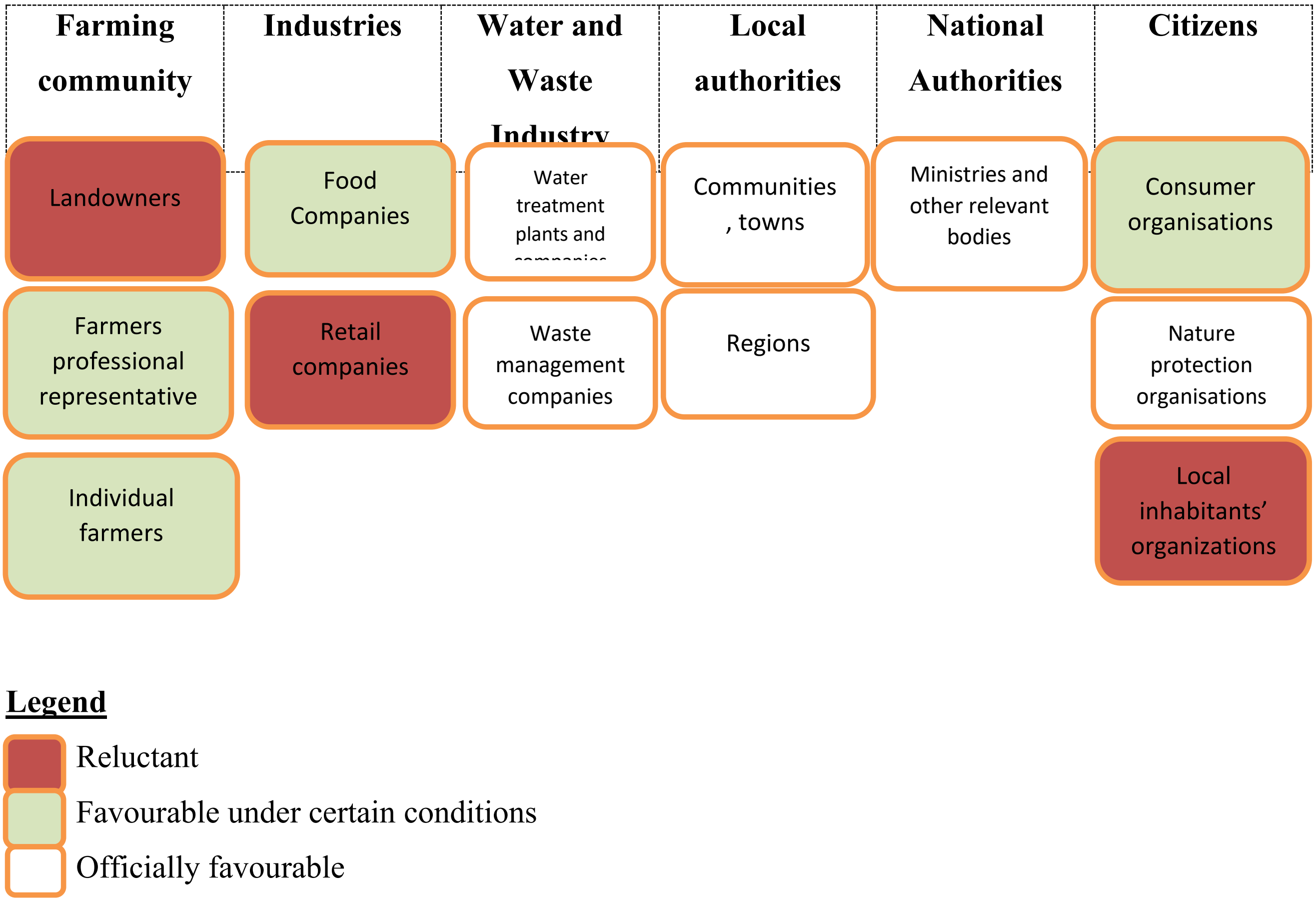


Figure 3: Stakeholders' position on the use of sewage sludge in Agriculture (European Commission, 2001)

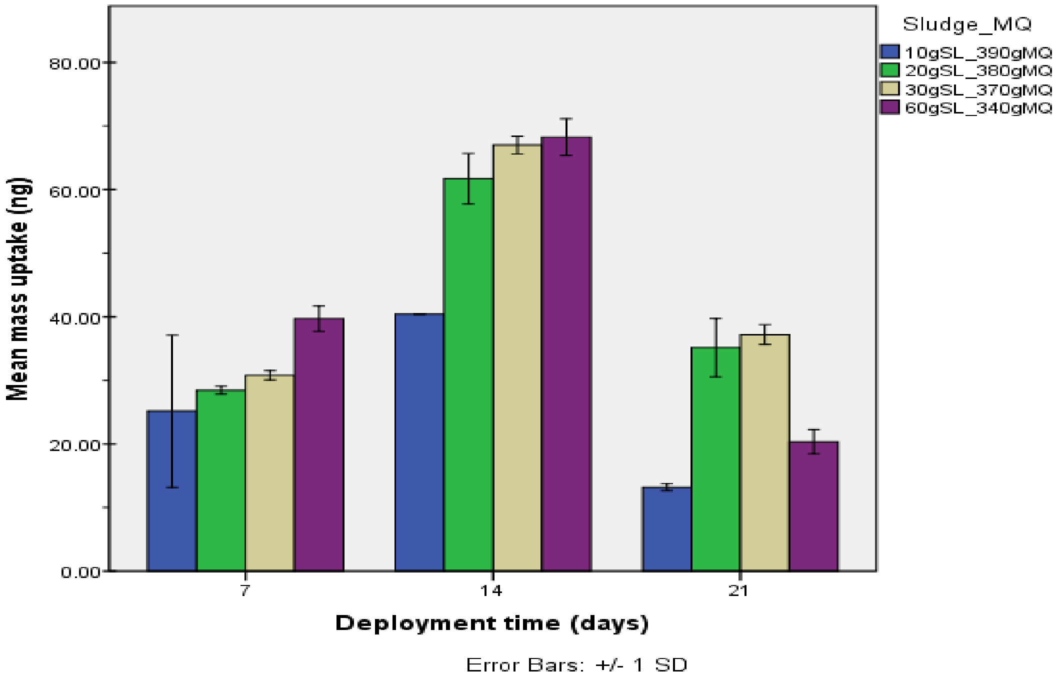
Selected Personal Care Product Ingredients

This study focused on a range of ingredients that are common in personal care products. This study focused on their fate (partitioning and bioavailability) in dewatered sewage sludge. 23 Personal Care Products ingredients were investigated out of which 18 compounds shall be discussed during this study due to their properties and their consistent presence in sludge. The reportable compounds are Methylparaben MEP, Ethylparaben ETP, 4-Hydroxybenzoic acid PHBA, Propylparaben PRP, Iso- Propylparaben i-PRP, Estriol E3, Butylparaben BUP, Benzylparaben BEP, iso- Butylparaben i-BUP, Tertiary butylhydroquinone TBHQ, Bisphenol-A BPA, Estrone E1, β-estradiol E2, 17α-Ethinylestradiol EE2, Heptyl paraben HEP, Ortho-phenylphenol OPP, Triclocarban TCC, and 4-tert-octylphenol 4-T-OP while Triclosan TCS, Diethylstilboestrol DES, Butylated hydroxyanisole BHA, Nonylphenol NP and Butylated hydroxytoluene BHT would not be discussed as there weren't clear patterns observed across the compounds or the system.

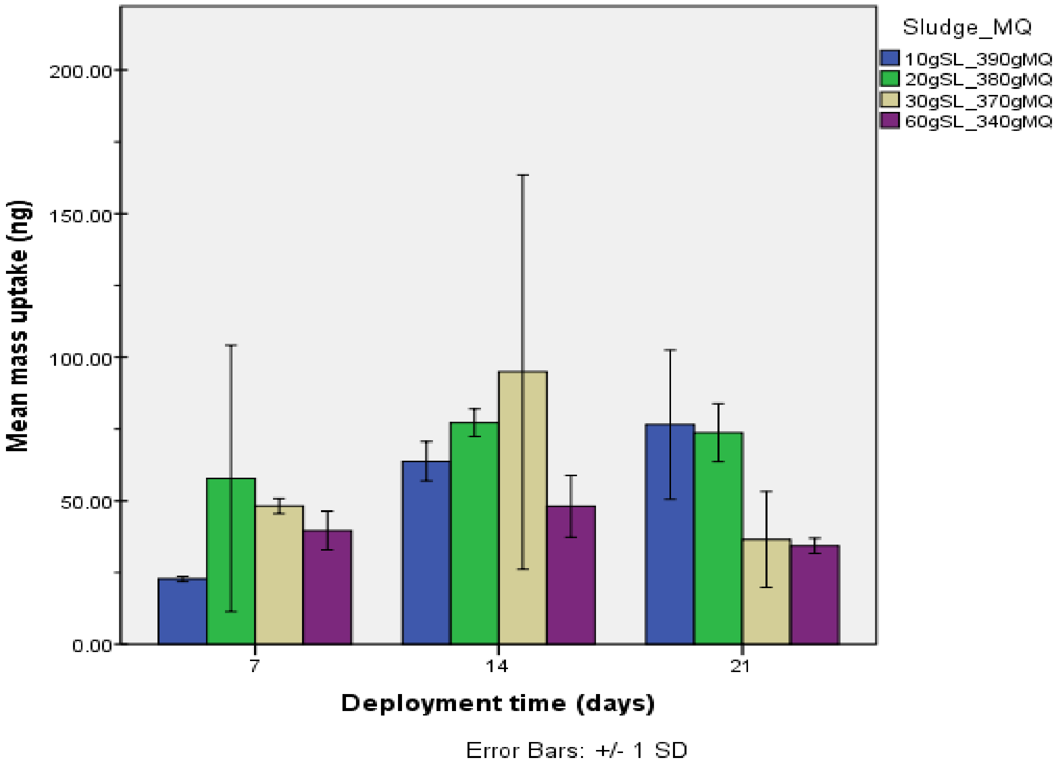
Experimental Study

Studies have shown that a wide range of PCPs can be detected within sludge (Xu et al., 2009; Yang et al., 2011; Verlicchi & Zambello, 2015). The initial experiment investigated the use of DGT samplers to determine the sorption/desorption and degradation of PCPs in the sludge/water system. DGTs were deployed in a sludge cake slurry and sampled after 7 days, 14 days and 21 days of contact time. The following compounds Methylparaben (MEP), Ethylparaben (ETP), 4-hydroxybenzoic acid (PHBA) and Propylparaben (PRP) were investigated. The study also investigated the use of NaN_3 to inhibit microbial activity and hence degradation. Sludge to water ratios was varied from 1/39 to 3/17 with a replicate of one of the experiments spiked with sodium azide to inhibit microbial activity. The preliminary study suggested that PCP ingredients are readily available in a soluble state for sorption into the binding gel. There was a noticeable reduction in mass uptake between 14 and 21 days for some compounds which could be attributable to degradation of the target analytes or desorption rate from the sludge and its mobility (Menezes-Blackburn et al., 2016). With these results in mind suggesting that bioavailable fraction of PCPs reduce overtime or degrade over the period suggest that PCPs might not pose a risk to the on-going use of sewage sludge for agricultural purposes. However, these preliminary results required a further investigation which led to this repeat study. The mass uptake for the compounds are shown in Figure 4a-d below;

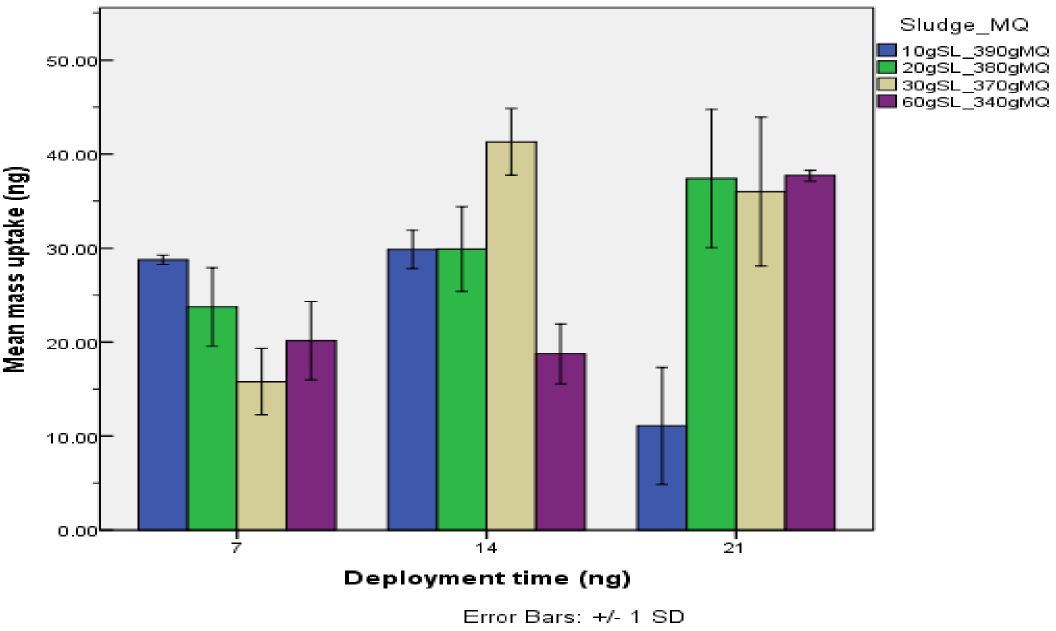
Mass uptake for MEP (ng) by o-DGT



Mass uptake for ETP (ng) by o-DGT



Mass uptake for PHBA (ng) by o-DGT



Mass uptake for PRP (ng) by o-DGT

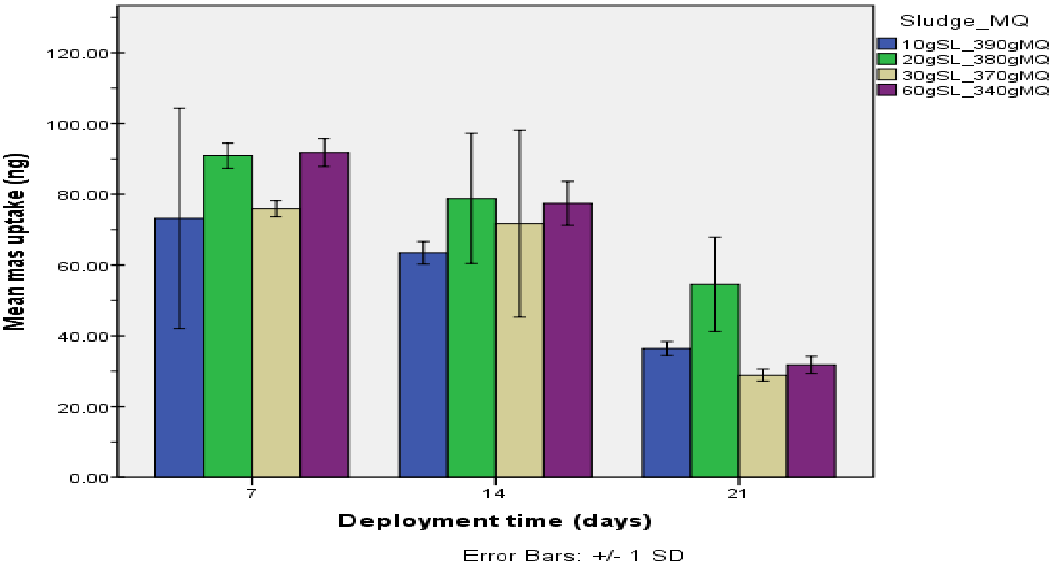


Figure 4(a-d): Mass uptake for MEP, ETP, PHBA, PRP (ng) by o-DGT at various sludge ratios; 10g Sludge: 390g MilliQ water, 20gSl:380gMQ, 30gSl:370gMQ, 60gSl:340gMQ, measured on 7th, 14th and 21st days.

Study Design

Sewage sludge cake was collected from LWWTW and prepared to form a slurry, with sludge to water ratio of 3:37 based on the water holding capacity earlier calculated for the preliminary experiment. 15g of sludge was used for each experimental design while 12 o-DGT samplers were deployed in each sampling pot and retrieved on the 7^{th}, 14^{th} and 21^{st} day in duplicates respectively. A control experiment was spiked with 0.03%v Sodium Azide NaN_3, mixed thoroughly to inhibit bacterial growth. This was aimed at controlling or reducing the biodegradation of the target compounds. A little paste of the slurry was put on the o-DGT open surface, before being concealed in the paste.

Upon removal, the DGT binding Gels were removed while the analytes of interest were extracted using organic solvent Acetonitrile before preparing the samples for Liquid Chromatography-Mass spectrometry (LCMS) instrumental analysis. As an extension to this, another set of experiments were run simultaneously to quantify chemical uptake by the o-DGT over 24 hours rather than allowing accumulation over 7, 14 and 21 days. o-DGTs were deployed in quadruplicate for 24 hours on the 6th, 13th and 20th days for retrieval on the 7th, 14th and 21st days respectively. It was expected that this approach would give us concentration that is available at the time rather than 7days /14days/21days time-weighted average concentration.

Materials and Methodology

Diffusive Gradients in Thin-film (DGT) Preparation

DGTs has been used for this study because of its characteristics which includes Time Weighted Average sampling, size, costs of production and sorption. They were prepared at the Lancaster University Laboratory using HLB resins for the 0.56mm binding gel, agarose powder to prepare 0.8mm diffusive gel while 0.45um, 25mm diameter GH Polypro (GHP) membrane filters were purchased from Pall Corporation, USA and the DGT mouldings from the DGT Laboratory. Diffusive gels were prepared by dissolving 0.9g agarose powder in 60 ml boiling Milli Q water to give a 1.5% agarose solution. HLB Hydrophilic-lipophilic balance binding and the diffusive gels were prepared following the standardized DGT preparatory procedure.

Sludge and MWHC

Data provided by the WWTP confirmed that the average percentage of dry solids of the sludge cake was (dried at 105^0C) was 23.3% with a pH of 7.90. Maximum Water Holding capacity was determined to indicate the sludge to water ratio to be used in the laboratory experiment. To determine the MWHC, the following method was used;

10g of oven-dried sludge at 105^0C was added into a filter (Whatman 54 hardened 110mm diameter) that has been placed in a plastic funnel. This funnel was soaked in a beaker for 2 hours using deionised water. The filter with the funnel was removed and placed in the cylinder for it to dry under the fume's cupboard for another 2hours. The wet sludge was weighed by weighing the filter paper containing the sludge while 3.5g weight was assumed for the filter paper. Maximum Water Holding capacity was then calculated using the simple equation.

$$\mathrm{MWHC} = \frac{\text{Mass of Wet Sludge} - \text{Mass of Dry Sludge}}{\text{Mass of Dry Sludge}} \qquad (1)$$

The above was repeated for 3hrs and the average MWHC was found to be 132%.

Deployments and Sampling

Laboratory Deployment

All three experiments were carried out in the laboratory at 20°C. Experiment A consisted of a mixture of sludge and milli-Q water at a ratio of 60:740. This sludge and water were mixed and left for 1 hour before DGT deployment. 12 o-DGTs were deployed and these were removed in multiples of four on days 7, 14 and 21 days respectively. A parallel system B was set up at the same time but with 240µL of Sodium Azide NaN_3 to inhibit bacterial growth and degradation over the period. 12 o-DGT samples were also deployed in this system. The third system C was set up with only sludge and milli-Q water and initially without DGT. Four DGTs were added only for 24 hours before retrieval of other samplers on the 6th, 13th and 20th days while they were retrieved at the same time with the other samplers. The essence of this system was to understand uptake over 24 hours compared continuous sampling over 7 days, i.e. to provide a spot sampling equivalent.

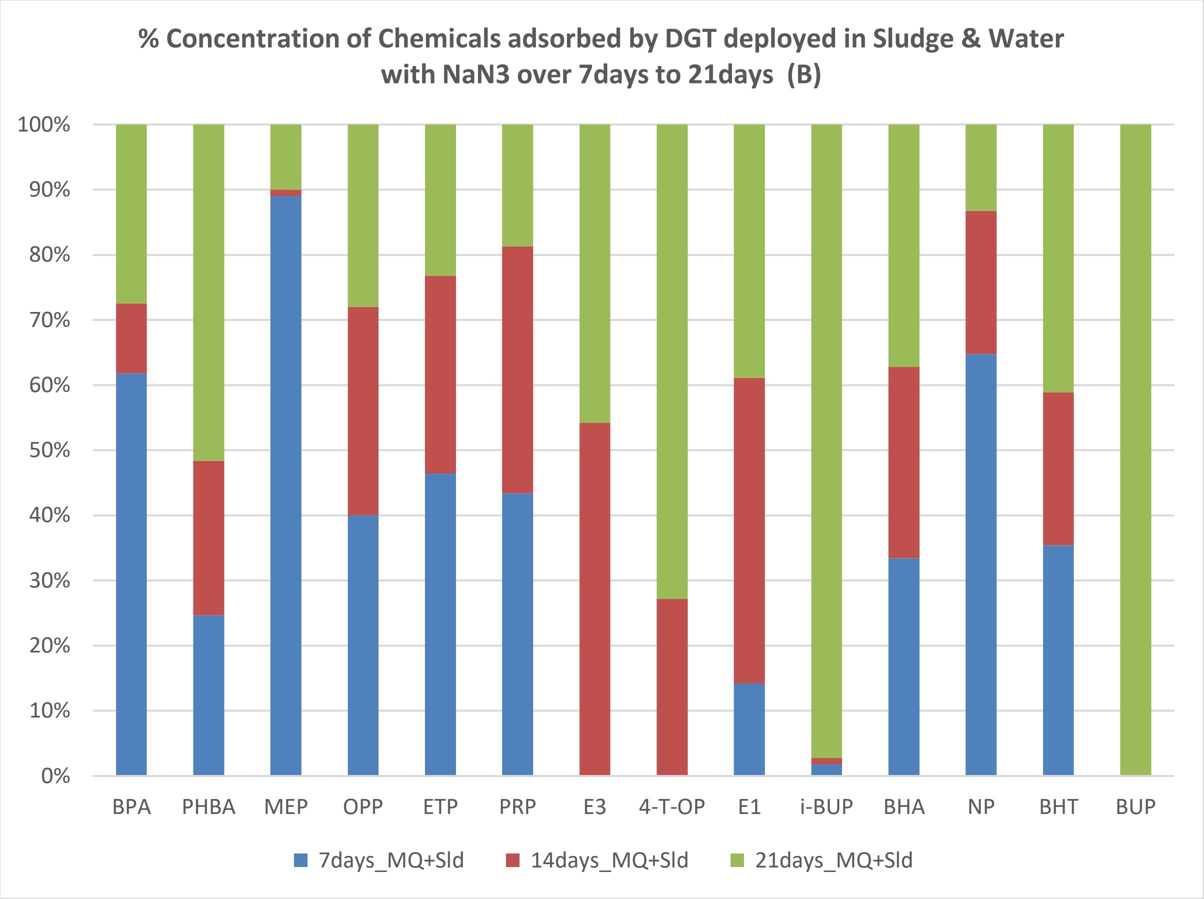
DGT Extraction Process

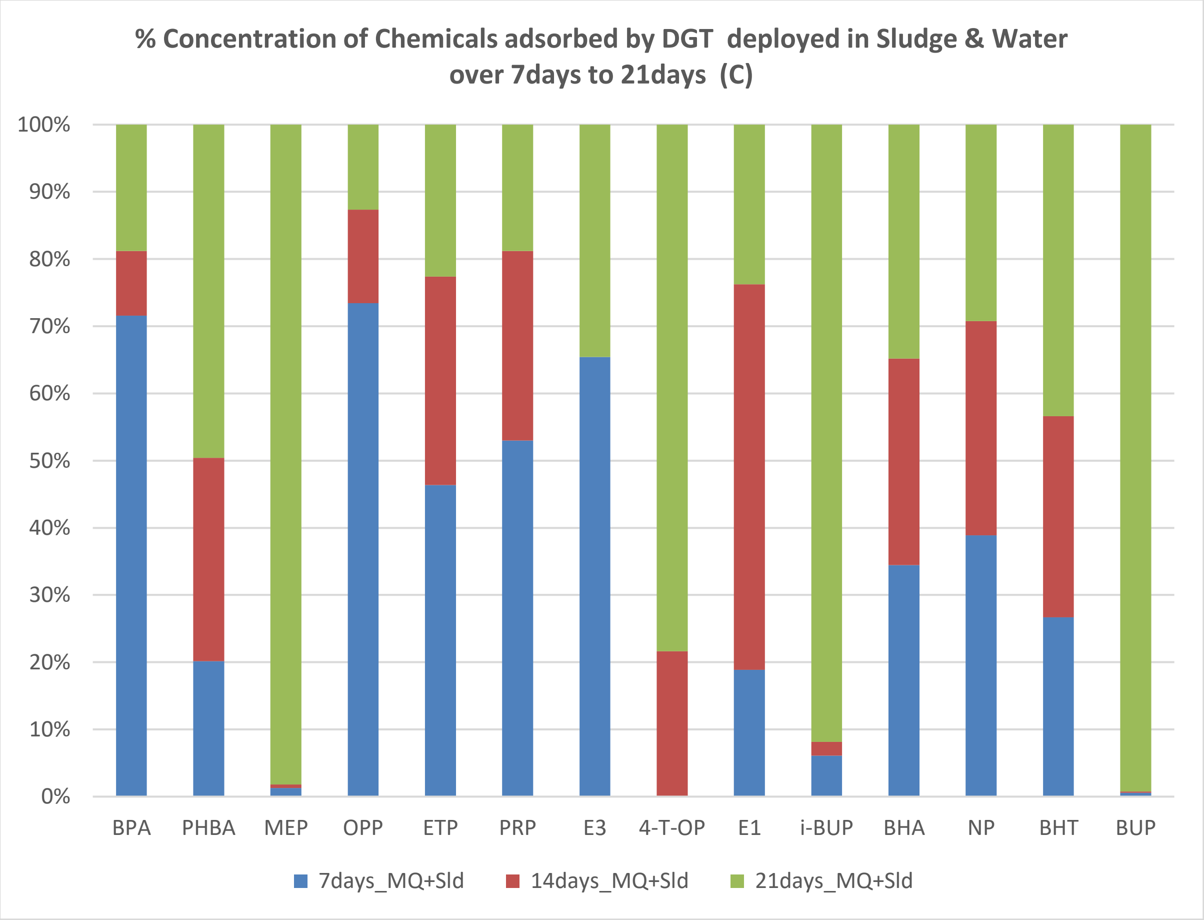
A standard DGT extraction and preparation process for analysis by LCMS was followed for all DGT samples. The resin gels were transferred into 15ml clear amber vials, 50µL of mixed internal standards added to each of the vials, while 5ml of organic solvent, Acetonitrile (ACN) was added. The solvent was decanted after centrifuging for 30 minutes. This process was repeated without the IS with 3ml of ACN and finally rinsed with 2ml Acetonitrile organic solvent. 10ml extract was dried under the nitrogen and reconstruct using 1ml Acetonitrile for storage and preservation. The 200µL aliquot of the extract was prepared for LCMS analysis with ACN in the ratio 80/20 for MQ_{sol}/ACN. The extraction and mobile phase for the LCMS analysis was Acetonitrile organic solvent but with 5mM NH_3OH for as a buffer during the analysis.

Results and Discussion

This laboratory study using sewage sludge cake with or without sodium Azide (NaN_3) was intended to provide insight into understanding the role of bioavailability and possible biodegradation during the treatment process but also after sludge use as an agricultural fertilizer.

Due to a range of affinities between the study chemicals and organic matter (i.e. range of solubilities in water), some of the compounds were detected within the first 7 days while some were not until the last week of the study. Figure 5 indicates the percentage of chemicals adsorbed into the o-DGT with the deployment time. From the experiment A where o-DGT were deployed for only 24 hours, only a few compounds such as BPA, PHBA, BHT, OPP and NP were detected in a visible percentage within the first 7 days while the majority of compounds did not dissociate from the sludge matrix until the 15th-20th day of the deployment. However, results from experiment B & C show that some of the compounds might have degraded rapidly and as such, impact negatively on their detection over the 24 hours DGT deployment period. There is also the possibility of o-DGT's inability to uptake a reportable concentration over 24 hours.


% Concentration of Chemicals adsorbed by DGT deployed in Sludge & Water with NaN3 over 7days to 21days (B)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
BPA
PHBA
MEP
OPP
ETP
PRP
E3
4-T-OP
E1
i-BUP
BHA
NP
BHT
BUP
7days_MQ+Sld
14days_MQ+Sld
21days_MQ+Sld


% Concentration of Chemicals adsorbed by DGT deployed in Sludge & Water over 7days to 21days (C)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
BPA
PHBA
MEP
OPP
ETP
PRP
E3
4-T-OP
E1
i-BUP
BHA
NP
BHT
BUP
7days_MQ+Sld
14days_MQ+Sld
21days_MQ+Sld

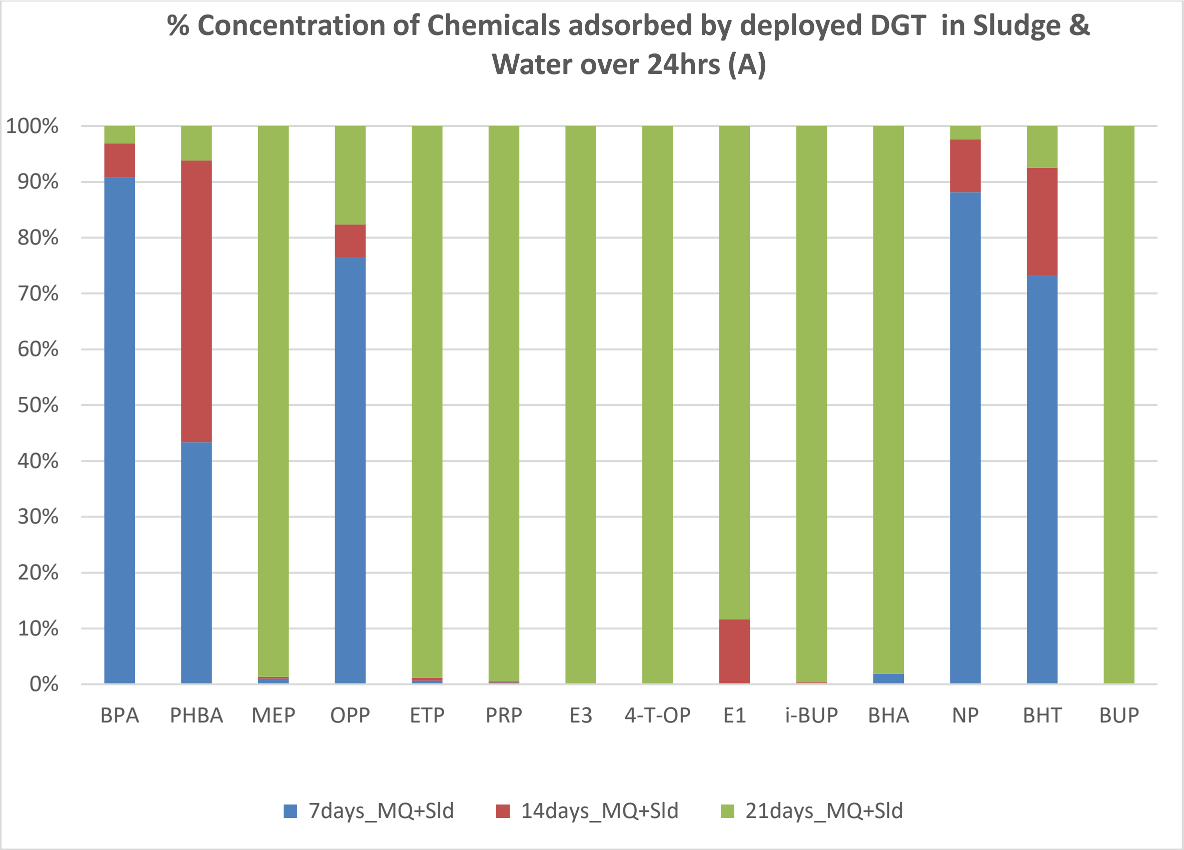


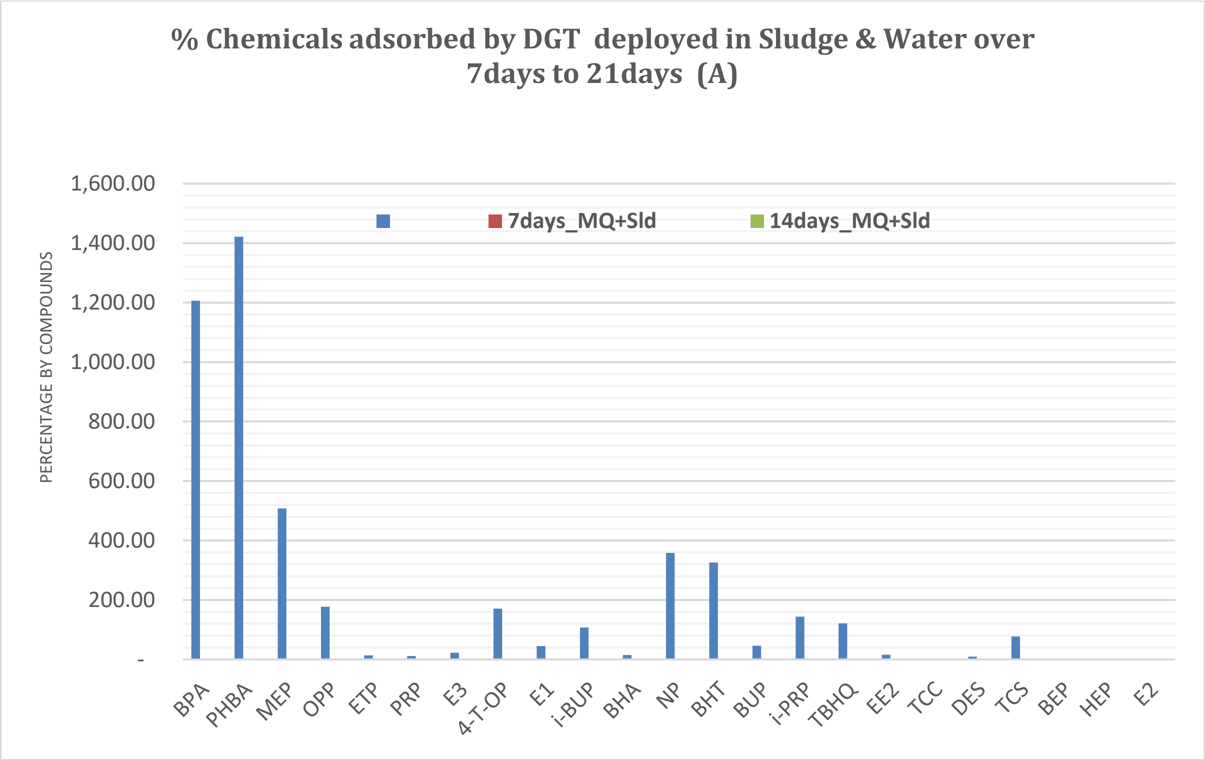
Figure 5: % Concentrations of chemicals in sludge cakes that are used by the farmer to treat the soil under various conditions. (A) All the DGTs were deployed on day 0 into 3:37 sludge/water ratios of 15g sludge cake (B)All the DGTs were deployed in a controlled medium with 0.03%v Sodium Azide NaN_3 to inhibit bacteria growth on Day 0 and retrieved on days 7, 14 & 21 (C) The DGTs were deployed for 24hours uptakes on the 6th, 13th and 20th day respectively.

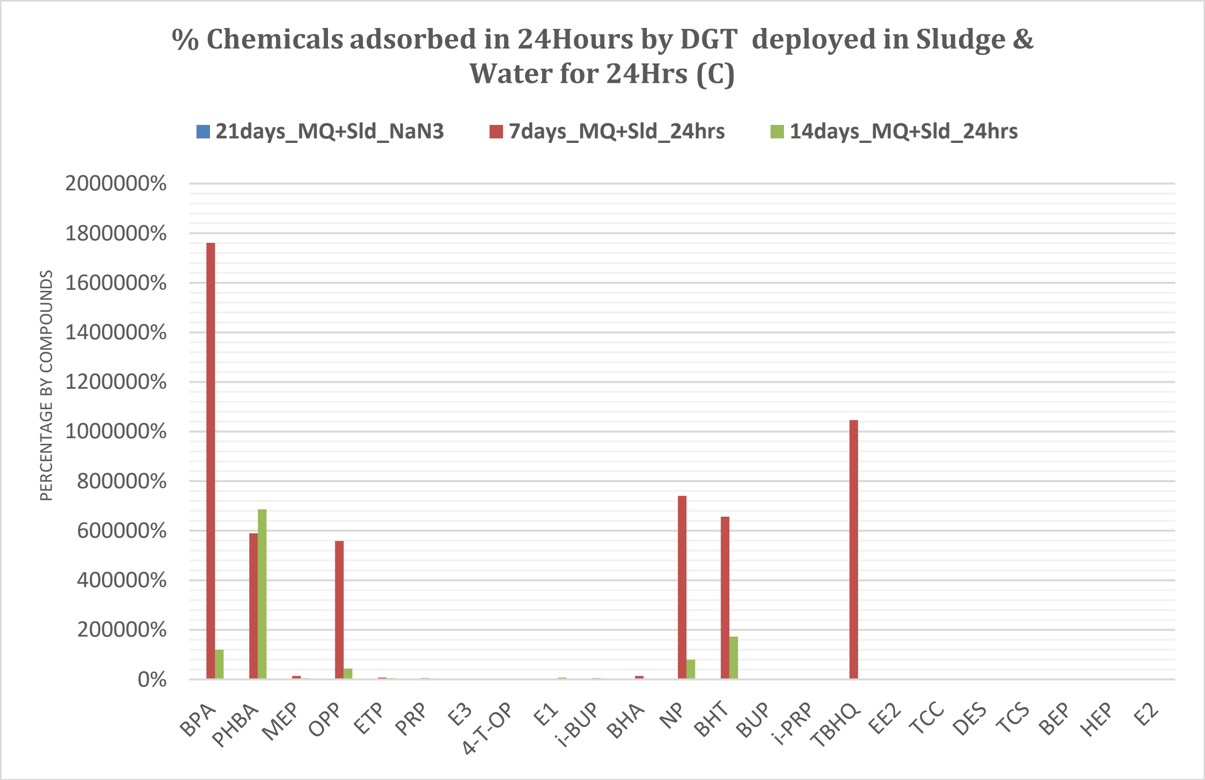
23 compounds were initially investigated out of which 8 were not been reported. These compounds TBHQ, EE2, BEP, HEP, E2, TCC, DES, and TCS were below detection limits. The chemical sorption to the DGT in the laboratory experiment is shown below across the spectrum of the sampled compounds over a 21-day period where the DGTs were deployed for 21 days and retrieved in batches on the 7th, 14th and 21st day. The mixture was in the 3:37 sludge/water ratios of 15g sludge cakes that have been prepared ready for agricultural use by the local farmers. DGT in the sludge slurry measures dissolved bioavailable compounds that is available for plant uptake. There is an expected depletion of concentration throughout the uptake period which would lead to chemical desorption from the sludge slurry. The idea is to

gain understanding into the behaviour of this chemicals post application for agricultural use and to have an idea of how long it could take for the amended soil to have substantially depleted the chemicals for safe land use by the farmers. However, some of these compounds could end up being transported into the rivers if not degradable.

Figure 6A shows 51% of the total BPA was adsorbed by DGT within 7 days of the deployment while 50% of PHBA was adsorbed over 14 days. This informed that desorption of BPA with log Kow 3.64 was rapid within the early days of the sampling than the PHBA with log Kow 1.39. However, reduction in the concentration of BPA over the remaining 14 days could have been as a result of depletion or degradation of the desorbed concentration from the sludge slurry. The results suggest that microbially mediated degradation was inhibited by the sodium azide during the experiment, which could also explain the low concentrations of some of these compounds detected in the activated sludge could have been a result of degradation during the treatment process. During the 24 hours deployment of DGT into the sludge, it was clear that sorption was chemical-dependent as compounds like BPA, NP, BHT, OPP tend to dissociate quickly while other compounds like E3, PRP, ETP, 4-T-OP, i-BUP, BUP did not dissociate until after 14 days. This is an indication of how the compounds would be dissociated from the sludge matrix once applied to the soil. Although some variation would be expected in the real-time environment due to temperature variations and various microbes in the soil that would aid biodegradation.

Figure 6C shows that most of the compounds were detected during the 24hr sampling on the 21st day of the sampling campaign which could be as a result of biodegradation or desorption from the sludge slurry. This could imply that a long time, might be needed for some of the compounds to fully desorbed from the cake before they could be used by the farmers after applying to their land. It also tells us that these compounds could have a strong affinity to organic matter while chemicals with least affinity to the organic matter will be desorbed early leading to degradation before the crops and plants begin to take up the bioavailable compounds.


% Chemicals adsorbed by DGT deployed in Sludge & Water over 7days to 21days (A)
7days_MQ+Sld
14days_MQ+Sld
PERCENTAGE BY COMPOUNDS
1,600.00
1,400.00
1,200.00
1,000.00
800.00
600.00
400.00
200.00
-
BPA
PHBA
MEP
OPP
ETP
PRP
E3
4-T-OP
E1
i-BUP
BHA
NP
BHT
BUP
i-PRP
TBHQ
EE2
TCC
DES
TCS
BEP
HEP
E2


% Chemicals adsorbed in 24Hours by DGT deployed in Sludge & Water for 24Hrs (C)
21days_MQ+Sld_NaN3
7days_MQ+Sld_24hrs
14days_MQ+Sld_24hrs
PERCENTAGE BY COMPOUNDS
2000000%
1800000%
1600000%
1400000%
1200000%
1000000%
800000%
600000%
400000%
200000%
0%
BPA
PHBA
MEP
OPP
ETP
PRP
E3
4-T-OP
E1
i-BUP
BHA
NP
BHT
BUP
i-PRP
TBHQ
EE2
TCC
DES
TCS
BEP
HEP
E2

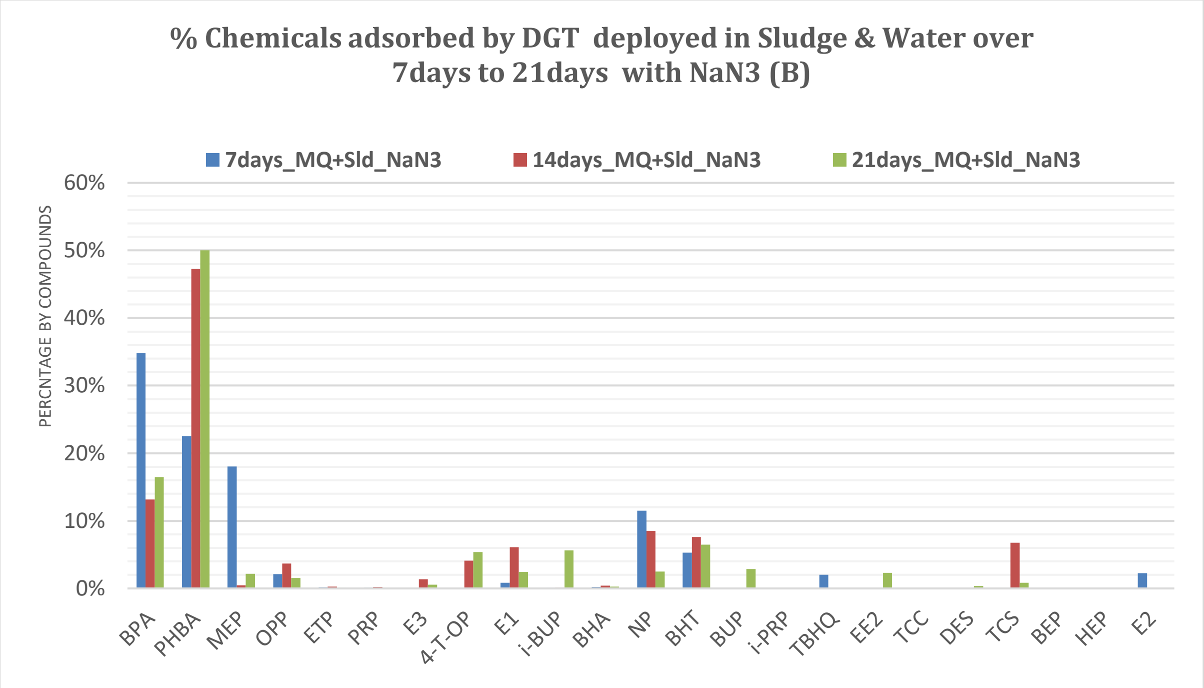


Figure 6(A-C): % chemicals adsorbed by DGT from sludge slurry from sludge cakes ready for agricultural use under various conditions (All the DGTs were deployed in 3:37 sludge/water ratios of 15g sludge cake)

Some of the detected chemicals are grouped below to further investigate their behaviour over the sampling period. These have also been selected to ensure that some of the chemicals investigated in the earlier experiments are included to be able to draw some inference and probably suggest when it would be safer for the farmers to commence planting of their crops once the soil has been amended using the sludge cakes. Figure 7 shows that Sludge slurry without any inhibitor had early desorption of most of the chemicals investigated except for PHBA and MEP. This suggests that more time might be required for these 2 compounds to fully desorbed from the sludge cake while applied to agricultural land considering the concentrations 2114 ngL^{-1} and 1497 ngL^{-1} respectively. However, other selected compounds show that the concentrations adsorbed by DGT was higher within the 7th deployment day and thence depletion in the concentrations which could be related to degradation of bioavailable concentrations. On the other hand, with the addition of NaN3 in Figure 8, PHBA behaviour could be explained similarly as in the case without the inhibitor only that the concentrations were higher because of controlled or reduced biodegradation. However, all the other compounds show depletion in chemical concentrations except for BPA which increased in concentrations between week 2 and week 3 from 470ngL^{-1} to 1202ngL^{-1} . This trend was exhibited by BPA under both conditions which suggest that NaN_3 inhibits degradation.

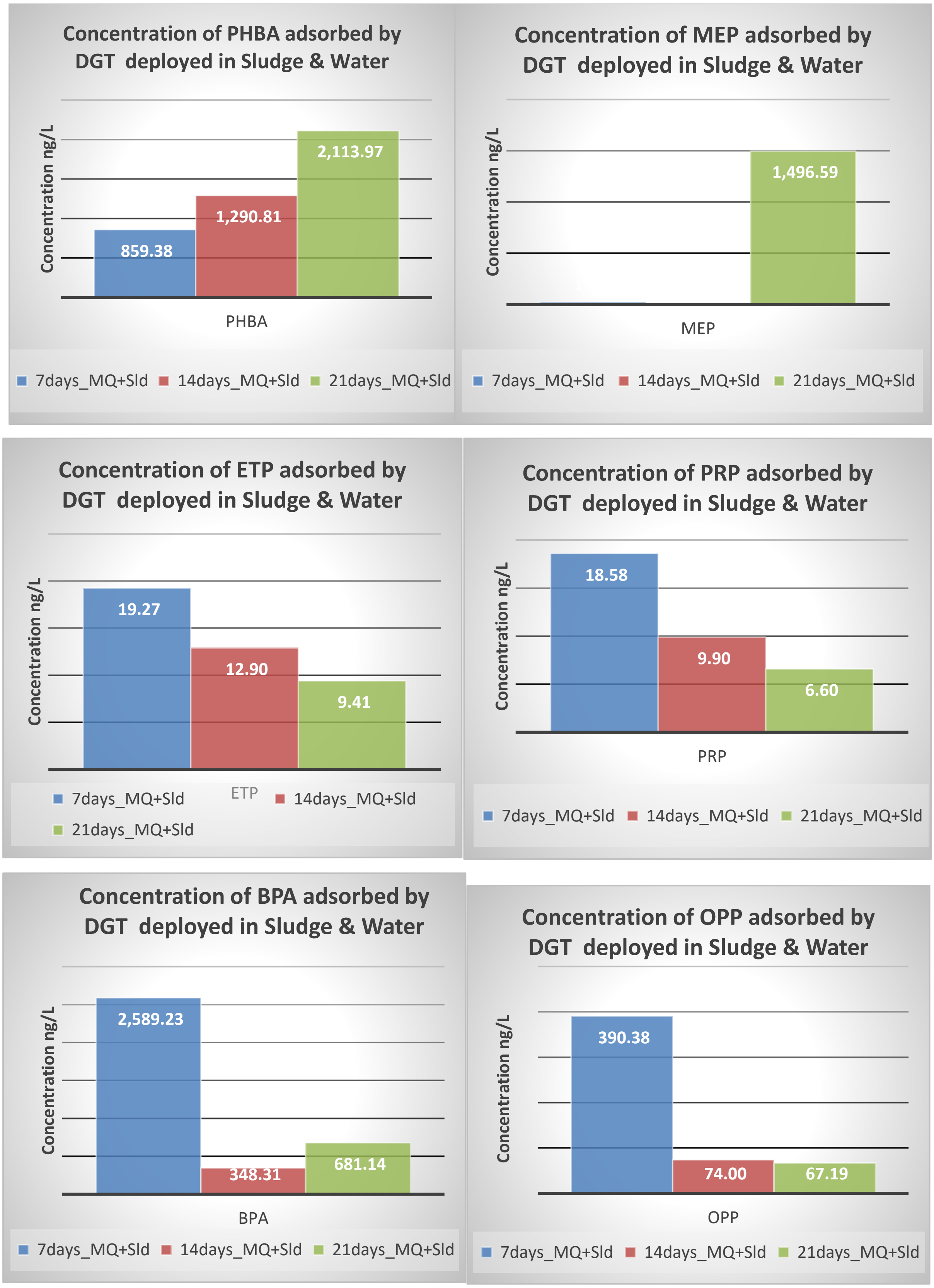


Figure 7: Time Weighted Average Concentrations (ngL^{-1}) **of chemicals in sludge cakes adsorbed by o-DGT in MQ water & Sludge mixture without the inhibitor** NaN_3

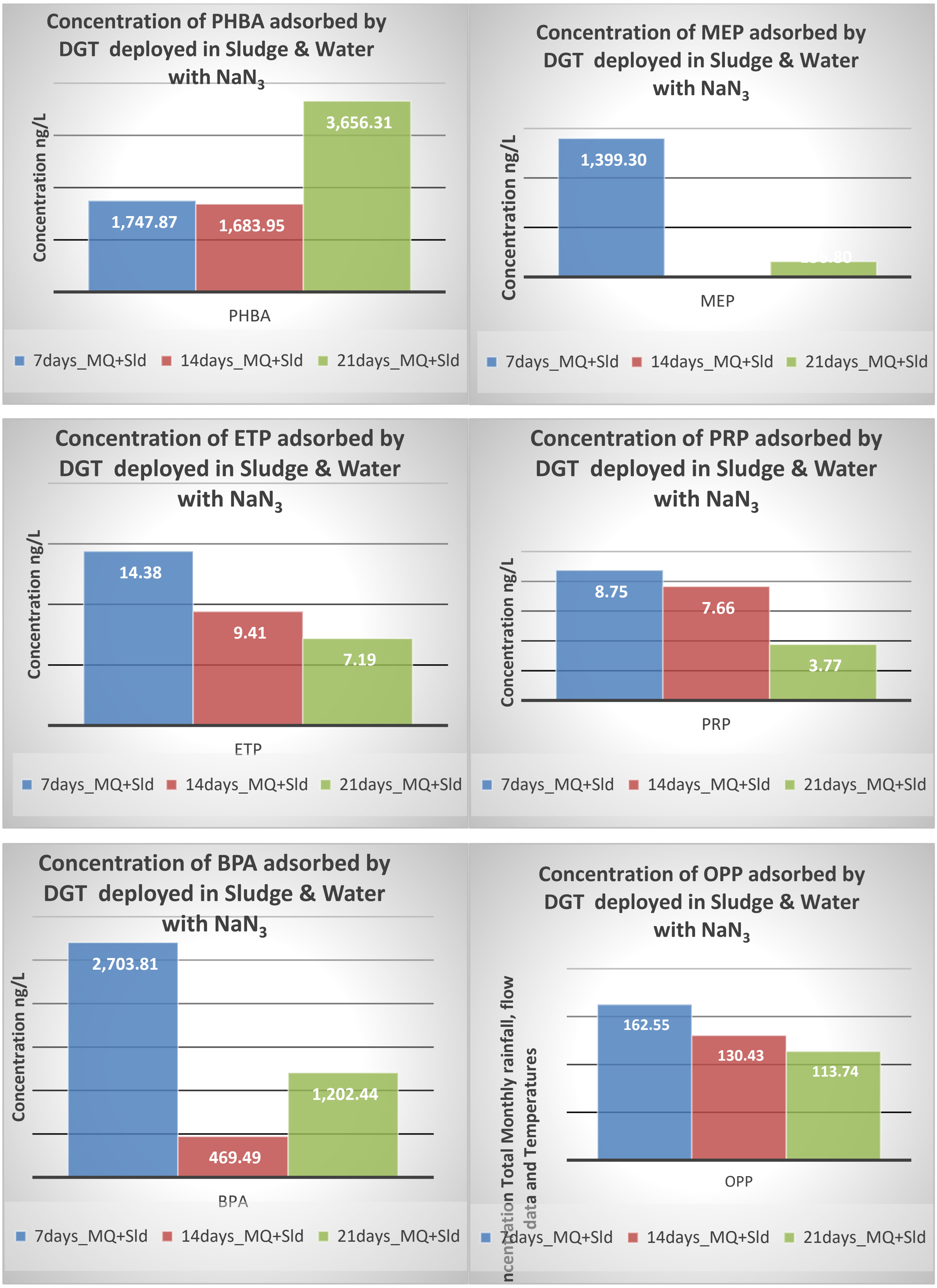


Figure 8: Time Weighted Average Concentrations (ngL^{-1}) **of chemicals in sludge cakes adsorbed by o-DGT in MQ water & Sludge mixture with the inhibitor** NaN_3

Figure 9: 24hrs Concentrations (ngL^{-1}) of chemicals in sludge cakes adsorbed by o-DGT in MQ water & Sludge mixture without the inhibitor NaN$_3$

BPA decreased in concentration from day 7 to 21 even though the concentration on the 14th day was lower than the 21st day. This pattern was also observed for MEP and BHT. However, PHBA increased over 7 days to 21 days while OPP, NP and TBHQ decreased across the period under both conditions. These trends are in response to the relative solubility and partition coefficients (Kow, Koc) of these compounds. For instance, PHBA has a $LogK_{ow}$ of 1.39 and solubility of 5000 mg L^{-1} shows an upward trend across the period. However, MEP also has a solubility of 2500 mg L^{-1} and a similar $LogK_{ow}$ of 2. This compound was observed reducing in concentration over 21 days with theNaN3 which inhibits biodegradation. However, as can be seen in Figure 7 where NaN_3 was not applied, concentrations of MEP was very low until 21 days had elapsed suggesting a combination of degradation if explained in the light of Figure 8 or slow desorption from the sludge slurry.

DGT samplers deployed for 24 hours show that MEP, ETP and PRP were more available on the 21st day compared to the other compounds while early desorption was observed in BPA and OPP and the adsorbed concentration suggest degradation over the period. All the above figure 9 tells us about the concentration of chemicals that were available at the beginning of the study and how quickly some degraded while some had slow desorption rate leading to having a higher concentration at the latter part of the study than the earlier prior. While those that desorbed early were observed to have degraded over the period. In comparison with the first study that Methylparaben (MEP), Ethylparaben (ETP), Propylparaben (PRP) and 4-hydroxybenzoic acid (PHBA) and concluded that PCP ingredients are readily available for plant uptake, the reduction was between the day 14 to 21, the 2nd study for similar compounds also suggest that reduction in PCP bioavailability, MEP and PHBA full desorption or degradation could take longer than 14 days. It implies that bioavailability of ETP and PRP reduced from week 1 to week 3 suggesting that these could fully degrade if allowed a week further or 2 under the influence of environmental climatic factors such as temperature or precipitations while available surface area resulting from the soil to sludge ratio also play an important role.

Conclusion

This study was conducted using prepared sludge cakes for agricultural use. There is a huge responsibility on the operators of the treatment plants to ensure that the sewage sludges are appropriately disposed to avoid reintroduction of the organic contaminants and the pathogens into the environment. However, there is a growing interest in its use for agricultural purposes, especially for the treatment of agricultural soils. End-of-waste criteria and Life Cycle Assessment was proposed (Kacprzak et al., 2017) to fully establish the environmental, economic and ecotoxicology impact of various disposal avenues where the current of the art-of-state method, agricultural reuse and incineration seems more favoured. This study used DGT as a tool to quantify the bioavailable fraction of chemicals present which will be available for plant uptake once the agricultural soils are amended with the sludge cakes.

Analysis of the total uptake over 21 days suggested that 72% of BPA uptake, 73% of OPP, 65% E3,53% PRP and 100% TBHQ were adsorbed by DGT within the first 7 days of the 21 days sampling. However, 50% of the total PHBA adsorbed by DGT was observed on the 21st day, 98% PHBA, 78% 4-T-OP, 92% i-BUP, 35% BHA, 43% BHT, 99% BUP, 98% i-PRP as well as 100% TCC, DES, TCS were potentially removed within the 21 days. The measured concentrations provide insights into the sorption/desorption behaviour along with biodegradation. This suggests that leaving the amended soil for a longer than 3 weeks period would greatly reduce organic contaminants before the farmers planting their crops which supports EU-27 recommendations of sludge reuse or incineration (Kelessidis & Stasinakis, 2012). Concerns by the food retailers, landowners and local inhabitants' organizations were also central in the decision making which would require an advance technology to reduce the level of pathogens in the sludge.

It can be concluded that contaminants dissociation from the sludge matrix is chemical dependent and the presence of microbial activities would aid biodegradation. The variation between the first and the subsequent studies could be because of using sludge cakes from different batches which have been subjected to different climatic conditions before commencing the laboratory studies on the cakes. It can thereby be concluded that DGT is a suitable tool to measure dissolved bioavailable chemicals for plant uptake, allowing a longer period between the application of sludge cakes and planting would reduce the risk of chemical contaminations. This outcome supports the notion of various researchers, while Werle and Wilk, (2010) also investigated the future thermal utilization of sewage sludge in obtaining some forms of energy to include gasification, pyrolysis, combustion, and co-combustion. While this is a noble idea, studies have shown that lands near the treatment work most especially the reed beds enhance eutrophication which supports other claims that the agricultural land yields where sewage sludge is applied are more than that of lands where inorganic fertilizers are applied (Singh & Agrawal, 2008).

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7. Conclusions and Recommendations

7.1. Summary of Papers and Conclusion

This study was aimed at gaining more insight into a passive sampler for water sampling as a tool for water quality standards investigations and monitoring of Pharmaceuticals and Personal Care Products PPCP. Following the EU Directives, there are two major areas of concern about the surface or underground water that Water framework Directive is meant to protect, and the members' states are expected to monitor to achieve "good ecological status" and "good chemical status". Annex V of WFD described good ecological status regarding the biological community, hydrological characteristics, and chemical characteristics. Good chemical status is centred around compliance with established quality standards. DGT has been validated in the laboratory with some field validation and coupled with its TWA capability, it was chosen as the sampling tool for this study. Four different studies have been carried out in France and England on commonly found pharmaceuticals personal care products ingredients.

The studies considered the investigative capacity and monitoring potentials of a passive sampler leading to the following outcomes. The Italian sampling campaign investigated the viability of nature-based treatment technology as a replacement for the Conventional treatment works for the removal of pharmaceuticals and personal care products ingredients. The removal efficiency of both systems is similar except for few chemicals which were prevalent at the effluent such as Nonylphenol and 4-tert-octyl phenol and Clarithromycin. 23 Personal care products were investigated and the preservatives which are commonly used shows an average removal rate of 41 % - 100% in the nature-based systems and 40 % to 100% in the Conventional treatment systems. Concentrations of antibiotics ranged from 6 ngL^{-1} – 960 ngL^{-1} in the influents and 4.5 ngL^{-1} – 410 ngL^{-1} in the effluents of the conventional WWTPs and 6.6 ngL^{-1} to 11,600 ngL^{-1} in the influents and 3.7 ngL^{-1} - 1,295 ngL^{-1} in the effluents of the nature-based plants. These two systems have demonstrated that they are comparable in terms of performance while the exception in terms of few compounds with high concentration at the effluent could be as a result of degradation of the parent forms which were not measured at the influent channels.

The studies at the treatment work in North West of England for 52 weeks confirmed the suitability of o-DGT for environmental monitoring of organic pollutants over a long period but with the aid of appropriate sampling devices such as cages, polyethene mesh etc. however, a further study to understand the partitioning of organic contaminants in the activated sludge also revealed that even though chemical desorption from the sludge cakes was observed within the first seven days, of deployment, 98% PHBA, 78% 4-T-OP, 92% i-BUP, 35% BHA, 43% BHT, 99% BUP, 98% i-PRP as well as 100% TCC, DES, TCS were achieved within 21 days of deployment. It was thence concluded that sludge amended soils should be allowed a minimum of 28 days before cropping to significantly reduce the presence of organic contaminants. It is therefore submitted that DGT could be suitable for long-term monitoring of environmental pollutants, investigative purposes, and treatment systems performance examination once the experimental design is appropriately formulated with due consideration to other relevant environmental parameters.

DGT is considered a suitable method for the monitoring of organic contaminants and hence is considered a viable tool kit that can help in cost reduction, efficiency and reduced operational challenges most especially with the use of a range of available deployment devices. These devices make it useful in any aquatic environment and the risk associated with sample loss is minimal.

7.2. DGT performance in this study

This study has been able to support some of the conclusions in the previous studies about the suitability of DGT as an investigative tool (Mengistu *et al.*, 2012) and a long-term monitoring passive tool in the environment(Zhang, et al., 2013). It is however important to mention that to be able to fully ascertain its suitability over a long period, an extensive study which would compare some of the common passive samplers in the market such as Chemcatcher, POCIS would be important. Also, for such an extensive study, it will be ideal to undertake a study of the full chemical spectrum which would help us to understand the activities of the parent compounds in the treatment process most especially in chemicals like Nonylphenol and other endocrine disruptive compounds (Gong *et al.*, 2009) which are prevalent at the effluent channels than in the influent channels.

Consideration should also be given to the water flow rate in future studies. For instance, the use of metallic cages as a deployment device in the Northwest of England sampling campaign helped in the recovery of all the samplers even where there was excess water influx into the treatment works. However, there were a very few instances where DGT samplers would be found directly in the cage rather than inside the polyethene mesh which was immersed into the cages. This informed that in an instance where a deployment without the use of the appropriate device is commissioned, the possibility of losing some of the samplers as well as being immersed in the organic matters cannot be underestimated. On the other hand, the samplers were excellently deployed in the nature-based treatment works as well as in the treatment works with the aid of 4kg weight to ensure that they are not swept away by the water or being relocated to the lower stream.

DGT has shown to withstand various seasonal fluctuations in weather with good performance rate in as much the samplers are kept immersed into the sampling medium to avoid dryness of the binding and diffusive gels.

7.3. Achievements of this study

- Design and construction of nature-based treatment system are chemically driven. The nature of prevailing chemicals in the locality should be considered while designing the system for effective removal of the pollutants. System design could then be single-stage up to multi-staged system for effective removal of the pollutants. It is important to have insight into the prevailing chemicals in the wastewater before the construction. For instance, if the wastewater is predominantly of nutrients, Nitrogen, Phosphorus and Potassium, operators and the project owner may need more plantation to use up the nutrients and in such a system, there may need to channel the wastewater to multi-staged beds.

- Removal and treatment performance of both nature-based system and conventional treatment works are comparable. However, the decision on the best system would be influenced by the population to be served, sources of the wastewater, and the available resources for the project. Constructing the nature-based system may be capital intensive at the start of the project. But there is a little operating cost of such a system compared to the conventional system and as such, it is a cheap way of treating wastewater in a small community or developing economy.

- DGT passive samplers can be stored for 6 months and the data obtained in the sampling campaign is at 95% confidence level compared to a freshly made DGT samplers. Once the DGT samplers are prepared, they are to be stored in a salt solution 0.01-0.1 MNacl. Once it is properly hydrated, it can stay active for up to 6 months. This was tested in a preliminary study to ascertain the performance of DGT that have been stored for some time.

- Sewage sludge cake application to agricultural soils requires more than 21 days to allow degradation of organic contaminants such as Methylparaben MEP, Ethylparaben ETP, Propylparaben PRP to degrade before cropping. There have been various concerns in the area of pathogens and contamination of vegetable. It therefore means that the longer the land is left the lower the risks of pathogens and contaminants.

- Compounds in the wastewater are partitioned into organic matters (sludge), removed by degradation (aerobic or anaerobic bacteria), while some are re-introduced into the environment through the effluent water. This means that soluble compounds degrades while some conjugates may deconjugate into other forms. It means that the parent form may have been detected at the influent while it is being found at the effluent in a different form. This is one of the reasons why many chemicals are found predominant at the effluent while they are not significantly detected at the influent channel masking as a negative removal in the treatment system.

7.4. Some of the challenges faced during the study

The study faced few challenges as highlighted below.

- A study was commenced in Ireland and the samplers were removed by the Irish Environment Protection Agency, EPA. This study did not continue until a later date. When the matter surrounding the study was resolved with the EPA by our Irish partner, TelLab, Ireland, our preliminary study suggested a very low concentration of chemicals of interest due to our inability to deploy the samplers within a reasonable distance from the sources of the contaminants. Some of these samplers were also removed by the passers-by. Following up on the poor chemical signals, the sampling campaign was discontinued.

- There were a series of LCMS machine breakdown during this study. The study was started using Walters However, the study was completed using Shimadzu LCMS-8040
- Due to high impurity in the samples, about 3 columns were blocked because of pressure due to column blockage due to impurities from the samples even though they have been filtered suing .22 µm filters.

7.5. Further Studies and Recommendations

- Further studies could be undertaken to broaden and understand more, the behaviours of nature-based treatment plants in all the seasons of the year. This study is aimed at giving insight into the performance of the system in all seasons. For instance, the wastewater inflow is low in the summer and maybe more in the spring. It means that the chemical concentration will vary over the year and a full assessment over the various season would be useful to create a better overview of the performance of the system across all seasons of the year.

- Sediment analysis in the nature-based treatment could help understand the chemical partitioning and gain more insight into their removal pathways. It is important to know the fate of compounds in the nature-based treatment system. Sediment analysis combined with water analysis will help us to understand how the chemicals are fully removed in the nature-based system. It is a usual practice that the organic matter is removed at some stages where they may be insitu for many years. It implies that a full study of the system would help in understanding how the organic matter should be disposed to avoid re-introduction into the environment.

- Chemical partitioning at the conventional treatment works could be better understood by simultaneously undertaking sampling campaigns of influent water, effluent water, activated sludge and sludge cakes over a short period. However, knowledge of the residence time is required so that the study design would map the influent water with the effluent and investigate the processes that over time

- An extensive in situ study can also be undertaking on soil samples from agricultural land where the sludge cakes have been applied over a period to investigate degradation rates under natural climatic conditions. It therefore means that samples of the sludge cake that has been applied will be analysed at the same time that the amended soil is being analysed. This is to help the researchers understand the concentration of the contaminants that were applied and their fate in the agricultural land over the period.

- A further study to investigate the presence of the parent forms of those compounds with negative removals at the influent channels would be imperative to fully establish their removal efficiency and compare both Nature-based and Conventional systems accordingly. It is understood that many of the processes in the treatment plant include deconjugation of compounds in their parent forms. This leads to higher concentrations of such chemicals at the effluent channels. A study of full chemical spectrums including all compounds in various forms would help to understand the deconjugation process which will help to have a conclusive discussion about negative removals that are observed by researchers.

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Appendix 1: Priority Substances and Certain Other Pollutants according to Annex I of Directive 2013/39/EC, which amended Annex X of the WFD

List of priority substances in the field of water policy

Number	CAS number	EU number	Name of priority substances	Identified as priority hazardous substance
(1)	15972-60-8	240-110-8	Alachlor	
(2)	120-12-7	204-371-1	Anthracene	X
(3)	1912-24-9	217-617-8	Atrazine	
(4)	71-43-2	200-753-7	Benzene	
(5)	not applicable	not applicable	Brominated diphenyl ethers	X
	32534-81-9	not applicable	Pentabromodiphenylether (congener numbers 28, 47, 99, 100, 153 and 154)	
(6)	7440-43-9	231-152-8	Cadmium and its compounds	X
(7)	85535-84-8	287-476-5	Chloroalkanes, C10-13 iv	X
(8)	470-90-6	207-432-0	Chlorfenvinphos	
(9)	2921-88-2	220-864-4	Chlorpyrifos (Chlorpyrifos-ethyl)	
(10)	107-06-2	203-458-1	1,2-Dichloroethane	
(11)	75-09-2	200-838-9	Dichloromethane	
(12)	117-81-7	204-211-0	Di(2-ethylhexyl)phthalate (DEHP)	
(13)	330-54-1	206-354-4	Diuron	
(14)	115-29-7	204-079-4	Endosulfan	X

(15)	206-44-0	205-912-4	Fluoranthene	
(16)	118-74-1	204-273-9	Hexachlorobenzene	X
(17)	87-68-3	201-765-5	Hexachlorobutadiene	X
(18)	608-73-1	210-158-9	Hexachlorocyclohexane	X
(19)	34123-59-6	251-835-4	Isoproturon	
(20)	7439-92-1	231-100-4	Lead and its compounds	
(21)	7439-97-6	231-106-7	Mercury and its compounds	X
(22)	91-20-3	202-049-5	Naphthalene	
(23)	7440-02-0	231-111-4	Nickel and its compounds	
(24)	25154-52-3	246-672-0	Nonylphenols	X
	104-40-5	203-199-4	(4-nonylphenol)	X
(25)	1806-26-4	217-302-5	Octylphenols	
	140-66-9	not applicable	(4-(1,1',3,3'-tetramethylbutyl)-phenol)	
(26)	608-93-5	210-172-5	Pentachlorobenzene	X
(27)	87-86-5	201-778-6	Pentachlorophenol	
(28)	not applicable	not applicable	Polyaromatic hydrocarbons (PAHs)	X
	50-32-8	200-028-5	(Benzo(a)pyrene)	X
	205-99-2	205-911-9	(Benzo(b)fluoranthene)	X
	191-24-2	205-883-8	(Benzo(g,h,i)perylene)	X
	207-08-9	205-916-6	(Benzo(k)fluoranthene)	X
	193-39-5	205-893-2	(Indeno(1,2,3-cd)pyrene)	X
(29)	122-34-9	204-535-2	Simazine	
(30)	not applicable	not applicable	Tributyltin compounds	X

	36643-28-4	not applicable	(Tributyltin-cation)	X
(31)	12002-48-1	234-413-4	Trichlorobenzenes	
(32)	67-66-3	200-663-8	Trichloromethane (chloroform)	
(33)	1582-09-8	216-428-8	Trifluralin	X
(34)	115-32-2	204-082-0	Dicofol	X
(35)	1763-23-1	217-179-8	Perflurooctane sulfonic acid and its derivatives (PFOS)	X
(36)	124495-18-7	Not applicable	Quinoxyfen	X
(37)	Not applicable	Not applicable	Dioxins and dioxin-like compounds	X ([9])
(38)	74070-46-5	277-704-1	Aclonifen	
(39)	42576-02-3	255-894-7	Bifenox	
(40)	28159-98-0	248-872-3	Cybutryne	
(41)	52315-07-8	257-842-9	Cypermethrin ([10])	
(42)	62-73-7	200-547-7	Dichlorovos	
(43)	Not applicable	Not applicable	Hexabromocyclododecanes (HBCDD)	X([11])
(44)	76-44-8/1024-57-3	200-962-3/213-831-0	Heptachlor and heptachlor epoxide	X
(45)	886-50-0	212-950-5	Terbutryn	