Occurrence and Effects of Pharmaceuticals in Rivers

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Abstract

Over the past twenty years or more, there has been increasing interest in the occurrence and effects of pharmaceuticals in the aquatic environment, particularly in Europe and North America. While the understanding of their occurrence is now relatively well understood in these parts of the world there remains a scarcity of data from many African countries. Thousands of pharmaceuticals are in use worldwide and the effects of these drugs, especially at environmentally relevant concentrations, are still unknown. Moreover, few ecotoxicity test species are recommended by Organisation for Economic Co-operation and Development (OECD) and so a dearth of information exists for many organisms. This PhD was thus carried out to determine the occurrence of pharmaceuticals in Africa rivers (Nigeria) and to seek to improve the understanding of the effects of prolong low-level exposure of *Gammarus pulex* and *Aquaticus aquaticus* to pharmaceutical contamination.

The occurrence of 37 pharmaceuticals belonging to 19 therapeutic classes was studied in surface water and effluent in Lagos State, Southwest Nigeria. Samples were collected quarterly between April 2017 and March 2018 from 22 sites, and 27 compounds were detected at least once, many in the microgram per litre range. Maximum concentrations for a range of compounds including sulfamethoxazole, paracetamol, cimetidine, fexofenadine, carbamazepine, metformin and diazepam ranged from 75 μg L^{-1} to 129.5 μg L^{-1}. Mean concentrations for 13 compounds were also in the μg L^{-1} order. These values are several orders of magnitude higher than most studies of pharmaceutical occurrence in Europe and North America but similar to some other peak concentrations measured in developing countries such as China and India. Multiple pharmaceutical compounds were found at all monitoring sites and there were

no clear spatial patterns. This may indicate that a variety of sources exist throughout the catchment, revealing that there are potentially many contributing sites. Studies in Europe and the US have found that sewage treatment plants (STPs) are the major source of pharmaceutical pollution (Hughes et al., 2013) but in the developing world it seems that there are a greater range of sources contributing to loads in rivers. These may include STPs, pharmaceutical manufacturing plants, urban waste collection areas and disposal of effluent by vacuum trucks. Seasonal trends in the data were complex with some compounds such as fexofenadine, carbamazepine, paracetamol, cimetidine, metformin and sulfamethoxazole being found at higher concentrations in the dry season and conversely, others such as paracetamol, sulfamethoxazole, tramadol and metformin being greater during the wet period. Seasonal usage is unlikely to explain this phenomenon as many compounds would be used equally over the year to treat persistent illnesses, e.g. carbamazepine and metformin. It may be that the multiple sources of pharmaceuticals in the catchment results in this complex picture with some that are associated with continuous effluent discharges (e.g. from STPs and manufacturing facilities) being diluted in the wet season but other sources (e.g. urban waste sites) which see pollutants mobilised in periods of rainfall.

Effect studies focused on the biological effects of erythromycin, diclofenac, ibuprofen and their mixtures on the growth, feeding and mortality of aquatic macro-invertebrates (*Gammarus pulex* and *Asellus aquaticus*). It was found that for erythromycin and diclofenac, growth rate decreased, feed intake was reduced, and mortality was significant for *G. pulex* but not significant for *A. aquaticus*. For ibuprofen, there was, however, no effect for both test species. For mixtures of erythromycin, diclofenac and ibuprofen growth rate decreased, feed intake was reduced but mortality was not significant for both *G. pulex* and *A. aquaticus.* The effects of these pharmaceuticals on

the growth, feeding and mortality of the test animals were a result of the actions of the drugs and not attributed to a more general stress response. Although pharmaceuticals are indispensable to human health their usage and discharge to the aquatic environment coupled with their ecotoxicity to aquatic life may lead to ecological problems in the near future. Furthermore, this research confirms the suitability of the test species (*G. pulex* and *A. aquaticus*) as ecotoxicological test species that is both amenable to laboratory culture and sufficiently sensitive to provide reliable quantification of environmental risk.

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List of Definitions

AOH - Adsorbable Organic Halogens

AOI - Adsorbable Organic Iodine

ASA - Acetylsalicylic Acid

ARGs - Antibiotics Resistance Genes

ARBs - Antibiotics Resistance Bacteria

CA - Concentration Addition

DMBs - Dewatered Municipal Biosolids

DT_{50} - Half Life

GMP - Good Manufacturing Practices

EC_{01} – Effect Concentration at 1%

EC_{10} – Effect Concentration at 10%

EC_{50} – Effect Concentration at 50%

ECx – Effect Concentration at x %

EMEA - European Medicines Evaluation Agency

ERA- Environmental Risk Assessment

EU - European Union

EQS - Environmental Quality Standards

HPLC-MS/MS–High-Performance Liquid Chromatography Tandem Mass Spectroscopy

HRT - Hydraulic Retention Time

IA – Independent Action

ICM - Iodinated Contrast Media

KOW – Octanol-Water Partition Coefficient

LC50 – Median Lethal Concentration

LMBs - Liquid Municipal Biosolids

LOD – Limit of Detection

LOEC – Lowest Observed Effect Concentration

LOQ – Limit of Quantification

Log Kow = Octanol: Water Partition Coefficient;

MEC – Measured Environmental Concentration

MFB - Multispecies Freshwater Biomonitoring

MOA – Mechanism of Action/Mode of Action

MQL=Method Quantification Limit

NA= Not Available

NOEC – No-Observed Effect Concentration

NR= Not Reported

NSAID – Non-Steroidal Anti-Inflammatory Drug

OECD - Organisation for Economic Co-operation and Development

OTC – Over-the-counter

PBO - Piperonyl Butoxide

PEC – Predicted Environmental Concentration

PNEC - Predicted No Effect Concentration

pKa - Dissociation Constant

PPCPs – Pharmaceuticals and Personal Care Products

PSU -Practical Salinity Unit

Q-TOF – Quadrupole Time-of-Flight

RQ – Risk Quotient

SSRI – Selective Serotonin Reuptake Inhibitor

SEM - Standard Error of the Mean

STP – Sewage Treatment Plant

TUs -Toxic Units

TCS - Trichlorosilane

WWTP—Wastewater Treatment Plant

WFD - Water Frame Directive

CHAPTER ONE

Introduction and research context

1.0 Pollution of freshwater ecosystems

Freshwater ecosystems comprise one-fifth of the earth's surface water and are essential for human survival; providing water for agricultural activities and most of the drinking water for human populations (Fent, 2008). More than 40 % of the fish species in the world are found in freshwater ecosystems and some fauna and flora that lives in freshwater habitats are unique to their environment. Freshwater ecosystem such as rivers, wetlands and lakes are degrading at a faster rate than terrestrial ecosystems because of the anthropogenic activities of man (Rodriguez et al., 2015). Human activities are significantly impacting and endangering these ecosystems. Watersheds, which catch precipitation and channel it to streams and lakes are highly vulnerable to pollution and human activities can pollute freshwater ecosystems via point or non-point sources. National Geographic reported that 70 % of industrial waste dumped into the water in developing countries is untreated (Ebele, et al., 2017). Runoff from agricultural land, which includes fertilisers, also pollutes freshwater ecosystems. The effects of this pollution are wide-ranging and can damage life in freshwater, which continues up the global food chain as animals that feed on aquatic life take in pollution. Sources and types of water pollution, especially those found in developing countries, are outlined in Table 1.

Table 1: Major water pollutants and their sources. (Adapted from Odiete, 1999).

Types and Effects	Examples	Major Sources
Infectious agents (pathogens) *Causes diseases*	Bacteria, viruses, protozoa, parasites	Human and animal wastes
Oxygen demanding wastes *Deplete dissolved oxygen needed by aquatic species*	Biodegradable animal wastes and plant debris	Sewage, animal feedlots, food processing facilities, pulp mills
Plant nutrients *Causes excessive growth of algae and other species*	Nitrates (NO_2^-) and phosphates (PO_4^{3-})	Sewage, animal wastes, inorganic fertilizers
Organic chemicals *Add toxins to aquatic systems*	Oil, gasoline, plastics, hydrocarbons, pesticides, cleaning solvents	Industry, farms, households
Inorganic chemicals *Add toxins to aquatic systems*	Acids, bases, salts, metal compounds	Industry, household, surface runoff
Sediments *Disrupt photosynthesis, food webs, other processes*	Soil, silt	Land erosion
Heavy metals *Cause cancer, disrupt immune and endocrine systems*	Lead, mercury, arsenic, chromium	Unlined landfills, household chemicals, mining refuse, industrial discharges
Thermal *Make some species vulnerable to disease*	Heat	Electric power and industrial plants

1.1 Emerging contaminants

There have been significant concerns in the last two decades about the presence of new types of environmental pollutants known as "emerging contaminants" of which pharmaceuticals have been the primary focus in the aquatic environment (Kümmerer, 2008a; Van De Steene et al., 2010). Pharmaceuticals are medicines specially design to interact with human and animal biological systems to produce a beneficial effect. They consist of a large number of compounds used to cure and prevent diseases and include:

analgesics, antibiotics, anti-inflammatories, cancer treatments, anti-depressants, and x-ray diagnostics amongst many others. Pharmaceuticals are consumed all over the world even in the poorest countries, however, the quantity consumed depends on many factors, such as the age of the population, level of industrialisation, money available to buy the drugs and access to medical care (Hughes, 2013; Daughton, 2001). Pharmaceuticals perhaps may have been in the environment as far back as drugs have been in use. Their detection in the early 1990's was as a result of technological breakthrough in analytical techniques needed to detect these compounds accurately in environmental samples (Kümmerer, 2009). As sensitivity of the instruments increases because of technological advances more compounds were found at lower concentrations in the environment. Hence, the occurrence of pharmaceuticals in freshwater ecosystems is well established in Europe and America, and is now a growing concern in developing countries, including those in Africa. Data on the environmental occurrence, fate and effects of drugs in the aquatic environments of developing countries is still very sparse.

There are many routes through which pharmaceuticals and resulting metabolites/ degradation products enter the ecosystems (Figure 1).

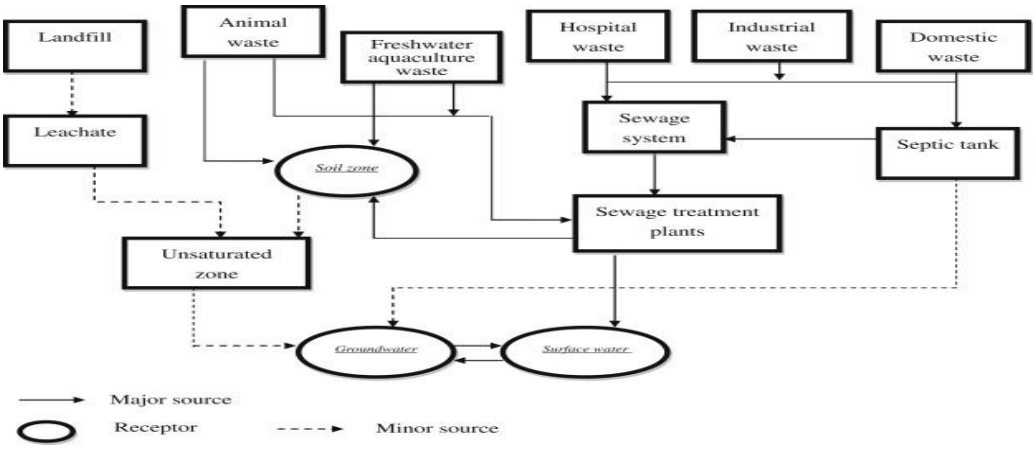

Figure 1: Sources and pathways of pharmaceutical residues in the aquatic environment. [Adapted from Heberer, (2002)]

Unused medicines are disposed of down the sink or toilet and pharmaceutical manufacturing and hospital effluents are disposed of directly to rivers or through wastewater plants. Furthermore, landfill leachates can reach rivers as can runoff from agricultural land where sewage sludge or manures have been applied (Kümmerer, 2009). There is no accurate quantitative data on the contribution from each of these sources to contaminant levels in aquatic environments (Roig and Touraud, 2010). However, the main route is through the excretion of the drug residues after human use either as parent compounds, metabolites in urine and faeces or water-soluble conjugates (Heberer, 2002; Crane et al., 2006 and Samuelsen et al., 2003). They get in the aquatic ecosystem through the sewage system or directly in untreated effluent where sewage treatment systems do not exist. Degradation of pharmaceuticals and their metabolites can take place at STPs but will vary significantly between pharmaceuticals. During sewage sludge treatment, the rate of chemisorption differs between pharmaceuticals and is determined by electrostatic interactions of the drugs, microorganisms within the activated sewage sludge and hydrophobicity of the drugs (Fent et al., 2006; Kummerer, 2013). Any left-over drug and metabolites in the effluents are diluted on reaching

surface waters. As a result, pharmaceuticals are present in low levels (μgL^{-1}) in aquatic ecosystems (Ashton et al., 2010; Choi et al., 2008; Escher et al., 2015). Many pharmaceuticals are not persistent or readily bioaccumulative but the endless discharge of pharmaceuticals into the ecosystem makes them pseudo-persistent. Although the concentrations of pharmaceuticals in receiving waters are quite low (Escher et al., 2005) they pose a potential ecological risk to aquatic fauna and flora. The impact of pharmaceuticals in the aquatic ecosystem is not well established but they are produced to have a unique mode of action for the benefit of humans. However, these impacts could also be seen in other aquatic organisms that possess identical receptors. In other aquatic species, these biological targets may be responsible for other metabolic functions (Goday et al., 2015; Seiler, 2002), hence, pharmaceuticals and their metabolites can act through other modes of action in aquatic species. The impact of pharmaceuticals may go unnoticed for a long period because of the low-level concentrations of the drugs in the aquatic environment (Escher et al., 2005; Cizmas et al 2015; Gurke et al., 2015). It is also possible that the effect of the pharmaceuticals may impact local population dynamics throughout the aquatic environment, from macroinvertebrates up to higher organisms. Indeed, it is established that pharmaceuticals concentrations could be higher in the sediment of river beds and as a result benthic macroinvertebrate animal that populate this niche may be exposed to higher levels than normal (Franzellitti et al., 2013; Halling-Sørensen et al., 1998; Pouliquen et al., 1992; Tauxe-Wuersch et al., 2005).

This effect can often disrupt key biological functions in aquatic organisms such as reproduction and growth (Fent et al., 2006). Despite the longevity of exposure of aquatic organisms to a wide variety of human drugs notable adverse effects are surprisingly rare. The reason for this may be that the concentrations in aquatic

ecosystems are far too low to show acute toxic effects. Acute effects data show that generally, an effect concentration of over 1 mg L^{-1} is required to induce mortality in aquatic organisms (Crane et al., 2006; Fent et al., 2006). It is now widely accepted that the route of exposure is of a continuous chronic nature and this is reflected in the ecotoxicological literature (Fent et al., 2006; Lei et al 2015; Fernandez et al., 2010; Santos et al., 2009). There are few examples of chronic effects on aquatic organisms at environmentally relevant concentrations. The presence of the synthetic hormone contraceptive 17α ethinylestradiol (EE2) in sewage effluent and surface waters has been linked with the endocrine disruption of fish and frogs (Anumol et al., 2016; Escher et al., 2017; DEFRA, 2006; Hedgespeth et al., 2012; Walker and McEldowney, 2013). However, it is still unknown exactly to what extent synthetic hormones such as EE2 effect feminisation of male fish compared with naturally occurring estrogens such as oestrone; yet, it is thought to play a significant role (Sumpter, 2010). The use of diclofenac in cattle caused a considerable decline in vultures in India and Pakistan (Oaks et al., 2004). The Gyps genus of vulture was surprisingly sensitive to residues of diclofenac in dead cows on which they fed, leading to acute renal failure and visceral gout (Oaks et al., 2004). Diclofenac has since been withdrawn as a veterinary medicine (Glassmeyer and Shoemaker, 2005). However, it is still used widely as an analgesic in human medicine; it is persistent through sewage treatment and is regularly detected in effluent and surface waters around the world (Hoeger et al., 2005).

Currently, the primary concern in pharmaceutical environmental exposures is the potential for chronic or long-term toxicity at environmentally relevant concentrations, the effects of pharmaceutical mixtures and impacts on populations, communities and ecosystem functioning (Fent, 2008; Hughes *et al*., 2013; Santos *et al*., 2010). Also, relatively little data on the occurrence of pharmaceuticals has been gathered in Africa,

Asia, and South America. However, over 1000 published papers on the occurrence of pharmaceuticals in aquatic environment exist in Europe and America (Santos *et al*. 2010). The need for more research into both the occurrence and effects of pharmaceutical compounds in river ecosystems cannot be overemphasised. Further knowledge on the effects of exposure at environmentally relevant concentrations on both water column dwelling, and sediment-dwelling aquatic macro-invertebrate animals will aid in prioritising the highest risk compounds for future interventions. Thus, this thesis will aim to provide first-hand knowledge on the occurrence of pharmaceuticals in Nigerian rivers and add substantially to current knowledge on Africa and the effects of such pollution on the aquatic environment.

1.2 Statement of Aim

Aim: The broad aim of this research is to quantify the occurrence of selected pharmaceuticals in African Rivers (Nigeria) and to seek to improve the understanding of the effects of prolonged low-level exposure of freshwater ecosystems to pharmaceutical pollution

1.2.1 The objectives

1. To evaluate the presence of drugs belonging to different therapeutic classes in previously unstudied surface water.
2. To evaluate the temporal and spatial patterns of pharmaceuticals in the Odo Iya Alaro river, Lagos Southwest Nigeria.
3. To assess the ecological effects of prolonged low-level exposure to pharmaceuticals on growth, feeding, and mortality of freshwater macro-invertebrate animals.

4. To determine the differences in effects between different invertebrate species i.e. water column dwelling animals-*G. pulex* and sediment dwelling animals- *A. aquaticus.*
5. To examine the biological effects of mixtures of pharmaceuticals on macro-invertebrates' relative to individual compounds.

1.2.2 Hypotheses to be tested

(H1): That pharmaceuticals will be consistently present in the Odo Iya Alaro river, Lagos Southwest Nigeria.

(H2): That exposure of *G. pulex* and *A. aquaticus* to low concentrations of pharmaceuticals will not have lethal effects.

(H3): That extended exposure to low concentrations of pharmaceuticals will cause significant reductions in sub-lethal endpoints (e.g. growth and feeding).

(H4): That the effects of mixtures will be more pronounced than compounds acting singly.

1.3 Thesis plan

Chapter One provides the rationale behind the research including gaps in the literature.

Chapter Two reviews the available evidence so far obtained and covers existing research on the occurrence and effects of human pharmaceuticals in freshwater biomes, with research gaps identified.

Chapter Three presents the results of the pharmaceutical field monitoring study conducted in Nigeria, examining frequency of detections, mean, maximum, minimum, and median values, as well as spatial and temporal variation in pharmaceutical concentrations in the Odo Iya Alaro river, Lagos Southwest Nigeria.

Chapter Four presents the results of the effects of long-term exposure of *Gammarus pulex*, a water column dwelling macro-invertebrate, to single and multiple mixtures of pharmaceuticals.

Chapter Five presents the results of long-term exposure of *Asellus aquaticus* (bottom dwelling macroinvertebrate) to single and multiple mixtures of pharmaceuticals.

Chapter Six presents a general discussion bringing the above experiments together.

Chapter Seven presents a research synthesis and highlights limitations of the current study and further research opportunities.

CHAPTER TWO

Literature Review

2.0 Pharmaceuticals in the environment

2.1 General overview

All over the world, pharmaceuticals have been known to be an increasing threat to aquatic life (Boxall et al., 2012). Concentrations in the aquatic environment varies from μg L^{-1} to ng L^{-1} and almost all the therapeutic class of drugs have been found in the milieu. Garrison et al., (1976) discovered clofibric acid with concentration levels between 0.8 and 2 μgL^{-1} (the first compound of pharmaceutical origin) in the US effluent treated wastewater treatment plants (WWTPs). And ever since, increasing number of drugs have been discovered in different water bodies such as rivers, lakes, streams and drinking water (Zorita et al., 2009). Main therapeutic classes of pharmaceuticals such as antibiotics, analgesics, anti-epileptics, β-blockers and lipid regulators has been found in the influents and effluents of WWTPs. It has been revealed that highest environmental concentration of pharmaceuticals are around WWTPs, implying that the WWTPs in use are not well-suited for the complete elimination of these pharmaceuticals. For an example 32 drugs were found in Germany around WWTPs; drugs such as gemfibrozil (a lipid regulator) and carbamazepine (a seizure drug) were found at maximal levels of 6.3 and 4.6 μgL^{-1} respectively (Ternes, 1998). Elevated levels of carbamazepine, diclofenac and naproxen was found in Canadian treated wastewater (Metcalfe et al., 2003) and almost 30 different drugs were found in treated WWTPs effluents across Italy by Castiglioni et al., (2005); ofloxacin was found at varying concentrations of 150-1081 ngL^{-1}, 27-1168 ngL^{-1} for atenolol while carbamazepine was between 33-1318 ngL^{-1} concentrations. Furthermore, studies

conducted in UK found propranolol (75 ngL^{-1}) in all the effluents, diclofenac (500 ngL^{-1}) in 86 % and ibuprofen (3000 ngL^{-1}) in 84 % WWTP effluents investigated (Ashton et al., 2011). Larsson et al., (2007) conducted a study in India and found high levels of ciprofloxacin, losartan and metoprolol in the final discharge of WWTPs treating wastewaters for pharmaceutical industries at concentrations 3100 μgL^{-1}, 2500 μgL^{-1} and 950 μgL^{-1} respectively, although the receiving water bodies often contain lesser compounds at lower concentrations. Boyd et al., (2003) conducted an experiment on the incidence of drugs in the US streams, he revealed that ibuprofen, triclosan and fluoxetine were found at lower concentrations (ngL^{-1} range) in WWTP effluents but not present in surface waters. Ternes (1998) found a similar contamination pattern in his investigation. However, studies have shown that other drugs are everywhere in the environment. A systematic investigation was carried out for antibiotics in surface, piped and ground waters in Mekong Delta, Vietnam in 2015, not one investigated antibiotics was found in ground water and piped water, however in surface water, sulfonamides such as sulfamethoxazole (SMX), sulfadiazine (SDZ), trimethoprim and enrofloxacin in concentrations of 21 ngL^{-1}, 4 ngL^{-1}, 17 ngL^{-1} and 12 ngL^{-1} respectively were found (Cohen et al., 2007). These concentrations were lesser than the predicted no effect concentrations (PNECs) implying low risk of antibiotics in the aquatic environment (Cohen et al., 2007). In addition, pharmaceuticals are detected at various concentration levels (ibuprofen 29.1 ngL^{-1}, salicylic acid 651 ngL^{-1}, naproxen 7.9 ngL^{-1}, diclofenac 45.5 ngL^{-1}, clofibric acid 26.5 ngL^{-1}, mefenamic acid 15.3 ngL^{-1}, carbamazepine 23.9 ngL^{-1} and gemfibrozil 11.4 ngL^{-1}) in effluent samples of wastewater treatment plants of the Pearl River in Southern China in 2010 (Zhao et al., 2010). Drugs such as clofibric acid 111 ngL^{-1}, ibuprofen 928 ngL^{-1} and diclofenac 125 ngL^{-1} were found in UK estuarine (Thomas and Hilton, 2007). More than 50

pharmaceuticals, illicit drugs and endocrine disruptive compounds were found in UK surface waters (Kasprzyk-Hordern et al., 2012) and among the most detected chemicals are naproxen (<146 ngL^{-1}), codeine (<813 ngL^{-1}), ketoprofen (<14 ngL^{-1}), diclofenac (<261 ngL^{-1}) and ibuprofen (<93 ngL^{-1}). Some pharmaceuticals are not easily degraded and found their way into aquatic environment and even drinking water (Heberer, 2002; Loraine and Pettigrove, 2006). Jones et al., (2005) found the NSAID phenazone at high concentrations of up to 400 ngL^{-1} in drinking waters.

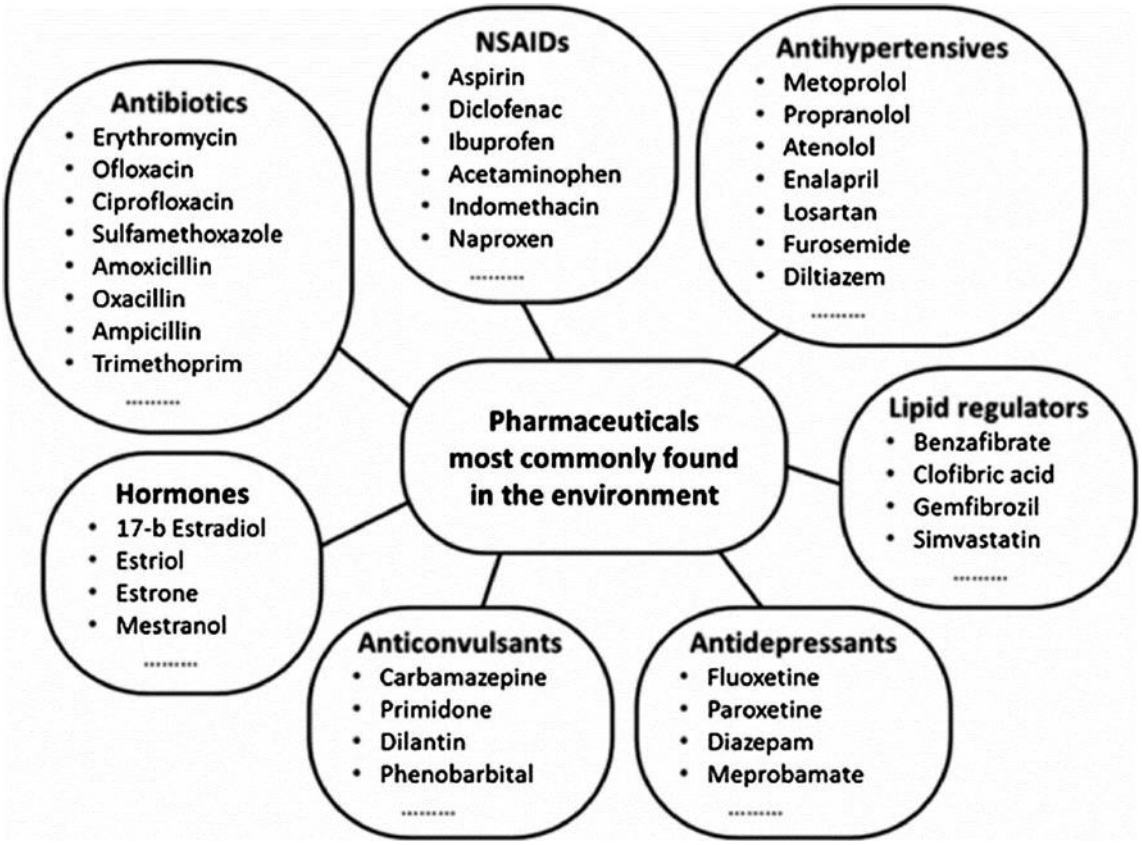

Figure 2.1: Pharmaceuticals commonly detected in the aquatic environment (**Source:** Cizmas et al. (2015))

2.2 Pharmaceutical sources and pathways in the environment

Every year, over 100,000 of both human and veterinary drugs are produced in Europe and America, there are data on the use of these pharmaceuticals (Kümmerer, 2009), however in Africa and some developing nations, data are limited (Wood et al., 2015). The usage of pharmaceuticals varies from one nation to another and some can be obtained on prescription and others can be sold over the counter (OTC) or on the internet, hence the difficulty in tracking drug usage and consumption. This problem is more complicated in developing nations, including Nigeria.

2.2.1 Primary and secondary sources. The pathway for drugs into the ecosystem can be categorized into two; primary and secondary. The primary source are majorly the manufacturing plants, hospitals, household and care homes (especially in Europe and America). Accumulation of pharmaceuticals into the secondary sources such as STPs and landfills is via the release of these drugs into the sewerage systems. These secondary sources can also be regarded as channels for the release of pharmaceuticals into the ecosystem. When drugs are administered orally (mouth), topically (onto the skin), subcutaneously (under the skin), nasally (nose), intramuscularly (into the muscle), intravenously (into a vein), pessary (genito-urinary tract) and/or as a suppository (rectally), the pathways for the environmental contamination is initiated. When these drugs are introduced into the body, different phases of hepatic metabolism acts upon the drugs, initiating therapeutic responses as the drugs elicit effects at the receptor sites (Triebskorn et al., 2004).

The pathway of drug excretion is primarily through the kidneys; however, drugs may also be excreted via faeces, skin and through the lungs. Drugs may be excreted as

conjugates, metabolites of the parent compound or unchanged as the parent compound (Crane et al., 2006; Luo et al. 2014). For example, fluoxetine can be metabolised or bio-transformed to norfluoxetine (main active metabolite) by demethylation reactions in the liver (Altamura et al., 1994). Diclofenac for instance is bio-transformed in man and excreted as sulphates and glucuronide conjugates from the body, primarily in the urine (65 %) and the bile (35 %) (Altenburger et al., 2015). Also, amoxillin is eliminated from the body majorly as unchanged parent compound (80-90 %) and as metabolites (10-20 %), chloramphenicol is passed out majorly as glucuronides (70-90 %) and as unchanged parent compound (5-10 %) (Hilton and Thomas, 2003). These excreted metabolites and parent compounds are released directly into the sewerage system and end up in STPs. The wastewater after treatment will be passed onto the water bodies (Kolpin et al. 2002) or may aggregate as biosolids, accumulating in agricultural land areas (Rooklidge 2004). Moreover, in developed economies, Western Europe and America, manufacturer recommends expired drugs be returned to pharmacies for controlled and proper disposal by landfill or incineration, however, most times this is not the case (Bound and Voulvoulis 2005). Figure 2.2 shows possible pathway of drug to the ecosystem.

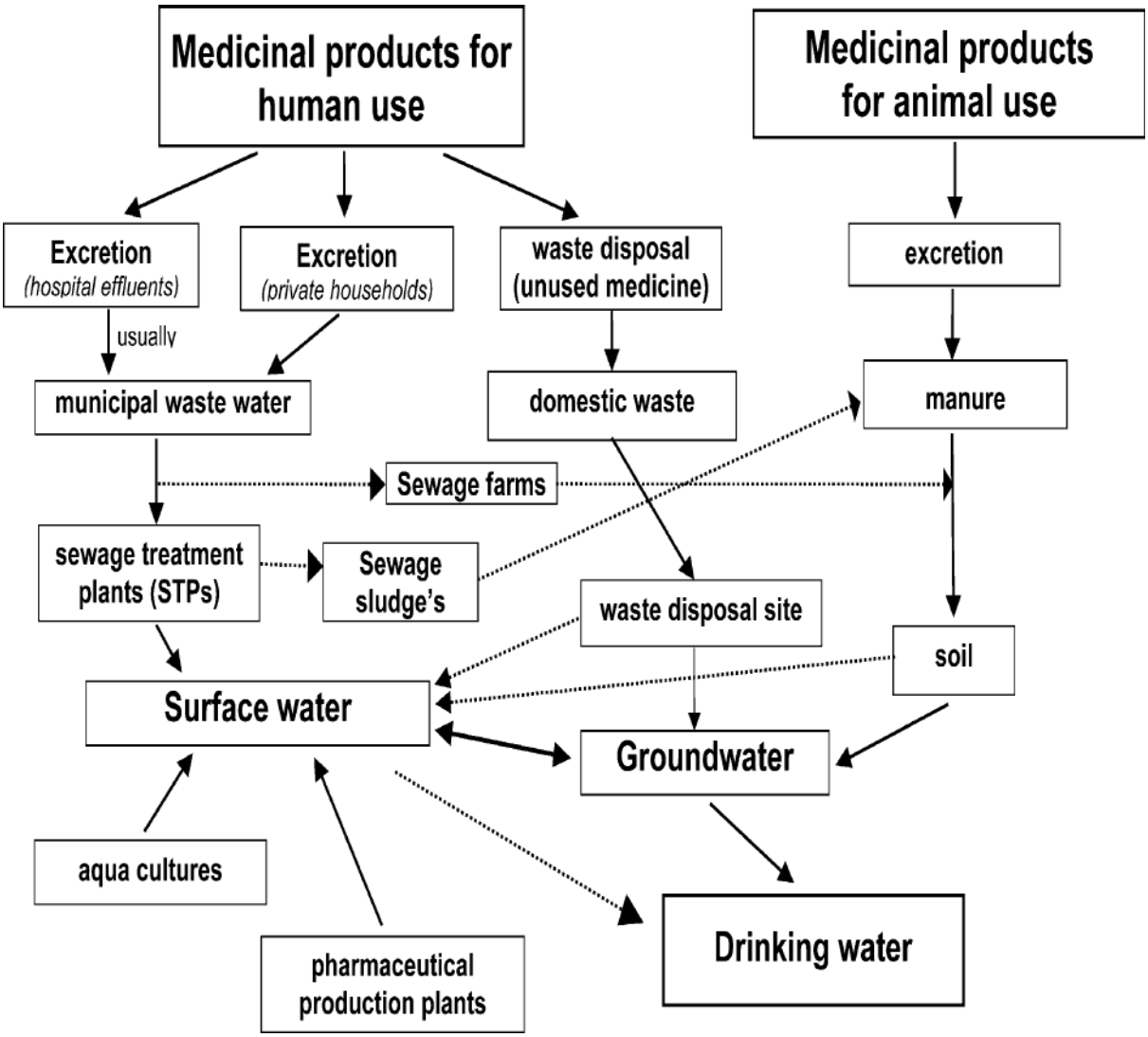

Figure 2.2: Scheme showing possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment. (**Source:** Thomas Heberer, 2002).

2.2.2 Primary sources

2.2.2.1 Residential households

Pharmaceuticals gets into the environment majorly through residential households (Kümmerer 2009c) as a result of consumption at homes. The parent compounds and the metabolites are released into the sewerage system (McClellan and Halden 2010; Pérez and Barceló 2007; Ternes 2000). Huggett et al., (2003) investigated the contribution of different sources to pharmaceutical pollution of the environment in Taiwan and found

that residential household contributions was highest especially for erythromycin and cephradine with concentrations of 705 ngL^{-1} and 128 ngL^{-1} respectively. However, 95 other drugs were found in elevated concentrations from erstwhile sources including hospitals, aquacultures, pharmaceutical manufacturing facilities, and sewage treatment plants implying that other sources maybe greater contributors than previously thought. Similarly, 24 compounds were found from 4 livestock STPs, 12 municipal STPs, 4 hospital STPs and 4 pharmaceutical manufacture STPs by Sim et al. (2011) when he investigated the presence of drugs in Korea. Furthermore, concentrations of livestock and pharmaceutical manufacturing STPs were highest compared to others. In addition to excreted compounds, careless dumping of unused or expired drugs may also increase the drug levels from residential households. In 2005, Bound and Voulvoulis interviewed 400 inhabitants of a household mainly in South-East of England and found that the major route of disposal of unused or expired medicines is via the sink, household waste or toilet. Further study by Seehusen and Edwards (2006) on patient disposal of medicines showed that many agreed to flush down expired and unused medicines down the sewer and only less than 23 % of patients reported to return drugs to pharmacy for proper disposal.

2.2.2.2 Hospitals

The hospital is known to be a place where all types of therapeutic drugs are dispensed and used, hence, found in hospital wastewater treatment plants at nanogram per litre to microgram per litre concentration levels (Kümmerer 2001; Lin and Tsai 2009). In the past, hospital wastewaters were thought to have lower levels of pharmaceuticals compared to household and industrial wastewaters (Kümmerer 2009). Though, the effluents are diluted by municipal wastewaters (Kümmerer and Helmers 1997, 2000)

therefore contributing less than 1 % of the total municipal sewage (Kümmerer 2008). However, studies using large scale and mass loading sampling methodology showed different results. Lin et al. in 2008 found elevated concentration of cephalexin (2457 ngL^{-1}) from hospital effluents than other sources including drug production facilities (27 ngL^{-1}), aquaculture (12 ngL^{-1}) and STPs (283 ngL^{-1}). A similar comparison study was done by Langford and Thomas (2009) at two Norwegian hospitals and found that 6% acetaminophen measured in STP influent came from Rikshospitalet and Ullevål hospitals. Nagarnaik et al. (2010, 2011) studied other compounds including metoprolol, sertraline and ibuprofen and deduced that they contribute less than 1 % while propranolol contributed about 7.2 %, indicating that hospitals contribute more to pharmaceutical contamination of the environment than nursing homes facilities, assisted living facilities and independent living facilities.

2.2.2.3 Care homes

Drugs are supplied in care homes to patients for the management of illnesses. Much of these medications given are often on refill prescriptions and prescribed for a long period of time and sometimes for the lifespan of the patient. In New Mexico, Brown et al. (2006) found 23.5 μgL^{-1} and 1.3 μgL^{-1} ofloxacin in wastewater from elderly homes and assisted living facility respectively. In addition, cardiovascular drugs were found by Nagarnaik et al., (2010) when the effluents from nursing homes, independent living facilities and assisted living facilities all in New Mexico were analysed. For the nursing homes the following drugs were found metoprolol (1584 ngL^{-1}), diltiazem (2708 ngL^{-1}), furosemide (1030 ngL^{-1}), desmethyl diltiazem (2118 ngL^{-1}) from independent living facilities: Hydrochlorothiazide (3636 ngL^{-1}), valsartan (4916 ngL^{-1}), atenolol (11326 ngL^{-1}), diltiazem (2886 ngL^{-1}), and gemfibrozil (1152 ngL^{-1}) and assisted living

facilities: norverapamil (2829 ngL^{-1}), atenolol (4783 ngL^{-1}), and valsartan (8727 ngL^{-1}) from nursing homes.

Additional investigation by Nagarnaik et al. in 2011, revealed the presence of neurological drugs in wastewater from the different homes. Fluoxetine and amitriptyline were only found in nursing home effluents at concentrations 180 ngL^{-1} and 290 ngL^{-1} respectively, oxycodone (8 ngL^{-1}), propoxyphene (26 ngL^{-1}), sertraline (110 ngL^{-1}), carbamazepine (30 ngL^{-1}), amitriptyline (190 ngL^{-1}), 10-hydroxy-amitriptyline (32 ngL^{-1}), fluoxetine (42 ngL^{-1}), desmethylsertraline (86 ngL^{-1}) and amphetamine (102 ngL^{-1}) were found in assisted living facilities wastewater and amphetamine (120 ngL^{-1}), oxycodone (14 ngL^{-1}), fluoxetine (81 ngL^{-1}), carbamazepine (110 ngL^{-1}), paroxetine (28 ngL^{-1}), amitriptyline (37 ngL^{-1}) and 10-hydroxy-amitriptyline (12 ngL^{-1}) were found in independent living facilities wastewaters.

2.2. 2.4 Manufacturing facilities

Pharmaceutical manufacturing facilities output are most likely to raise the concentration levels in water bodies. Manufacturing industries may directly discharge treated or untreated effluents into water or indirectly via STPs. These discharges may vary in concentrations depending on the production method, capacity, facilities cleaning and the type of pharmaceuticals produced (Scheytt et al., 2005). Although in some countries, the release of pharmaceutical effluents into the environment is not monitored by law, Larsson and Fick in 2009 proposed that Good Manufacturing Practices (GMP) and the country's specific environmental policy would help reduce the discharge into the environment. Sirtori et al. (2009) established that biological treatment is frequently carried out as an end-of-pipe clean-up strategy, and Hoerger et

al. (2009) and Zühlke et al. (2004) infer this as the reason for reduced level of concentrations in European and American manufacturing wastewaters, falling within the limits of Environmental Risk Assessment (ERA) guidelines. However, the reverse is the case outside of Europe and the US, where tremendously high levels of pharmaceuticals have been detected from different sources including STP effluents, manufacturing plants and receiving water bodies as seen in Nigeria (our recent monitoring report-in press).

2.2.2.5 Agriculture

Agriculture over the years have been a vital source of veterinary antibiotics to both aquatic and terrestrial milieu (Boxall et al. 2003). Lee et al., in 2007 reported an increase in livestock production over the past few decades, increased environmental contamination with pharmaceuticals (most especially antibiotics) is therefore a consequent of the increased livestock production. For instance, chlortetracycline use for the treatment of enteritis and leptospirosis in cattle and also, as a growth enhancer was found in fresh manure containing 14 $\mu g\ g^{-1}$ by Elmund et al., (1971). However, in 2010 Furtula et al., revealed that poultry litter contributes to the environmental weight of some antibiotic since they are given as feed additives at ranges of 0.07 to 66 mgL^{-1}. Malintan and Mohd had in 2006 reported eight sulphonamide antibiotics with concentrations between 5.03 ngL^{-1} and 94.95 ngL^{-1} in swine wastewater from three sites in Malaysia. Flaherty and Dodson also in 2005 examined a large area of Australian grasslands and its neighbouring lands for antibiotics and discovered noticeable concentrations (ngL^{-1}). However, there were inconsistencies among result, and they explained that these inconsistencies may be due to the variability in the topography of the catchment area.

2.2.2.6 Aquaculture

Veterinary pharmaceuticals are directly introduced into the aquatic ecosystem via aquaculture. In aquaculture, very few compounds are allowed for the treatment of fish's diseases. Some of these compounds include drugs such as oxytetracycline, amoxicillin, flumequine, sulfamerazine and thiamphenicol. They are regularly administered as feed additives (Bloor 2005) and a greater percentage of these antibiotics (70-80 %) are released through the urine and excreta, others are released from the uneaten medicated feed (Abedini et al. (1998); Haug and Hals, (2000); Martinsen and Horsberg (1995); Samuelsen et al. (2003)). These compounds settle at the bottom of the fish farming structures (e.g. ponds) at low mg kg^{-1} levels (Jacobsen and Berglind 1988; Björklund et al. 1991; Coyne et al. 1994). However, the concentrations may varies depending on sediment types, bacteriological composition and locality (Palmer et al., 2008).

2.2.3 Secondary sources

2.2.3.1 Sewage Treatment Plants

In Europe and North America, buildings are built with adequate sanitation systems connected to sewers to easily collect wastewaters. In these developed economies, industrial, domestic and commercial waste are combined and treated in STPs/WWTPs and the resulting effluents discharged into receiving water bodies. However, the treated waste effluents may still contain pharmaceuticals (Jones et al. 2007; Schultz and Furlong 2008; Togola and Budzinski 2008; Zhang et al. 2007). A very high percentage of pharmaceutical inputs into aquatic ecosystem is from STP discharges, which are dependent on the sources. For instance, hospitals specific drugs will be amplified by the number of hospitals in the STP catchment area (Orti et al. 2010) and the population

covered by the STP will decide the number of drugs in the wastewater (Lin et al. 2009). In many Africa countries buildings have their own soak away systems and when filled vacuum trucks are hired to drain and empty into various water bodies. Wastewater treatment plants are not built to remove pharmaceuticals, hence traces of pharmaceuticals are detected in water bodies (Camacho-Munoz et al. 2010). The treatment employed in STPs can decrease the concentration level between influents and effluents. Furthermore, the number of days spent in the sedimentation tanks, the hydraulic retention time (HRT) and tertiary treatments may impact on the breakdown process of the compounds (Jones et al., 2007).

2.2.3.2 Biosolids

In STPs, pharmaceuticals may congregate and sorbed to biosolids and used as substitutes to agricultural fertilizers. In agriculture fields in England and Wales, annually loads of dewatered municipal biosolids (DMBs) and liquid municipal biosolids (LMBs) are used according to DEFRA (2005). Therefore, Rooklidge (2004) observed that many compounds after accumulating in the soil, percolate or leach into ground water and run off to water bodies and courses. For example, neutral and acidic drugs, antibiotics and bacteriocides, sulphonamide, beta-blockers were applied to soil microplots for 266 d rainfall induced runoff, all studied compounds were found in runoff after the first day of rainfall application (Han et al., 2006).

2.2.3.3 Landfill sites

In landfill sites, where industrial, household and agricultural waste are dumped, pharmaceuticals may accumulate and percolate/leach into the ground water. For instance, Eckel et al. (1993) found an industrial landfill site receiving hospital waste,

was responsible for contaminating a shallow groundwater with phensuximide, pentobarbital and meprobamate. Similarly, a landfill, in Denmark receiving effluents from pharmaceutical manufacturing plant produced a large variety of compounds down a leachate gradient (Barnes et al. 2004; Holm et al. 1995; Scheytt et al., 2005).

2.3 Occurrence of pharmaceuticals in the aquatic environment

2.3.1 Occurrence of analgesics and anti-Inflammatory drugs in STPs / WWTPs

Several analgesics drugs also have anti-inflammatory and antipyretic effects. Tens of thousands of NSAIDs are prescribed for pain management worldwide but much more are sold without prescription as over-the-counter (OTC) drugs. In UK, NSAIDs can be purchase in stores in limited quantities while in Nigeria no limit on the quantity and types of drugs that can be bought. In Germany, paracetamol and acetylsalicylic acid (ASA) are the most common painkiller (Ternes et al., 2013) and are mostly OTC drugs. Ternes, (2010), found 0.22 μgL^{-1} of acetylsalicylic acid in Germany sewage effluents and easily degraded into metabolites and active forms. These metabolites (salicylic acid, ortho-hydroxyhippuric acid and gentisic acid) were found in measured concentrations of 54, 6.8, and 4.6 μgL^{-1} respectively. All were well removed by the municipal STPs, and only salicylic acid was detected at very low concentrations in the sewage effluents. In a similar study Heberer, 2012b, Garcia-Galan et al., 2010, Subedi et al., 2014 reported a mean concentration of 0.04 μgL^{-1} for salicylic acid in sewage effluents, surprisingly the mean influent concentrations of 0.34 μgL^{-1} were recorded. In contrast, much elevated concentrations of salicylic acid 13 μgL^{-1} were found in sewerage effluents in Greece and Spain (Heberer and Feldmann, 2008; Farre et al.,

2011). Residues of salicylic acids are also found in food preservatives and its natural formation may be responsible for its prevalence in the environment.

Other NSAIDs were also detected in STPs, paracetamol for example was found at < 10 % in all the monitored effluent samples in Germany (Ternes, 2008). Kolpin et al (2002a) investigated 142 contaminated streams by municipal sewage effluents in the US, more than 17 % of analysed samples are paracetamol at measured concentration of 10 μgL^{-1}. In Berlin, (Heberer et al., 2011), monitored pharmaceuticals in STP and surface water and found diclofenac as most commonly detected (17 % removal rate) with average concentration of 3.02 $\mu gL^{-1}$and 2.51 μgL^{-1} both in effluent and influent respectively. Diclofenac unlike paracetamol and ASA has low removal rate hence, its persistence in the environment. In a similar report by Zhang et al., (2012), 69 % removal rate was reported in sewage treatment plants for diclofenac (Table 2.1) Surface water and sewage effluents from the US, UK, Sweden, Spain, Brazil, Canada, Italy, Austria, France, Germany, Czech Republic, Greece, and Switzerland, were monitored, concentrations up to μgL^{1} of diclofenac was reportedly detected (Ahrer et al., 2009; Andreozzi et al., 2013a; Buser et a1.,2008; Daughton and Ruhoy, 2009a; Drewes et al., 2009, 2013; Farre et al., 2011; Heberer, 2012b, 2012a; Koutsouba et al., 2003; Maltby et al., 2002; Gunnarsson et al., 2008; Oilers et al., 2011; Kasprzyk-Horden and Baker, 2012; Soulet et al., 2002; Stumpf et al., 2009; Ternes, 2008; Tixier et al., 2003; Tran et al., 2016,2017, 2018; Watkinson et al., 2009). When sewage effluents samples in UK, Italy Austria, Canada, Germany, Brazil, France, Greece, and Switzerland were analysed, ibuprofen was detected generally at low concentrations when compared with diclofenac (Andreozzi et al., 2013a; Buser et al., 2008; Gans et al., 2012; Heberer et al., 2011; Maltby et al., 2002; Oilers et al., 2011; Ternes, 2008; Tran et al., 2017;

Samuelsson et al., 2006; Witer et al., 2013). This may be due to faster degradation of ibuprofen during sewage processing (Zwiener and Frimmel, 2013). The concentration of ibuprofen in Switzerland sewage influents was >3 μgL^{-1} and in the effluents was 0.5 μgL^{-1} (Kasprzyk-Horden and Baker, 2012) and < 1.3 μgL^{-1} in STP effluent and <100 ngL^{-1} in surface water (Tixier et al., 2013). In sewage effluent in Sweden, ibuprofen (7.11 μgL^{1}) was detected but diclofenac was not found (Andreozzi et al. 2013a). Ibuprofen was also monitored in sewage effluent samples collected from the US, it was detected at concentrations range of <300 ngL^{-1} - 3.38 μgL^{-1} (Drewes et al., 2012, 2013). Farre et al. (2011), found higher value of 85 μgL^{-1} for ibuprofen in sewage samples obtained in Spain, (2.81 μgL^{-1} - 5.77 μgL^{-1}) in influent and (0.91 μgL^{-1} - 2.10 μgL^{-1}) in effluent samples from Spanish municipal STPs (Rodriguez et al. 2003). However, higher value of 2.7 μgL^{-1} was also recorded in the same study for surface water.

Ibuprofen can be biologically transformed into its metabolites in human body (hydroxy- and carboxy-ibuprofen and to carboxy-hydratropic acid). These metabolites are detected together with ibuprofen and as a mixture (Stumpf et al., 2008) in raw sewage (Buser et al., 2009). Substantial quantity of ibuprofen, particularly the metabolite (carboxy-ibuprofen), was removed (96 % - 99.9 %) in the sewage treatment plants (Buser et al., 2009). Hughes et al. (2013) reported mean detection frequency of 63.0 % for ibuprofen.

In addition, other group of NSAIDs has been found in wastewater and/or sewerage. Some of these compounds include indomethacin, ketoprofen, 4-aminoantiyrine, propyphenazone, phenylbutazone, fenoprofen, codeine, aminophenazone, hydrocodone, phenazone, naproxen, mefenamic acid, flurbiprofen and their metabolites (Aydin and Talini, 2012; Ahrer et al., 2001; Andreozzi et al., 2003a; Amdany, et al., 2014; Boyd

and Grimm, 2009; Brook et al., 2003a; Desbrow et al., 2012; Farre et al., 2010; Gans et al., 2002; Gogoi et al., 2018; Heberer, 2012b, 2011a; Miao et al., 2010; Ollers et al., 2011; Rodriguez et al., 2013; Schmidt and Soulet et al., 2002; Kasprzyk-Horden and Baker, 2012; Stumpf et al., 2009, 2008; Ternes, 2010, 2008; Tixier et al., 2013; Yang et al., 2017).

Table 2.1: Concentration range of selected pharmaceuticals in raw influent (ng/L) and treated effluent (ng/L) from WWTPs in different geographical regions of the world. (**Source:** Tran et al., 2018)

Selected pharmaceuticals	Asia			North America			Europe		
	Influent	**Effluent**	**Reference**	**Influent**	**Effluent**	**Reference**	**Influent**	**Effluent**	**Reference**
Antibiotics									
Amoxicillin	<MQL 6516	<MQL 1670	Trans et al., (2016); Mutiyah & Mittal, (2013); Matsuo et al., (2011); Minh et al., (2009)	NR	<MQL	Palmer et al. (2008)	<MQL	<MQL-190	Papageorgiou et al. (2016); Zuccato et al. (2005); Dinh et al. (2017)
Azithromycin	1537 303,500	60.1-980	Trans et al., (2016) Mohapatra et al., (2016)	61-2500	57 -1300	Guerra et al. (2014)	77 1139	38-784	Senta et al. (2013); Gobel et al. (2007); Miege et al. (2009)
Ceftazidime	<MQL	<MQL	Trans et al., (2016)	NA	NA	NA	NA	NA	NA

Chloramphenicol	<MQL 2430	<MQL-1050	Trans et al., (2016); Minh et al., (2009); Sui et al. (2011); Peng et al.,(2006)	NA	NA	NA	<MQL - 319	<MQL	Gracia-Lor et al. (2012); Kasprzyk-Hordern et al. (2009),
Chlortetracycline	2333 - 15,911	<MQL - 1986	Trans et al., (2016); Minh et al., (2009); Yang et al. (2017); Behera et al.(2011);Choi et al. (2008)	<MQL-310	<MQL-420	Guerra et al. (2014); Karthikeyan, Meyer (2006)	NR	<MQL	Dinh et al. (2017)
Ciprofloxacin	15.5-6453	<MQL 524.1	Trans et al., (2016); Mohapatra et al. (2016); Tewari et al., (2013); Yang et al., (2017)	<MQL 246,100	<MQL – 620	Guerra et al. (2014); Karthikeyan, Meyer (2006);Brown et al. (2006); Miao et al. (2004)	<MQL - 13,625	<MQL - 5692	Papageorgiou et al. (2016); Senta et al. (2013); Miege et al. (2009); Rosal et al. (2010)
Clarithromycin	26 – 1854	4.79-637.1	Trans et al., (2016); Yang et al. (2017)	<MQL - 8000	130 - 7000	Guerra et al. (2014); Miao et al. (2004)	0.4-647	25-359	Senta et al. (2013); Gobel et al. (2007); Miege et al. (2009)

Clindamycin	23.8-26.6	2.94-4.24	Trans et al., (2016)	NA	NA	NA	<MQL-101	10-180	Gracia-Lor et al. (2012); Gurke et al. (2015)
Enrofloxacin	<MQL	<MQL	Sun et al. (2016)	5.9-250	3.5-270	Guerra et al. (2014); Karthikeyan, Meyer (2006)	<MQL-18	<MQL-636	Dinh et al. (2017); Kishida and Furusawa (2004)
Erythromycin	111.4-403.3	70-186.6	Trans et al., (2016)	NA	NA	NA	<MQL-2130	<MQL-290	Papageorgiou et al. (2016); Miege et al. (2009); Rosal et al. (2010)
Erythromycin - H_2O	226 20,600	194.5-14,400	Trans et al., (2016); Minh et al., (2009); Yang et al. (2017); Gulkowska et al. (2008)	<MQL - 3900	<MQL – 838	Guerra et al. (2014); Karthikeyan, Meyer (2006); Miao et al. (2004)	24 - 6755	15-2841	Senta et al. (2013); Gobel et al. (2007); Kasprzyk-Hordern et al. (2009)
Lincomycin	<MQL 19,401	3.92e21,278	Trans et al., (2016); Yang et al. (2017); Subedi et al. (2015); Behera et al. (2011)	<MQL-360	4.9-510	Guerra et al. (2014); Karthikeyan, Meyer (2006); Brown et al. (2006); Yang et al. (2011)	<MQL 281	<MQL	Papageorgiou et al. (2016)

Meropenem	264.8-433.6	27-67.9	Trans et al., (2016)	NA	NA	NA	NA	NA	NA
Minocycline	730.9-3808	<MQL	Trans et al., (2016)	<MQL	<MQL	Guerra et al. (2014)	NA	NA	NA
Ofloxacin	54.8-1274	13.3-7870	Minh et al., (2009); Yang et al. (2017); Sun et al. (2016); Lin et al. (2009)	470-1000	<MQL-506	Brown et al. (2006); Miao et al. (2004)	NR	71-8637	Dinh et al. (2017)
Oxytetracycline	<MQL-30049	<MQL-2014	Trans et al., (2016); Minh et al., (2009);Yang et al. (2017); Sun et al. (2016)	<MQL-47,000	<MQL-4200	Guerra et al. (2014); Karthikeyan, Meyer (2006); Miao et al. (2004)	<MQL-7	<MQL-5	Pailler et al. (2009)
Sulfamethazine	<MQL-1814	<MQL-260.8	Trans et al., (2016); Minh et al., (2009); Yang et al. (2017); Behera et al. (2011)	<MQL 300	<MQL-363	Guerra et al. (2014); Karthikeyan, Meyer (2006); Miao et al. (2004)	<MQL-680	<MQL	Miege et al. (2009); Gracia-Lor et al. (2012); Pailler et al. (2009)

Sulfamethoxazole	3.0-1389	<MQL-562	Trans et al., (2016); Minh et al., (2009); Yang et al. (2017); Behera et al. (2011); Choi et al. (2008)	<MQL-4200	<MQL - 1800	Palmer et al. (2008); Guerra et al. (2014); Karthikeyan, Meyer (2006); Miao et al. (2004)	<MQL-11555	<MQL-544	Papageorgiou et al. (2016); Senta et al. (2013); Gobel et al. (2007); Miege et al. (2009); Gurke et al. (2015); Kosma et al. (2014)
Tetracycline	<MQL-12340	<MQL-1536	Trans et al., (2016); Minh et al., (2009); Yang et al., (2017); Sun et al., (2016); Gulkowska et al., (2008)	<MQL-48000	<MQL-3600	Guerra et al. (2014); Karthikeyan, Meyer (2006); Miao et al. (2004)	<MQL-790	<MQL-850	Dinh et al. (2017); Miege et al. (2009); Pailler et al. (2009)

Trimethoprim	19.5-570	3.7-772	Trans et al., (2016); Sui et al., (2011); Tewari et al. (2013); Yang et al. (2017); Gulkowska et al. (2008)	<MQL-6796	<MQL-37000	Palmer et al. (2008); Guerra et al. (2014); Karthikeyan, Meyer (2006); Brown et al. (2006)	<MQL-4342	<MQL-3052	Papageorgiou et al. (2016); Senta et al. (2013); Gobel et al. (2007); Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Gurke et al. (2015); Kosma et al. (2014)
Tylosin	<MQL	<MQL	Trans et al., (2016); Minh et al., (2009)	<MQL-1500	21-720	Guerra et al. (2014); Karthikeyan, Meyer (2006)	<MQL	<MQL-173	Dinh et al. (2017); Gracia-Lor et al. (2012)
Vancomycin	962 43,740	<MQL	Trans et al., (2016)	NA	NA	NA	NR	<MQL-8514	Dinh et al. (2017)
Antimicrobials									
Miconazole	<MQL-597	<MQL	Yang et al. (2017); Sun et al. (2016)	5.2-43	1.6-27	Guerra et al. (2014)	<MQL-337.9	<MQL-35.7	Casado et al. (2014); Van De Steene et al. (2010)
Thiabendazole	<MQL-1.29	<MQL	Yang et al. (2017); Sun et al. (2016)	6.8-220	6.2-140	Guerra et al. (2014)	NA	NA	NA

Triclocarban	341.1-8880	8.4-5860	Trans et al., (2016); Subedi et al. (2015); Ryu et al., (2014)	340-4644	64-617	Guerra et al. (2014); Hedgespeth et al. (2012)	97-140	NR	Gasperi et al. (2014)
Triclosan	1.3-2500	49.1-263.9	Trans et al., (2016); Subedi et al. (2015); Ryu et al. (2014); Anumol et al. (2016)	14-6817	3.1-360	Guerra et al. (2014); Hedgespeth et al. (2012); Lee et al. (2005)	<MQL-5260	<MQL-430	Miege et al. (2009); Kosma et al. (2014); Gasperi et al. (2014)
NSAIDs									
Acetaminophen	67-147700	<MQL-2568	Tewari et al. (2013); Yang et al. (2017); Sun et al. (2016); Choi et al. (2008); Tran and Gin (2017)	21000-500000	<MQL-62000	Mohapatra et al. (2016); Guerra et al. (2014); Hedgespeth et al. (2012)	<MQL-482687	<MQL-24525	Papageorgiou et al. (2016); Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Kosma et al. (2014); Kosma et al. (2010),
Codeine	<MQL-242	<MQL-208	Sun et al. (2016); Subedi et al. (2015)	77-5700	80-3300	Guerra et al. (2014)	150-32295	9.7-15593	Kasprzyk-Hordern et al. (2009); Rosal et al. (2010); Baker and Kasprzyk-Hordern (2013)

Diclofenac	13-445	<MQL-69.2	Yang et al. (2017); Sun et al. (2016); Behera et al. (2011); Anumol et al. (2016); Tran and Gin (2017)	140-2450	<MQL-359	Yang et al. (2011); Lee et al. (2005); Metcalfe et al., (2003)	<MQL-4869	<MQL-5164	Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Kosma et al. (2014); Kosma et al. (2010); Clara et al. (2005a,b)
Fenoprofen	<MQL-2260	<MQL-23.4	Sun et al. (2016); Tran and Gin (2017)	<MQL	<MQL-405	Metcalfe et al. (2003); Lishman et al. (2006)	NR	<MQL-280	Andreozzi et al. (2003)
Ibuprofen	34.8-55975	<MQL-1890	Tewari et al. (2013); Yang et al. (2017); Sun et al. (2016); Subedi et al. (2015)	2500-45000	16-14600	Palmer et al. (2008); Guerra et al. (2014); Yang et al. (2011); Lee et al. (2005); Metcalfe et al. (2003)	<MQL-83500	<MQL-24600	Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Kosma et al. (2014); Kosma et al. (2010); Clara et al. (2005a,b)
Indomethacin	<MQL-449.4	<MQL-61.4	Sun et al. (2016); Tran and Gin (2017)	<MQL-640	<MQL-507	Lee et al. (2005); Metcalfe et al. (2003); Lishman et al. (2006)	<MQL-297	<MQL	Papageorgiou et al. (2016)

Ketoprofen	<MQL-286	<MQL-183	Sun et al. (2016); Subedi et al. (2015); Behera et al. (2011)	60-150	40-90	Lee et al. (2005)	<MQL-5700	<MQL-1620	Papageorgiou et al. (2016); Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Andreozzi et al. (2003)
Naproxen	<MQL-7762	<MQL-159	Tewari et al. (2013); Sun et al. (2016); Behera et al. (2011); Tran and Gin (2017)	1700-25000	<MQL-3500	Mohapatra et al. (2016); Guerra et al. (2014); Metcalfe et al. (2003); Lishman et al. (2006); Boyd et al. (2003)	<MQL-61100	<MQL-33900	Papageorgiou et al. (2016); Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Kosma et al. (2014); Kosma et al. (2010)
Salicylic acid	167-16900	<MQL-1426	Yang et al. (2017); Tran and Gin (2017)	2820-27800	<MQL-320	Lee et al. (2005); Lishman et al. (2006)	<MQL-164400	<MQL-10100	Papageorgiou et al. (2016); Kasprzyk-Hordern et al. (2009); Kosma et al. (2014); Kosma et al. (2010)
Beta-blockers									

Atenolol	<MQL-294700	<MQL-518.6	Tewari et al. (2013); Sun et al. (2016); Behera et al. (2011);Tran and Gin (2017)	500-2642	<MQL-14200	Palmer et al. (2008); Mohapatra et al. (2016)	<MQL-33106	<MQL-7602	Papageorgiou et al. (2016); Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Gurke et al. (2015
Metoprolol	<MQL-79500	<MQL-268	Mohapatra et al. (2016); Sui et al. (2011); Sun et al. (2016); Behera et al. (2011)	16-154	15-212	Mohapatra et al. (2016)	<MQL-4148	<MQL-5762	Papageorgiou et al. (2016); Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Rosal et al. (2010); Gurke et al. (2015
Propranolol	<MQL-9.56	<MQL-8.3	Sui et al. (2011)	NR	NR	NR	<MQL-1962	<MQL-615	Papageorgiou et al. (2016); Miege et al. (2009); Kasprzyk-Hordern et al. (2009); Rosal et al. (2010); Gurke et al. (2015)
Anticonvulsants									

Carbamazepine	<MQL-18500	<MQL-900	Yang et al. (2017); Sun et al. (2016); Subedi et al. (2015); Behera et al. (2011); Tran and Gin (2017)	<MQL-440	28-551	Palmer et al. (2008); Mohapatra et al. (2016); Yang et al. (2011); Metcalfe et al. (2003); Glassmeyer et al. (2005)	<MQL-3110	<MQL-4596	Papageorgiou et al. (2016); Miege et al. (2009);Kasprzyk-Hordern et al. (2009); Gurke et al. (2015); Kosma et al. (2014); Clara et al. (2005a,b);Kahle et al. (2009)
Gabapentin	4825.5-15359	213-8855	Tran and Gin (2017)	NR	1000 ± 900	Writer et al. (2013)	6442-25079	7651-56810	Kasprzyk-Hordern et al. (2009); Gurke et al. (2015)
Sulpiride	64.9-15,358.8	70.7-322.4	Sui et al. (2011); Tran and Gin (2017)	NR	3.30E+138	Gagne et al. (2006)	113-1100	110-294	Bollmann et al. (2016)
Lipid regulators									

Bezafibrate	16.8-159	<MQL-51.4	Sui et al. (2011); Sui et al. (2009)	NR	65-359	Metcalfe et al. (2003)	100-7600	<MQL-4800	Miege et al. (2009); Rosal et al. (2010); Gurke et al. (2015); Kosma et al. (2014); Clara et al. (2005a, b);Clara et al. (2005a,b),
Clofibric acid	<MQL-65	<MQL-44.9	Sui et al. (2011); Yang et al. (2017); Sun et al. (2016); Behera et al. (2011); Tran and Gin (2017), Sui et al. (2009)	<MQL	<MQL-44	Metcalfe et al. (2003); Lishman et al. (2006)	<MQL-265.9	<MQL-91	Papageorgiou et al. (2016); Kasprzyk-Hordern et al. (2009); Rosal et al. (2010); Kosma et al. (2014)
Gemfibrozil	<MQL-453.4	<MQL-535.2	Sui et al. (2011); Yang et al. (2017); Sun et al. (2016); Behera et al. (2011); Sui et al. (2009); Sui et al. (2010)	<MQL-36530	<MQL-1493	Metcalfe et al. (2003); Lishman et al. (2006)	<MQL-17055	<MQL-5233	Papageorgiou et al. (2016); Rosal et al. (2010); Kosma et al. (2014); Kosma et al. (2010)
Hormones									

Estrone	<MQL-132.5	<MQL-51.2	Behera et al. (2011); Tran and Gin (2017); Huang et al. (2013); Chang et al. (2011)	8-52	<MQL-56	Palmer et al. (2008); Hedgespeth et al. (2012); Lee et al. (2005); Lishman et al. (2006)	2.4-670	<MQL-95	Miege et al. (2009); Clara et al. (2005a, b); Migowska et al. (2012)
Estriol	<MQL-802	<MQL-30.2	Behera et al. (2011); Tran and Gin (2017): Huang et al. (2013)	<MQL-217	<MQL	Hedgespeth et al. (2012); Yu and Chu (2009)	<MQL-660	<MQL-275	Miege et al. (2009); Clara et al. (2005a, b);Migowska et al. (2012)
17a-ethinylestradiol	<MQL-26.1	<MQL-13.1	Huang et al. (2013)	<MQL-242	<MQL	Yang et al. (2011); Yu and Chu (2009)	0.4-70	0.5-106	Miege et al. (2009); Clara et al. (2005a, b)
X-ray contrast media									
Iohexol	63.8-124,966	2100-8700	Yang et al. (2017); Ryu et al. (2014); Anumol et al. (2016); Tran and Gin (2017)	NR	8623-9237	Nelson et al. (2010)	18000 ± 2000	1200 ± 100	Kormos et al. (2011)
Iopromide	47.7-12,200	<MQL-7140	Yang et al. (2017); Ryu et al. (2014)	NA	NA	NA	<MQL-7500	<MQL-9300	Miege et al. (2009);] Clara et al. (2005a, b)

Iopamidol	82.8-45,611	<MQL-6520	Yang et al. (2017); Ryu et al. (2014); Tran and Gin (2017)	NA	NA	NA	4300 ± 900	4700 ± 1000	Ternes and Hirsch (2000)
Stimulant									
Caffeine	759-60500	13-51700	Sui et al. (2011); Tewari et al. (2013); Yang et al. (2017); Subedi et al. (2015); Sui et al. (2010)	5809-82882	<MQL-37200	Palmer et al. (2008); Mohapatra et al. (2016); Hedgespeth et al. (2012)	102-113200	30-13900	Papageorgiou et al. (2016); Kosma et al. (2010); Buerge et al. (2003)

NA= not available in the literature
NR= not reported
MQL= method quantification limit

2.3.2 Occurrence of antibiotics/bacteriostatic drugs in STPs / WWTPs

In the last 30 years, antibiotics (Table 2.1) has been acknowledged as a contaminant of the river system because of their negative effects on aquatic systems (Kummerer, 2009). Though, the greatest challenge of discharging antibiotics to the environment is perhaps related to the development of the antibiotics resistance genes (ARGs) and antibiotics resistance bacteria (ARB), which decreases the therapeutic potential against animal and human pathogens. Studies has shown that 50% to 90% of antibiotics dispensed by man are excreted through urine and faeces (Kummerer, 2009; Tran et al., 2016).

Experiments have been done in Germany (Christian et al., 2003; Ternes et al., 2013; Hirsch et al., 1999; Steger-Hartmann et al., 1997), UK (Kasprzyk-Horden et al., 2017), Singapore (Tran et al., 2018), Sweden, (Andreozzi et al., 2003a), Austria (Gans et al., 2002), Italy, France, Greece, and the U.S. (Lindsey et al., 2001; Kolpin et al., 2012a) to study the occurrence of antibiotics in WWTPs. Many therapeutic classes of antibiotics have been detected in influent and treated effluents, although at low μgL^{-1} levels from samples collected from WWTPs from different countries of the world. Examples of such antibacterial drugs include sulfonamides (sulfadimidine, sulfamethazine and sulfathiazole), macrolide antibiotics (dehydroerythromycin, clarithromycin, azithromycin, roxithromycin, tylosin, clindamycin, and lincomycin) (Table 2.1). These agents are released into WWTPs from whence they can be removed or passed into the environment. The number of pharmaceuticals found in influents and effluent samples vary depending on factors such as the compound, usage pattern, sewer systems, weather conditions, river catchment features, persistency in the environment and the efficiency of the elimination process in the WWTPs (Luo et al., 2014; Tran et al.,

2016). Other factors include sampling methodology, sampling dates and sites and the type of wastewater sampled. Furthermore, from the Table 2.1, the level of antibacterial agents in the influent and effluent samples was greatly affected by the region, from less than the method quantification limit (MQL) to a few tens of micrograms per litre. Trimethoprim, macrolides, fluoroquinolones and sulphonamides were often found in both influent and effluent sampled. However, the presence of ceftazidime, amoxicillin, and meropenem (beta-lactams); minocycline, chlortetracycline and oxytetracycline (tetracyclines); vancomycin and chloramphenicol are nearly absent from influents and effluent samples assayed in European and North American, although they were present in samples from Asian continents (Minh et al., 2009; Mutiyar and Mittal, 2013; Tran et al., 2016).

In hospital effluents for example, Alder et al., (2011) and Hartmann et al., (2008) both detected elevated levels of antibiotics in hospital effluents. Hartmann et al. in 2008 also found 3-87 μgL^{-1} of ciprofloxacin and fluoroquinolone in hospital effluents. However, no traces of penicillin and tetracyclines were found when different sewage and groundwater samples were examined by Hirsch et al. (1999). The difference might be due to penicillin and tetracyclines precipitating and hydrolysing easily (Daughton and Ternes, 2007; Santos et al., 2010). A study conducted by Kolpin et al. (2002a) and Glassmeyer et al., (2005) reported the presence of tetracyclines in effluents and surface water samples in the US while Christian et al. (2010) reported a contrasting result from river water samples from North Rhine-Westphalia, although tetracyclines were not observed, five beta-lactam antibiotics (piperacillin, amoxicillin, ampicillin, mezlocillin, and flucloxacillin) were found only at minimal levels of less than 10 ngL^{-1}. Fluoroquinolone antibiotics was assayed in wastewater effluents in Switzerland, Golet

et al. (2011) found that ciprofloxacin and norfloxacin occurred at concentration levels of 249-405 ngL^{-1} and 45-120 ngL^{-1} respectively in the collected samples. A year later, Golet et al. (2012), also detected ciprofloxacin and norfloxacin in surface water at concentrations <19 ngL^{-1}. Christian et al. (2010) agrees with these results when he intermittently found ofloxacin and ciprofloxacin in German river water samples at amounts <21 ngL^{-1}. Christian et al., (2010) also conducted a similar study where he sampled surface water and frequently found macrolide antibiotics (clarithromycin, azithromycin, roxithromycin and clindamycin) and at similar concentrations to what was reported for the quinolones. Whereas tylosin, a drug used exclusively in animal medicine in the U.S., U.K. and Germany, was found only in one fresh water sample at a 90 ngL^{-1}concentrations. The most ubiquitous antibiotic was dehydroerythromycin and was recorded to occur at 300 ngL^{-1} peak concentrations. The moderately elevated amounts of erythromycin and its metabolites when compared to the other macrolide was attributed to its common use as topical agents against acne and other skin infections (Christian et al., 2010), because topical applications of this compound may cause reduced metabolism and reabsorption of the drug. In another experiment, sulfamethoxazole and trimethoprim were other compounds detected frequently at less than 100 ngL^{-1}.

2.3.3 Occurrence of antiepileptic and antipsychotic drugs in STPs / WWTPs

Epileptic patients are often depressed, antiepileptic and antipsychotic drugs such as carbamazepine, gabapentin, and sulpiride are administered to such patients to combat depression. Studies conducted in Europe and America have shown that these compounds are the mostly found in the influents and effluents of WWTPs (Behera et al., 2011; Gurke et al., 2015; Kasprzyk-Hordern et al., 2009; Subedi et al., 2015; Sun et

al., 2016; Tran and Gin, 2017; Writer et al., 2013; Yang et al., 2017). Antiepileptic and antipsychotic drugs are the third highest most prescribed and consumed drugs by Americans especially people within the age bracket of 18 years to 44 years (Pailler et al., 2009). They are detected in varying levels in sewage (influents and effluents) and surface water samples (Ahrer et al., 2011; Andreozzi et al., 2013a; Desbrow et al., 2012; Gans et al., 2012; Tixier et al., 2013; Tran et al., 2018). Table 2.1 also shows the various concentrations of antiepileptic drugs found in influents and effluents of WWTPs in different geographical regions of the world and variations from <MQL to upper ten thousand of ngL^{-1} (Bollmann et al., 2016; Gurke et al., 2015; Kosma et al., 2014). Investigations conducted by Gans et al. in 2012, Heberer in 2012b and Ternes in 2008 revealed that during sewage treatments, less than 10 % of carbamazepine was removed in influent and effluent samples collected from various municipal STPs. Carbamazepine was also found to be slightly persistent in contaminating surface waters in Switzerland and Germany (Heberer, 2012b; Tixier et al., 2013) and at elevated levels of <1075 ngL^{-1}. Another epileptic drug, gabapentin, was also found in surface water samples (635 ngL^{-1}) from STPs influents and effluents in Germany and UK (Gogoi et al., 2018; Hughes et al., 2013). Gabapentin has equally been found in secondary and tertiary treated effluents in the US at concentrations ranging from 100 - 200 ngL^{-1}(Desbrow et al., 2012 and Tran et al., 2018).

2.3.4 Occurrence of beta blockers in STPs / WWTPs

Beta blockers have been found in wastewater (influent and effluent) samples in all geographical region of the world in varying quantities (Table 2.1). They are used in the treatment of blood pressure, examples include acebutolol, metoprolol, betaxolol, propranolol, and nadolol (Andreozzi et al., 2013a; Huggett et al., 2013; Mohapatra et

al., 2016; Kasprzyk-Horden and Baker, 2012; Sun et al., 2016; Tran and Gin, 2017; Wilkinson et al., 2016). Mohapatra et al. (2016), for example reported the concentrations of atenolol in the WWTPs in India to be up to 294700 ngL^{-1}, which he attributed to the disposal of unused drug.

2.3.5 Occurrence of blood lipid regulators in STPs / WWTPs

Kasprzyk-Hordern et al., (2009); Kosma et al., (2010, 2014) and Rosal et al., (2010) had previously reported blood lipid regulators such as gemfibrozil and bezafibrate were often found in influents and effluents of WWTPs. The varying quantities of lipid regulating drugs in influent and effluent of WWTPs are shown in Table 2.1. The concentration levels vary from <MQL - 17055 ngL^{-1} and from <MQL to 5233 ngL^{-1} respectively, depending on the compound and sampling areas (Kasprzyk-Hordern et al., 2009; Kosma et al., 2010, 2014; Papageorgiou et al., 2016). For example, in the mid-seventies, the first compound of pharmaceutical origin (clofibric acid) was discovered in the US influents and effluents samples collected from wastewater treatment plants (WWTPs) by Garrison et al. (1976) and Hignite and Azarnoff, (1977). However, the concentrations of clofibric acid found was relatively low when juxtaposed with bezafibrate or gemfibrozil (Behera et al., 2011; Kosma et al., 2010; Papageorgiou et al., 2016). This stern from its infrequent use when compared with other lipid regulating drugs such as gemfibrozil, simvastatin and bezafibrate. Table 2.1 shows the different geographical regions of the world with different lipid regulating drugs and their levels. The differences that exist may be consequent of demographical patterns and rate of obesity in the region. Lipid regulators are used in the prevention of heart diseases that may arise due to consumption of high sugar foods and junks.

2.3.6 Occurrence of contrast media in STPs / WWTPs

Gagne and Andre, (2006) identified iodinated x-ray contrast media that are applied majorly in the hospitals and during surgeries as key addition to the total adsorbable organic halogens (AOH) in hospital wastewaters. Oleksy-Frenzel et al. in 2000 found high levels of adsorbable organic iodine (AOI) (130 µgl L^{-1}) in influent and effluent samples from municipal WWTPs in Berlin and a higher level of 10 mgL^{-1} was found in clinical wastewater, with no degradation or only small dilution during sewage purification. They presumed that the aquatic contamination was due to the occurrence of iodinated x-ray contrast media. 39 % of the AOI has been identified in sewage effluents as contrast agents. However, in the U.K., raised AOI values have been detected in surface waters and sewage, but also in bank filtrate and even raw drinking water samples (Putschew and Jekel, 2001). In AOI measured in surface waters, only 18 – 33 % can only be identified (Putschew et al., 2001; Putschew and Jekel, 2001). Although, it was assumed that the bulk of the AOI may consists of numerous other unknown metabolites of iodinated contrast media. Among the presumed metabolites that were analysed only one were identified in the samples (Putschew et al., (2000) and Tran et al., (2017)). The common X-ray contrast agents which include diatrizoate iopamidol, iohexol, iomeprol and iopromide were detected up to μgL^{-1} concentrations in influents and effluents of wastewater (Table 2.1), ditto for the surface water samples in diverse parts of the world (Gogi et al., 2018; Putschew et al., 2001; Ternes et al., 2013). Sometimes, ioxithalamic and iothalamic acids have been found at ngL^{-1} levels in influents and effluents of surface waters and STPs (Ternes and Hirsch 2000). The quantity of the X-ray contrast media in the aquatic environment are significantly increased on weekdays, because X-rays examination in hospitals and radiological

practices are only done from Monday to Friday (Ternes and Hirsch, 2000) and hence, appreciable increase on weekdays as pollutants. Ternes and Hirsch (2000) reported that the iodinated X-ray contrast media are found in large quantities in STP effluents when compared with other drugs. Nevertheless, when the maximal levels were considered, the pollution was not as great as expected. The x-ray contrast median concentration was measured by Ternes and Hirsch in 2000 to be <0.75 μgL^{-1} and are at least one order of magnitude less than the corresponding maximum concentration levels. Ternes and Hirsch (2009) reported finding iothalamic and ioxithalamic acids in some samples at low ng L^{-1} concentrations. Several other studies also reported the presence of iopromide, iopamidol, and diatrizoate in influents and effluents samples (Putschew et al. 2010). Many contrast media are not biodegradable in the aquatic environment, however, an investigation conducted by Steger-Hartmann et al. (2002), revealed that iopromide can be oxidized by conventional ozonation. Ternes et al. (2003), however, found iodinated x-ray contrast media iopamidol, iopromide, iomeprol and diatrizoate, at substantial concentrations. The ionic diatrizoate only demonstrated removal efficiencies of less than 14 % while 80 % removal was exhibited by non-ionic iodinated X-ray media (ICM) (Ternes et al., 2013). Gadolinium (Gd), is used in organic magnetic resonance in hospitals and is discharged via hospital wastes and public sewerage systems (Kummerer, 2001). It is a rare earth element, detected in hospital effluents at high concentrations up to 100 μgL^{-1}(Bau and Dulski, 1996).

2.3.7 Occurrence of cytostatic drugs in STPs / WWTPs

Drugs used during chemotherapeutic management of diseases are called cytostatic drugs. Consequent of their usage in the hospitals, Steger-Hartmann et al., (2007) found recurrently the residues of such drugs at μgL^{-1} concentration levels. Cytostatic drugs

have also been found although, in trace levels in effluent samples from hospital municipal STPs (Kummerer et al., 2007; Kasprzyk-Horden et al., 2007; Ternes, 2008). Steger-Hartmann et al. (2006) in a study on the university hospital sewage samples, found both cyclophosphamide and ifosfamide at 146 and 24 ngL^{-1} concentrations respectively. Kummerer et al. (2007) detected ifosfamide at average concentrations of 109 ngL^{-1}in an oncology hospital effluent while Ternes, (2008) detected cyclophosphamide at maximal levels of 20 ngL^{-1} in 4 out of 16 sampled STP effluents. However, he found ifosfamide in only 2 samples and the concentration of one sample was 2.9 μgL^{-1}. The predicted environmental concentration (PEC) value for ifosfamide was 0.8 ngL^{-1} in surface waters. These class of drug are highly mutagenic, embryotoxic and carcinogenic due to their high pharmacological potency (Kasprzyk-Horden et al., 2007).

2.3.8 Occurrence of oral contraceptives in STPs / WWTPs

Oral contraceptives are synthetic steroids with high pharmacological potency. They are often prescribed, however, the sale of this class of drug annually is low and hence they are only found in trace amounts (low ngL^{-1} range) in sewage effluents (Tran et al., 2018). This assumption has been supported by several studies carried out on STPs in Western Europe and South America (Adler et al., 2010; Baronti et al., 2000; Belfroid et al., 2009; Bruchet et al., 2002; Heberer, 2012b; Huang and Sedlak, 2011). Mestranol was only found intermittently at concentrations <4 ngL^{-1} (Spengler et al., 2009 and Ternes et al., 2009a). EE2 was found at mean concentration of 17 ngL^{-1} by (Santos et al., 2010) in a study of 20 sewage effluent samples. Several other investigations had been conducted by numerous authors across Europe and the US and the EE2 values ranges between 1-3 ngL^{-1} or less, below the analytical detection limit (Table 2.1)

(Bueno et al., 2012; Tran et al., 2018; Verlicchi et al., 2012; Yang et al., 2011; Zhang et al., 2012). The Canadian sewage effluent sample for EE2 was reported to be higher with a mean level of 9 ngL^{-1} by Ternes et al., (2009). However, much higher values for EE2 detected in streams in the US (Kolpin et al. 2012a) at <831 ngL^{-1} median value; 73 ngL^{-1} excluding non-detects. The contraction has been the subject of discussion (Ericson et al., 2010; Kolpin et al., 2012b). Six activated sludge STPs near Rome, Italy, was studied by Baronti et al. (2010), the mean concentrations of 3.0 ngL^{-1} was found for EE2 influent samples and 0.45 μgL^{-1} was detected for effluent samples. Baronti et al. (2010), still in the same study, recorded the removal rate of 85 % for EE2 while Zucchi et al., 2014 observed removal rates of 75.7 % and an average level of 2.0 ngL^{-1} in the effluents of STP in Berlin, Germany after secondary treatment including nitrification and denitrification. Above the analytical limit of quantitation of 0.4 ngL^{-1}, EE2 was not detected in effluents from another municipal STP in Berlin, Germany, operating with similar technology (Verlicchi et al., 2012).

2.3.9 Occurrence of pharmaceuticals in surface water

Effluent from STPs is the principal route through which pharmaceuticals enter surface water in the UK (Gardner et al., 2012; Wilkinson et al., 2017), the US (Spongberg et al., 2011), Italy (Meffe and De-Bustamante, 2014), and Africa (Wood et al., 2015) and congregate in the aquatic system (Luo et al., 2014) (Table 2.2).

The presence of 36 pharmaceuticals in surface water samples from Beijing, Changzhou, and Shenzhen in China was investigated by Wang et al. 2015 and 28 compounds were found. Sulfadimethoxine (164 ngL^{-1}), sulpiride (77.3 ngL^{-1}), atenolol (52.9 ngL^{-1}), and indomethacin (50.9 ngL^{-1}), had the maximum average concentrations. 86 samples of

surface water were collected from coastal sites that receives treated and untreated sewage by Spongberg et al. (2011) in Costa Rica. 34 compounds were analysed, doxycycline (77%), sulfadimethoxine (43%), salicylic acid (41%), and trichlorosilane (TCS) (34%) were most frequently detected. In a similar study of surface water samples collected from Elbe river in Czech, paracetamol was detected at <106 ngL^{-1} (Amdany et al., 2014). However, samples collected from Elbe and Saale rivers in Germany, paracetamol was found at <20 ngL^{-1} (ARGE, 2003). In the same study, acetylsalicylic acid (ASA) median concentration was found below the detection limit. Studies of Czech surface water samples found ibuprofen at 146 ngL^{-1}, ronidazole and metronidazole periodically found at 16 and 44 ngL^{-1} respectively (Amdany., 2014). The metabolites of ibuprofen (carboxy-ibuprofen and hydroxy-ibuprofen) has also been found in surface waters in UK, Germany and the US at median concentration of 0.02-0.34 μgL^{-} (Stumpf et al., 2008). Other compounds found at lower concentrations in surface water samples are metoprolol, bisoprolol and propranolol (Hirsch et al., 2008; Ternes, 2008). The beta-blockers, however, were found regularly in the surface waters samples in Switzerland, with concentrations of up to ng L^{-1} (Alder et al., 2010). Kim et al. (2007) conducted a research on the prevalence of 22 pharmaceuticals in 3 major rivers in South Korea receiving effluents from secondary STPs situated in manufacturing areas. All the test pharmaceutical compounds were detected at the upstream and downstream sampling sites with frequencies of detection varying from 17 % to 90 %. Baker et al., (2013), found EE2 at 0.04 ngL^{-1} in water samples from Tiber River, Italy. A maximal level of up to 4.3 ngL^{-1} was found in another study but most of the samples were below the detection limits (Adler et al., 2010; Belfroid et al., 1999; Zhang et al., 2012). Purdom et al., (1994) in his investigations, revealed that exposure

of fish to only 0.1 ngL^{-1} levels of EE2, could cause feminization in some species of male.

Table 2.2: Concentration range of some commonly detected pharmaceuticals in surface water (**Adapted from** Wilkinson et al., 2016a, 2017)

Therapeutic Class	Geographical Region	Contaminant	Surface Water (ng/L)	Reference
Analgesic	Europe	Ibuprofen	1-237	Petrie et al., 2015
	Europe	Diclofenac	<0.5-253	Petrie et al., 2015; Wilkinson et al., 2017
	Europe, North America	Paracetamol	110-10000	Kolpin et al., 2002; Boyd et al., 2004; Wilkinson et al., 2017
	North America	Codeine	12-1000	Kolpin et al., 2002; Boyd et al., 2004
	North America	Naproxen	<1-81	Kolpin et al., 2002
Antibiotic	Europe	Amoxicillin	<2.5-245	Petrie et al., 2015
	Europe	Erythromycin	<0.5-159	Petrie et al., 2015
	North America	Triclosan	140-2300	Kolpin et al., 2002; Boyd et al., 2004
	North America	Trimethoprim	<1-2	Van Ginneken et al., 2017
	North America	Sulfamethoxazole	<1-46	Van Ginneken et al., 2017
Antidepressant	Europe	Amitriptyline	66-207	Petrie et al., 2015
	Europe, North America	Fluoxetine	5.8-120	Petrie et al., 2015; Kolpin et al., 2002
	North America	Venlafaxine	1.1-35	Petrie et al., 2015
Antineoplastic	Europe	Ifosfamide	0.05-0.14	Wilkinson et al., 2017
	Europe	Cyclophosphamide	0.05-0.17	Wilkinson et al., 2017
	Europe	Tamoxifen	<0.05-25	Coetsier et al., 2009

Beta Blocker	Europe	Metoprolol	<0.5-10	Petrie et al., 2015; Relic et al., 2017
	Europe, North America	Atenolol	<1-48	Petrie et al., 2015; Van Ginneken et al., 2017
Hormones/Steroids	Europe, North America	17α ethinylestradiol	73-831	Wilkinson et al., 2017
	Europe, North America	17β estradiol	0.1-200	Petrie et al., 2015; Kolpin et al., 2002
	Europe, North America	19 norethisterone	48-872	Petrie et al., 2015; Kolpin et al., 2002
	Asia	Coprostanol	<1-2717	Peng et al., 2008
Liquid Regulator	Europe, North America	Bezafibrate	<10-60	Petrie et al., 2015
	North America	Gemfibrozil	48-790	

2.3.10 Occurrence of pharmaceuticals in groundwater

The frequencies and concentrations levels of pharmaceuticals are lower in groundwater than in surface water (Vulliet and Cren-Olivé, 2011). Anti-inflammatories and analgesics are the most popular drugs found in groundwater e.g. diclofenac, ibuprofen, paracetamol and salicylic acid because they are extensively consumed. At landfill leachates, different painkillers such as ketoprofen, diclofenac, ibuprofen, aminophenazone, propyphenazone and phenazone, together with their metabolites have been detected in underground water samples of UK, Denmark, Croatia and Germany at levels of up to ngL^{-1} (Ahrer et al., 2001; Brausch and Rand, 2011; Radke et al., 2010). The results of a national survey of pharmaceuticals conducted by Barnes et al., 2008 across 18 states in the US, showed that the most frequently detected drugs in 47 groundwater samples were sulfamethoxazole (23 %).

In a similar investigation conducted by Loos et al. (2010), 164 groundwater samples were collected from 23 European countries, carbamazepine was reportedly found in 42 % of the samples with a maximum concentration of 390 ngL^{-1}. Therapeutic group like, lipid regulators and its metabolites, such as gemfibrozil, bezafibrate were not detected in the ground water sampled. However, frequencies of detection of clofibric acid (3 %) in a later study by Peng et al., 2014 were lower than those of antibiotics and anti-inflammatory drugs. Holm et al. (1995) in his report, suggested that groundwater near landfill locations could pose a severe ecological risk because of pharmaceutical contamination. In groundwater samples collected in Berlin, Germany clofibric acid discovered at 4 μgL^{-1} (Heberer and Stan, 1997) from an abandoned sewage irrigation field. Again, Sacher et al. (2011) reported the presence of antibiotics in groundwater samples collected near Baden-Wurttemberg, Germany at concentration of 410 ngL^{-1}

(sulfamethoxazole) and 49 ngL^{-1} (dehydroerythromycin). Heberer and Feldmann, (2008) found sulfamethazine and sulfamethoxazole at low levels in selected underground water samples in Germany and the US. In Denmark, Holme et al., 1995 also found sulphonamides and their metabolites at low concentrations in underground water samples collected down slope of a landfill. Similar reports by Sacher et al (2011) revealed the presence of sotalol at elevated levels of 560 ngL^{-1} in three groundwater samples in Germany.

In nations with highly developed healthcare systems such as Western Europe and the US, large X-ray contrast media may be present in the sewage effluents, consequently, increased environmental contamination with X-ray contrast media. Amidotrizoic acid, diatrizoate, iopamidol, and iopromide (iodinated contrast agents) were detected up to the μgL^{-1} level in groundwater samples because of the recurring presence in the aquatic ecosystem. Hence, may easily percolate into the groundwater aquifers (Peng et al., 2015). Comparing groundwater with reservoirs, the later were considerably more polluted, showing greater frequencies of detection and elevated level of concentrations (Peng et al., 2014). In underground water, drugs do not display substantial trends or seasonal variations, but the concentration of drugs in reservoirs are higher throughout spring than in other seasons.

Groundwater samples across 14 countries in 4 continents was investigated by Lapworth et al. (2012) compounds such as carbamazepine: 5 μgL^{-1} (n = 23), sulfamethoxazole: 252 ngL^{-1} (n = 15), ibuprofen: 1.5 μgL^{-1} (n = 14), caffeine: 9.8 μgL^{-1} (n = 14), and diclofenac: 121 ngL^{-1} (n = 11), were detected. The existence of drugs and the relationship between their presences in groundwater with possible pollution causes in Taiwan was investigated by Lin et al. (2015). Nearly all the 50 analytes targeted were

found at ngL^{-1} level excluding 17α-ethinylestradiol (1822 ngL^{-1}), sulfamethoxazole (1820 ngL^{-1}) and acetaminophen (1036 ngL^{-1}). Sui et al. (2015) reported that analgesics, antibiotics, anti-inflammatories and lipid regulators, were frequently detected in groundwater.

2.3.11 The fate and removal of pharmaceuticals in STPs/ WWTPs

The volatility of pharmaceutical compounds is low implying the distribution in the environment will happen mostly through liquid medium and food chain dispersion. Regardless of the route into the aquatic environment, however, the concentration of drugs is controlled by similar physical, chemical and biological methods (Rosal et al., 2010). In wastewater treatments (WWT), two types of removal procedures are important, and these are adsorption and biodegradation. Studies by various researchers (El-Gindy et al. 2007; Kim et al. 2005; Liu et al., 2004; Liu and Williams 2007; Williams et al., 2013) had shown that drugs can be transformed by photodegradation, biodegradation, hydrolysis or sunk as deposits into the bottom of the river. However, the resistant of several drugs to photodegradation causes increase persistency of such compounds in the aquatic ecosystem (Boreen et al., 2003; Calisto et al., 2011), hence, may remain in the bottom of the river as deposits for years (Klaminder et al., 2015). Adsorption of pharmaceuticals is a function of both hydrophobic and electrostatic interactions of the drugs with microbes and particulates (Williams et al., 2013). Acidic drugs with pKa values ranging from 4.9 to 4.1, are NSAIDs like ketoprofen, diclofenac, naproxen, acetylsalicylic acid, ibuprofen, indomethacin, clofibric acid, fenoprofen, as well as bezafibrate (pKa 3.6) and gemfibrozil, occurs as ion at neutral pH ((Klaminder

et al., 2015). Adsorption increases with lower pH. At neutral pH, these negatively charged drugs therefore occur mainly in the dissolved phase in the wastewater.

Sorption of acidic drugs to slurry is not significant or the removal of drugs from wastewater and surface water. Suggested not to be very important in the elimination process of pharmaceuticals from wastewater and surface water. Hence, the level of drugs in digested slurry and residues are relatively low, as showed in numerous monitoring studies (Ternes et al., 2004; Urase and Kikuta, 2005). However, Golet et al., (2002) demonstrated that fluoroquinolone antibiotics can adsorb to sludge to a large extent. For the hydrophobic EE2 (logKow 4.0) sorption to sludge is likely to play a role in its removal from wastewater.

Some pharmaceuticals are well eliminated during sewage treatment process while others are not. The removal rate for drugs such as ASA, salicylic acid, carbamazepine propranolol, diclofenac and naproxen in STPs are 81 %, 91 %, 7-8 %, 96 %, 26 % and 81 % respectively (Carballa et al., 2004; Clara et al., 2004) and that of bezafibrate (51 %) vary from one STP to another. Table 2.3 shows the removal range of selected pharmaceuticals in wastewater treatment plants. Thomas and Foster (2004) investigated the removal efficiencies of three STPs in United States of America and found 94-100 % elimination for diclofenac ibuprofen, ketoprofen and naproxen. More than 80 % elimination took place during secondary treatment and <50 % in the primary treatment.

In another study on elimination rate of pharmaceuticals during sewage treatment process, Roberts and Thomas, (2005) observed that tamoxifen was not removed. However, because pharmaceuticals comprise of diverse group of chemicals with varied physico-chemical properties, the differences in removal rate is expected. Once the effluents are discharged into rivers, biotransformation through abiotic process takes

place. Hence, drugs like carbamazepine and clofibric acid undergo photodegradation because of their slight removal during treatment.

Table 2.3: Apparent removal of selected pharmaceutical in WWTPs (**Source:** Tran et al., 2018)

Selected pharmaceuticals	Removal range (%)	Reference
Antibiotics		
Amoxicillin	69.9-99.7	Tran et al. (2016), Mutiyar and Mittal. (2013)
Azithromycin	<0-99	Tran et al. (2016), Guerra et al. (2014)
Chloramphenicol	11.8-73.8	Zhou et al. (2013)
Chlortetracycline	31.4-97.8	Tran et al. (2016), Zhou et al. (2013)
Ciprofloxacin	<0-100	Tran et al. (2016), Guerra et al. (2014), Zhou et al. (2013), Mohapatra et al. (2016), Gros et al. (2010)
Clarithromycin	<0-99	Tran et al. (2016), Guerra et al. (2014), Zhou et al. (2013), Mohapatra et al. (2016)
Clindamycin	<0-88.9	Tran et al. (2016), Gurke et al. (2015), Kovalova et al. (2012)
Enrofloxacin	0-67	Guerra et al. (2014)
Erythromycin-H_2O	<0-100	Tran et al. (2016), Zhou et al. (2013), Mohapatra et al. (2016)
Lincomycin	<0-100	Tran et al. (2016), Guerra et al. (2014), Zhou et al. (2013), Sim et al. (2010)
Meropenem	80.7-92.6	Tran et al. (2016)
Minocycline	44.8-86.9	Tran et al. (2016)
Ofloxacin	<0-99	Guerra et al. (2014), Zhou et al. (2013), Gros et al. (2010), Radjenovic et al. (2007)
Oxytetracycline	54.6-96.3	Tran et al. (2016), Zhou et al. (2013)
Sulfamethazine	<0-96.2	Tran et al. (2016), Guerra et al. (2014), Zhou et al. (2013), Behera et al. (2011).
Sulfamethoxazole	<0-99	Tran et al. (2016), Guerra et al. (2014), Zhou et al. (2013), Mohapatra et al. (2016), Gros et al. (2010), Radjenovic et al. 2007, Sim et al. (2010), Behera et al. (2011)
Tetracycline	34-97	Tran et al. (2016), Guerra et al. (2014), Zhou et al. (2013), Gros et al. (2010)
Trimethoprim	<0-97	Tran et al. (2016), Guerra et al. (2014), Zhou et al. (2013), Mohapatra et al. (2016), Sim et al. (2010), Behera et al. (2011).
Vancomycin	96.6-99.9	Tran et al. (2016),
Antimicrobials		
Miconazole	<0-99	Guerra et al. (2014)
Thiabendazole	<0-88	Guerra et al. (2014)
Triclocarban	<0-99	Tran et al. (2016), Guerra et al. (2014)
Triclosan	<0-100	Tran et al. (2016), Guerra et al. (2014), Mohapatra et al. (2016), Behera et al. (2011), Ying et al. (2009), Kosma et al. (2010), Tran and Gin. (2017)
NSAIDs		

Acetaminophen	<0-100	Guerra et al. (2014), Mohapatra et al. (2016), Gros et al. (2010), Radjenovic et al. 2007, Sim et al. (2010), Behera et al. (2011), Kosma et al. (2010)
Codeine	<0-98	Guerra et al. (2014)
Diclofenac	<0-98	Mohapatra et al. (2016), Gros et al. (2010), Radjenovic et al. (2007), Behera et al. (2011), Ying et al. (2009), Kosma et al. (2010)
Fenoprofen	98.6-100	(15) Kosma et al. (2010)
Ibuprofen	<0-99.8	Guerra et al. (2014), Gros et al. (2010) Radjenovic et al. (2007), Sim et al. (2010), Behera et al. (2011)
Indomethacin	7-98.6	Kovalova et al. (2012), Radjenovic et al. (2007), Kosma et al. (2010)
Ketoprofen	51.5-91.9	Gros et al. (2010), Radjenovic et al. 2007, Sim et al. (2010), Behera et al. (2011), Ying et al. (2009)
Naproxen	<0-99.3	Guerra et al. (2014), Mohapatra et al. (2016), Gros et al. (2010), Radjenovic et al. (2007), Behera et al. (2011), Clara et al. (2005)
Salicylic acid	9-95.4	Gros et al. (2010), Kosma et al. (2010, Tran and Gin. (2017)
Beta-blockers		
Atenolol	<0-96	Mohapatra et al. (2016), Gurke et al. (2015), Kovalova et al. (2012), Radjenovic et al. (2007), Behera et al. (2011), Kosma et al. (2010)
Metoprolol	<0-58.7	Mohapatra et al. (2016), Gurke et al. (2015), Kovalova et al. (2012), Radjenovic et al. (2007), Behera et al. (2011).
Propranolol	<0	Gurke et al. (2015), Kovalova et al. (2012)
Anticonvulsants		
Carbamazepine	<0-83	Mohapatra et al. (2016), Gurke et al. (2015), Radjenovic et al. 2007, Sim et al. (2010), Behera et al. (2011), Ying et al. (2009), Kosma et al. (2010, Tran and Gin. (2017)
Gabapentin	<0-95.6	Gurke et al. (2015), Kovalova et al. (2012), Tran and Gin. (2017),
Sulpiride	<0-73.5	Tran and Gin. (2017), Bollmann et al. (2016), Sui et al. (2011)
Lipid regulators		
Bezafibrate	48.4-95.8	Gros et al. (2010), Radjenovic et al. (2007), Sui et al. (2011)
Clobric acid	27.7-71.8	Radjenovic et al. (2007), Behera et al. (2011).
Gemfibrozil	0-100	Mohapatra et al. (2016), Gros et al. (2010), Radjenovic et al. 2007, Sim et al. (2010), Behera et al. (2011), Ying et al. (2009), Kosma et al. (2010), Tran and Gin. (2017)
Hormones		
Estrone	0-100	Behera et al. (2011), Clara et al. (2005), Gabet-Giraud et al. (2010), Joss et al. (2004)
Estriol	18-100	Behera et al. (2011), Clara et al. (2005)
17α-ethinylestradiol	33-100	Clara et al. (2005), Joss et al. (2004)

2.4 Ecotoxicological effects of pharmaceuticals in aquatic environments

2.4.1 Toxicity testing methods

Toxicity tests are bioassays in which test organisms are exposed in a laboratory setting, to various concentrations of chemical toxicants, or dilutions of whole effluents using single species or multispecies (Odiete, 1999). Basically, four types of conventional toxicity tests are known, i.e. *ex-situ* static, static with renewal, continuous or intermittent flow and *in-situ* (Odiete, 1999).

Toxicity tests are used to assess the concentration of compounds and the length of exposure needed to cause an effect (Odiete, 1999). Therefore, toxicity testing is to measure pollutant concentration so as to evaluate the risk posed by a chemical and to protect the entire ecosystems by reducing the effect of sporadic and incessant pollution of the ecosystems (Bloor and Banks 2005). Thus, the impacts of toxic chemicals on non-target organisms in the environment are predicted and this represent the conventional method for toxicity measurement (Odiete, 1999). The dose-response correlation provides the foundation for evaluation of risk and threat pose in the environment during toxicity testing of chemicals. Toxicity testing is done following the guidelines for assessing toxicants by Organisation for Economic Co-operation and Development (OECD). The OECD regulation entail the use of various plants and animals, endpoints and different stages of developments to consider while conducting toxicity test.

There are two types of toxicity testing methods.

2.4.1.1 Acute toxicity test: They are test designed to assess the toxicity of compounds to selected organisms in the aquatic environment for a short period of exposure (usually

24-h, 48-h or 96-h) to different concentrations of the test compounds. Usual effective criteria (end points) for invertebrates are loss of equilibrium, immobility, and mortality.

They are cheap and less costly to perform; consequently, the majority of the toxicological database is skewed in favour of acute responses (Pascoe and Edwards, 1989) (Figure 2.3).

Various researchers had used different types of exposure conditions such as static, static renewal, re-circulatory and flow-through. The determination of which exposure to use in acute and chronic toxicity tests usually depends on test substance characteristics, test duration, and regulatory requirements.

2.4.1.2 Sub-lethal test: Sub-lethal test is meant for the assessment of the potential negative impacts of substances, under a longer period of exposure. The test specie may be exposed for between weeks to entire reproductive life cycle (Pascoe *et al.,* 1994). This testing method provides significant data for water quality criteria (Bloor et al., 2010 and Hellawell, 1986). Various investigators had used various methods such as biochemical, physiological, reproductive and behavioural as a measure of toxicity. Sub-lethal tests are meant to determine biologically non-toxic concentrations in which the long-term survival of organisms exposed to the chemicals are assured by monitoring the common effective criteria (Pascoe and Edwards, 1989) (Figure 2.3).

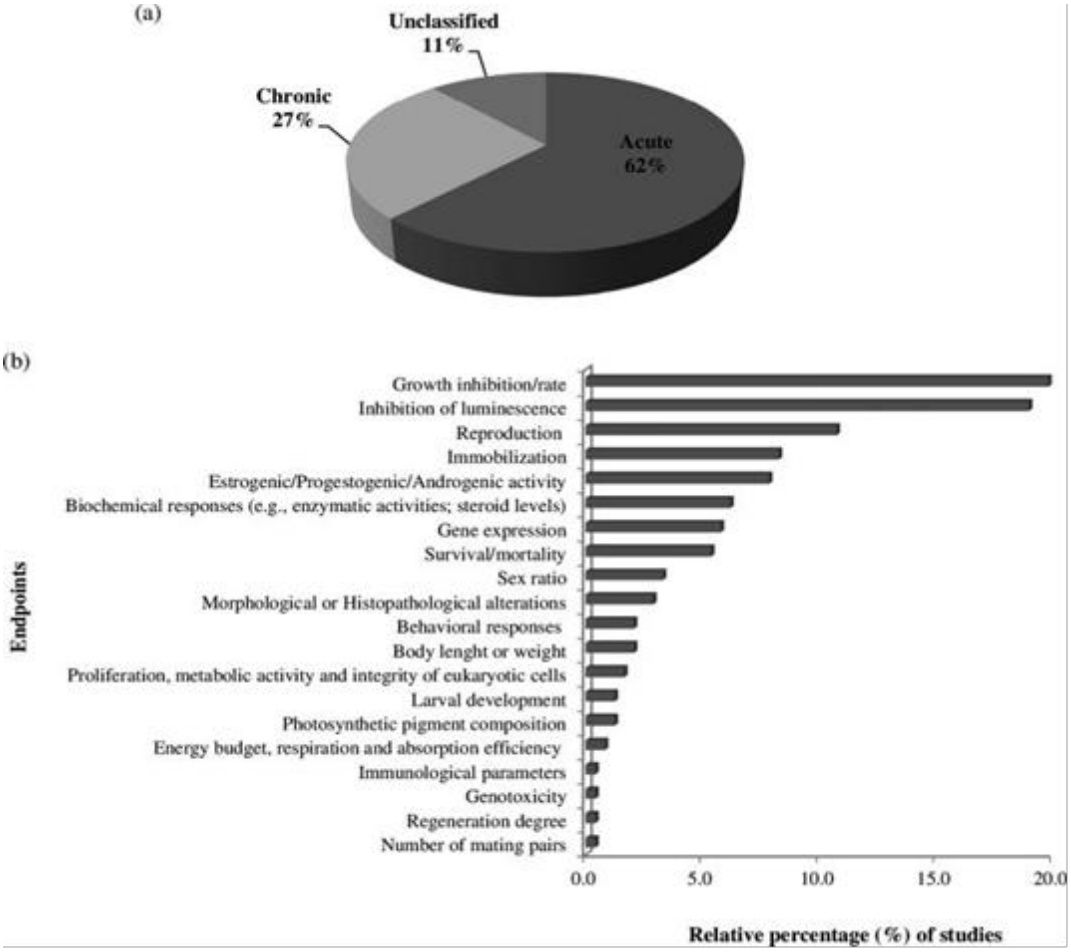

Figure 2. 3: (a) Percentage of acute x chronic studies obtained from the 194 assessments of the toxicity of pharmaceutical mixtures retrieved from 65 articles published between 2000–2017. Those studies not possible to be classified into these two categories according to international protocols were referred to as unclassified. (b) Principal endpoints used in the retrieved studies, expressed in relative percentage (**Source:** Godoy and Kummrow, 2017).

2.4.2 Mixture toxicity of pharmaceuticals.

Due to incomplete removal from treatment plants, organisms in water environment are continually exposed to pharmaceuticals. Not many investigations had been done on toxicity of drugs to organisms in water and understanding the impacts of pharmaceuticals in mixture compared to their effects when acting alone is significant in toxicity testing of pharmaceuticals. Sacher et al., (2011), defines interaction as the grouping of two or more compounds in a way to exert a greater collaboration (synergy)

or weakened (antagonistic or inhibitive) reaction in a mixture toxicity test. Interactions can consequently differ depending on the concentrations, the timing, the exposure pathway and length of exposure, and the target receptor. Different likely connections are possible in mixture of pharmaceuticals, all can either increased or reduced the potency of the drug mixture. Although from environmental scientists' perspective, synergistic interaction of drugs is a cause of worries compared to antagonism. However, in the past efficiency of plant protection produces has been increased with the principle of synergism e.g. pyrethrins in combination with piperonyl butoxide (PBO) has been used for many years to decrease the quantity of pyrethrins needed to attain the potency required for insecticides after use (Gabet-Giraud et al., 2010).

Ecotoxicologist had adopted two pharmacological models advanced by pharmacologists in early twentieth century (Bliss, 1939) used for the projection of toxicity of mixtures known as independent action and concentration addition**.** Table 2.4 shows the method used in predicting the effects of mixtures of drugs.

Table 2.4: Models/approaches used to assess/predict the mixture toxicity effects of pharmaceuticals retrieved from 65 international articles from 2000-2017 **(**Source: **Godoy and Kummrow, 2017)**

Model/Approach used	References
Concentration addition (CA) model, including its graphical representation (Isobologram)	Thorpe et al., (2003); Daughton and Ruhoy, 2009b; Runnalls et al., (2015); Zhao et al., (2015); Hinfray et al., (2016)
Independent action (IA) model, Parrella et al., (2014). Both CA and IA models, various CA and Toxic Unit (TU) approach	Richardson and Ternes, (2014); Kasprzyk-Horden et al., 2017
Combination-Index Isobologram (CI) model only	Rodea-Palomares et al., (2010)
All the three: CA, IA and CI models	Gonzalez-Pleiter et al., (2013); Di Nica et al., (2017); Geiger et al., (2016)
Principal Component Analysis/Cluster Analysis	Pomati et al., (2008); Franzellitti et al., (2013); Gonzalez-Rey et al., (2014); Zucchi et al., (2014); Ding et al., (2016)
Specific equation based on Toxic Units (TU) of the mixture	Zou et al., (2012)
Additive Index and the Modified TU approach	DeLorenzo and Flemming, (2008)
Comparison between observed and predicted additivity from the effects caused by 1 TU for each individual component	Borgmann et al., 2007
Statistical comparison between individual and mixture effects using statistical methods such as students T test, Analysis of Variance (followed by post-hoc test) or the Fisher method	Brain et al., (2004); Eguchi et al., (2004); Flaherty and Dodson (2005); Dietrich et al., (2010); Gust et al., (2012); Lang and Kohidai (2012); Melvin et al., (2014); Safholm et al., (2015); Wolfe et al., (2015); Gonzalez-Ortegon et al., (2016); Hua et al., (2016); Orn et al., (2016); Richardson and Ternes, (2014); Liange et al., (2017); Kasprzyk-Horden et al., 2017
Overlap analysis of the 95% confidence intervals of the individual and the mixture effects	Luna et al., (2013;2015)

Comparison of the toxicity threshold values (calculated from the square root of the product between the NOEC[a] and LOEC[b]) between the mixture and the individual effects of each component	Quinn et al., (2009)
Comparison between mixture and single effects of each component by means of simple present calculation	Parolini and Benelli (2012)
Empiric comparison between mixture and single effects of each component without using a mathematical approach or a direct statistical comparison	Ericson et al. (2010); Galus et al. (2013); Li and Lin (2015); Connolly et al., (2017)
The mixture toxicity was statistically compared to the individual toxicity of just one of the mixture compounds (the parental compound)	Alder et al. (2010)
The whole-mixture approach was used. The mixture toxicity was not compared to the individual effects of the components	Brain et al. (2005); Borgmann et al. (2007); Pomati et al. (2007); Gust et al. (2013); Melvin (2016)

[a]NOEC – no observed effect concentration
[b]LOEC – Lowest observed effect concentration

2.4.2.1 Concentration Addition (CA)

Concentration addition is founded on the principle that if the entire components in a drug mixture exhibit the same mechanism of action (MoA) such as acting on the same molecular receptor, they will individually behave in the same way so that each can be substituted by an equal effective concentration (EC_{50}) of another without causing a change in the effect of the final mixture. This is called the dilution principle as propounded by (Loewe and Muischneck, 1926). The effect of such mixture can easily be extrapolated from the total of all the effect of each component at their normal potencies (Backhaus, 2014). This method is also known as the Toxic Units (TUs) and mathematically it can be expressed as: $\sum_{i=1}^{n} \frac{ci}{ECxi} = 1$

Where n = the number of components in the mixture.

Ci = the concentration of the single chemical in the mixture that elicits the effect x, ECxi = the concentration of the same chemical that individually provokes the same effect x.

Ci/ECxi = Toxic Unit (TU)

When there are no interactions between the components, the sum of TUs will be equal to 1. However, (Deneer *et al*., 1988 and Van Hecken et al., 2000) demonstrated that the CA is also valid for compounds that display no particular mechanism of action but whose toxicity towards aquatic organisms is governed by hydrophobicity e.g. unionized and unreactive compounds. Van Leeuwen et al., (1992) suggested that the non-specificity of mechanism of action of those chemicals are called narcosis or baseline toxicity.

2.4.2.2 Independent Action (IA)

This concept was grounded on the believed that chemicals in mixtures have dissimilar effects i. e. individual compounds differ in their mode of action and receptor targets. Hence, an individual effect of each toxicant will remain unaltered and occur irrespective of the occurrence of other compound.

Mathematically, it can be expressed as:

$$E(cmix) = 1 - [\,(1 - E(c1))(1 - E(c2))]$$

Or in general

$$E(cmix) = 1 - \prod_{i=1}^{n}(1 - E(ci))$$

E(c1), E(c2) = the effects of single substances

E (cmix) = the total effect of the mixture.

2.5 Design of experiments for mixture toxicity studies

Several types of experimental designs had been used in the past to assess ecotoxicological effects of mixtures of pharmaceutical. Table 2.5 is a summary of review of types and percentage frequency of experimental designs employed in 194 assessments of the toxicity of pharmaceutical mixtures retrieved from the international literature.

Table 2.5: Types and percentage frequency of experimental designs employed in the assessments of the toxicity of pharmaceutical mixtures retrieved from International literatures (**Source:** Godoy and Kummrow, 2017)

Types of experimental design	Number of experimental data	Percentage frequency (%)	References
Fixed ratio design based on the NOEC[a]/EC_{01}[b] values of each compound	2	1	Backhaus et al. (2000b; 2011)
Fixed ratio design based on the LOEC[c] values of each compound	1	0.5	Borgmann et al., 2007Jr et al. (2016)
Fixed ratio design based on the EC_{10}[d] values of each compound	10	5.2	Di Nica et al. (2017)
Fixed ratio design based on the EC_{50}[e] values of each compound (including the isobologram method)	77	39.7	Backhaus et al. (2000b); Thorpe et al. (2003); Gobel et al., (2007); DeLorenzo and Fleming (2008); Schnell et al. (2009); Rodea-Palomares et al. (2010);Lang and Kohidai (2012); Zou et al. (2012); Gonzalez Pleite et al. (2013); Parrella et al. (2014); Vulliet and Cren-Olive, (2011); Geiger et al. (2016); Bialk-Bielinska et al. (2017)
Fixed ratio design based on different ECxf values besides the EC50c of each compound (including, e.g., EC5, EC10, EC20, EC80 and EC90)	22	11.3	Cleuvers (2003; 2004; 2005); Brezovsec et al. 2014; Godoy et al. (2015b); Nieto et al. (2016); Rossier et al. (2016); Siegenthaler et al. (2017)
Fixed ratio design based on the individual predicted no-effect concentration (PNEC) values	1	0.5	Di Nica et al. (2017)

Fixed ratio design based on the maximum aquatic environmental concentration of the compounds reported in the literature	3	1.5	Watanabe et al. (2016)
Fixed ratio design based on a specific exposure modeling	3	1.5	Zucchi et al. (2014); Runnalls et al. (2015); Guo et al. (2016)
Two-factor fractional-factorial design	2	1	Pomati et al. (2008)
Ray design consisting of multiple ratios based on the effective concentrations of the single compounds	13	6.7	Christensen et al. (2006; 2007); Hinfray et al. (2016)
Multiple combination ratios (based on the EC_{50}[d] of the single compounds) equidistantly distributed on the additivity line of the isobologram	8	4.1	De Liguoro et al. (2009; 2010)
Multiple ratios based on the 0.05, 1, 10, 20, 25 and/or 50% value of the maximum effect concentration of the standard compound established (reference)	12	6.2	Fent et al. (2006b)
The concentration of one of the components was fixed at their NOEC[a] value while the concentration of the other compound was altered	3	1.5	Eguchi et al. (2004)
The concentrations of the components were based on available data for aquatic environments and/or on those able to elicit measurable toxic responses	29	14.9	Brain et al. (2004; 2005); Flaherty and Dodson (2005); Borgmann et al. (2007); Pomati et al. (2007); Kahle et al., (2009); Dietrich et al. (2010); Parolini and Binelli (2012); Franzellitti et al. (2013); Galus et al. (2013); Gust et al.(2012; 2013); Luna et al. (2013; 2015); Gonzalez-Rey et al. (2014); Safholm et al. (2015); Wolfe et al. (2015); Zhao et al. (2015)

The concentrations of the components were based on available data for aquatic environments and/or on those able to elicit measurable toxic responses corresponding paper	8	4.1	Ericson et al. (2010); Melvin et al. (2014; 2016); Li and Lin (2015); Ding et al. (2016); Liang et al. (2017)

[a]NOEC – No observed effect concentration, [b]EC_{01}- Effect concentration at 1%, [c]LOEC – Lowest observed effect concentration, [d]EC_{10} – Effect concentration at 10%, [e]EC_{50} – Effect concentration at 50%, [f]EC_x- Effect concentration at X%

2.5.1 Measurable effects

After the use of pharmaceuticals, its entrance into human body will elucidate a response and cause an effect after interacting at the receptor or an enzyme. Roberts and Thomas, (2005) discussed the four groups of drug targets which are transporters, receptors, ion channels and enzymes.

Williams, (2006) highlighted the three basic conditions upon which a drug will act after administration. These are biological activity of the drug structure, target organ in the organism and finally channel between the drug target and mechanism of response. All the existing pharmaceuticals have been manufactured to meet these conditions. The enzymes and receptors in fish and humans have been shown to have a similarity of about 31 – 88 % (Huggett et al., 2003b). Hence, human medicines may elicit relative responses when they come in contact with fishes in the aquatic environment.

Further studies, by several author (Eccles, 2009 and Laville et al., 2004) have shown that fishes are not the only aquatic animals that can be affected by human drugs but other aquatic invertebrates can be affected such as *Gammarus pulex*, *Asellus aquaticus* and host of other aquatic animals and plants may also elicit a response similar to those expressed by mammals as a result of exposure to pharmaceuticals.

There is a continuous exposure of aquatic organisms to myriads of human pharmaceuticals which are manufactured to elicit pharmacological responses in the human body (Hughes et al., 2013; Kay et al., 2017; Subedi et al., 2017). However, this response frequently causes biological responses in aquatic organisms (e.g. growth disruption and reproduction alterations) (Fent et al., 2006). Little is known about the toxic levels of human drug in the environment, however, the effects of contraceptive pills, 17α ethinylestradiol (EE2) causing endocrine disruption of fishes and frogs has

been reported. The probable mechanism of action was described by Jobling et al., (2003) to be the binding and activation of the oestrogen receptors of fishes and frogs, thereby disrupting the endocrine system. As far back as 1976, Sumpter and Johnson, (2008) detected the presence of intersex fish in lagoons in the UK. The hormone is understood to cause male fish feminisation, however the degree to which the naturally occurring oestrogens such as oestone causes this feminisation process is unclear (Sumpter, 2010). Belfroid et al., (1999) detected EE2 in the River Rhine, Germany, at concentrations of 4.3 ngL^{-1}. The sexual reproduction of *Pimephales promelas* was severely damaged when in contact with EE2 (Lange et al., 2001). Even the fertilised eggs and sex ratio in fathead minnows was reduced according to Parrot and Blunt, (2005).

In a study by Gomez et al., (2006), he found that there were spermatogenic activities in ovarian tissues. He studied the effects of estogens on the intersex brown trout gotten from two different rivers in Switzerland and found 14 out of 57 ovarian tissues had spermatogenic activities, also 13 out of 64 females exhibited the same effects. Fishes are not the only aquatic organism that experiences reproductive issues due to estrogenic exposure, aquatic snails upon exposure to EE2, has eggs production level stimulated at 25 ngL^{-1}. The receptors responsible for mechanism of action are highly conserved in other vertebrates apart from man and fish (Christen et al., 2014).

The pathway of exposure of drugs to the environment has been recognised as a continuous chronic type and various studies has been done in this line. Hence, information is now on the increase about the chronic effects of drugs at relevant environmental concentration levels on the aquatic milieu (Qin et al., 2015). Boxall, (2012) and Kummerer, (2013) found a beta-blocker (propranolol) to cause toxic effect

in aquatic vertebrates due to the presence of identical beta-adrenergic receptors in some fishes. Verlicchi et al., (2012) found that exposing rainbow trout to diclofenac for 28 days causes renal lesions and gill alterations at a concentration level of 5 μgL^{-1}. The growth rate of *D. magna* after chronic exposure to concentrations levels of (0, 20, 40 and 80 mgL^{-1}) was reduced: the organism could not survive at the highest concentration and reproduction was altered at the lowest level of concentration after only 14 d (EC50) at 13.4 mgL^{-1}. There was a decrease from 55 % to < 20 % in time spent on activities when *G. pulex* was exposed to 10 and 100 ngL^{-1} at realistic environmental concentrations of fluoxetine.

2.5.2 Test animals (Bioassay species)

Flexibility is important in aquatic ecotoxicological studies; the studies should be governed by the type of aquatic pollutants and its established environmental pattern of behaviour. The species that are easily influenced by feeding habits, habitat requirements and behavioural characteristics are then chosen for testing. The use of a specie in testing are the commonest; they are simple involving less expenses but Bloor (2010) and Boyle, (1983) regarded this type as unrealistic. The sensitivities of macro-invertebrates of aquatic life to pollution vary and the degree of abundance of these organisms in the aquatic milieu has often been used to deduce the degree of pollution in a specific water body. For this reason, mixed species tests should be undertaken, such as, a comparison of key biotic indices species, which enable pollution boundaries to be established (Bloor *et al*., 2005 and MacNeil, 2002). Among crustaceans, *Daphnia magna* and *Ceriodaphnia dubia* are the most widely used species in the bioassays.

Macro-invertebrates are a sundry group of aquatic life that are important in the ecology of aquatic habitats. Examples include *Asellus aquaticus*, a non-swimming detritivore which is widely found in urban receiving watercourses and *Gammarus pulex*, which is generally classified as an omnivorous shredder. They serve as food to many fish and also play vital role in particulate organic matter decomposition in the aquatic ecosystem. They are frequent swimmers but are easily affected by water quality and they generally disappear with elevated levels of urbanisation. They serve as a bio-indicator of the aquatic environment and have been successful when used in various toxicity testing (Williams et al., 2013; Taylor *et al*., 1991) for mixed species bioassays as they have differing responses to several classes of chemical pollutant, enabling a relative tolerance index to be calculated (Sloof, 1983; Bloor *et al*., 2005; Williams et al., 2013). *G. pulex*, for example, is sensitive to a range of toxicants (Taylor *et al*., 1991; Bloor *et al*., 2005; Williams et al., 2013), such as ammonia (Hermanutz *et al*., 1987; Gammeter and Frutiger, 1990; McCahon *et al*., 1991; Bloor *et al., 2*005; Williams *et al*., 1986; Thomas *et al*.,1991) and phenol (Davies and Anderson, 1997; McCahon *et al*.;1991; Oksama and Kristoffersson, 1979) which are less toxic to *A. aquaticus* (Bloor *et al*., 2005; Maltby, 1995; De Nicola Giudici et al., 1988).

The two species are present in all aquatic milieus. *G. pulex* (Plate 1) dwells in water column and it's considered 'pollution sensitive', whereas *A. aquaticus* (Plate 2) is a sediment dweller and is classed as 'pollution tolerant' (Bloor, 2010; Maltby, 1995). Bloor, (2010) and Mac Neil, (2002) considered *A. aquaticus* to increase as *G. pulex* decreases as a result of reduced water quality.

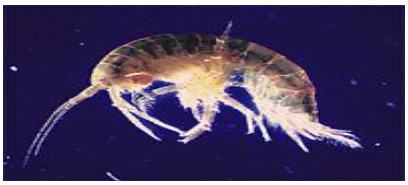

Plate1. G. pulex (pollution sensitive)

Plate 2 A. aquaticus (pollution tolerant)

The American gammarids *G. lacustris*, were sometimes recommended for the analysis of the contaminated sediments, however, they are not appropriate since they are not always in frequent contact with the sediments or residues (since they are water column) unlike the isopod *A. aquaticus* (Bloor, 2010; Gomez et al., 2006).

In the regular acute toxicity testing (LC_{50}), *G. pulex* has been suggested to be added to Daphnia for testing process. The feeding habit of this organism can be used as a determinant of sub-lethal toxicity. The high sensitivities of the juveniles have been verified from experiments involving cultured animals of different ages; hence their use in acute toxicity should be embraced. The effect of exposing the animals during its moult cycle and when parasitized has also been investigated. It was found that *Gammarus* normal behaviour was influenced in the presence of larvae and cystacanths of acanthocephalan parasites. Infected males do not guard females while infected females would not normally breed and the males would be castrated (McCahon and Pascoe, 1988b). In addition, Bloor *et al*., (2005); McCahon and Pascoe, (1988a) explained that the growth rate, reproduction behaviour/success and the respiration rate are all aspects of the *G. pulex* biology that may be included in the sub-lethal toxicity testing. The main demerit of employing *Gammarus spp.* as laboratory bioassay is that the organism has a low survival rate, particularly in static testing (Bloor, 2010; McCahon and Pascoe, 1988a).

It is often advisable to use laboratory cultured animals in toxicity testing due to overwhelming merits. For example, the animals used are grown under standard conditions without prior exposure to any toxic agent. Another merit is the lack of excessive handling, which could interfere with their sensitivity (Pascoe and Beattie, 1979; Holdich and Tolba, 1981). *G. pulex* and *A. aquaticus* were, therefore, chosen as test animals for the toxicity testing programme.

2.5.3 Dietary requirements of G. pulex and A. aquaticus

Baby food, compounded feed, different types of leaves and so on are various types of feed that exists in literature for the feeding of animals. However, G. *pulex* and *A. aquaticus* are detritivores, macro-invertebrates that have preference for 'conditioned' rather than 'unconditioned' leaf material (McCahon and Pascoe, 1988b). From his experiment, Graca *et al*., (1993) demonstrated that 'unconditioned' leaves reduce the growth of *A*. *aquaticus*, however, leaf 'conditioning' does not influence the growth of *G. pulex*. This is due to the fact that *G. pulex* can compensate its low energy intake by reducing its energy expenditure. Although the mechanism that explains this is unclear, but Graca, (1990) proposed that it could be as a result of reduced activity. In 1976, Willoughby and Sutcliffe researched and explained that bacteria and fungi are essential constituent of gammarid's diet. *A. aquaticus* preferentially feed on the mycelia; whereas *G, pulex* prefer the leaf material when they were allowed to choose between fungal mycelia and fungally 'conditioned' leaf material.

2.5.4 Factors affecting an organism's response during standard toxicity testing

During toxicity testing a lot of factors affect organism's response to toxicant. Factors such as water quality/physico-chemical parameter e.g. temperature, hardness, pH, and dissolved oxygen are of paramount importance (Pascoe *et al*., 1986; Samuelsen et al., 1992; Cairns *et al*., 1995). In addition, physical and biological factor such as the stage in the life cycle (Bloor, 2010; McCahon and Pascoe, 1988), parasitism (Pascoe *et al*., 1994; McCahon *et al.,* 1989) also plays a role in the response of the test species to the toxicant (figure 2.4). For example, the fish eggs are normally more tolerant to pollutants than hatched larvae (Beattie and Pascoe,1978) and the response of crustaceans to a toxicant is dependent upon its moult cycle (Bloor, 2010), developmental stage and age of the animals is important in the response to the toxicant (Davies and Anderson, 1997; McCahon and Pascoe.,1988). The animal past history is also important in determining its future response.

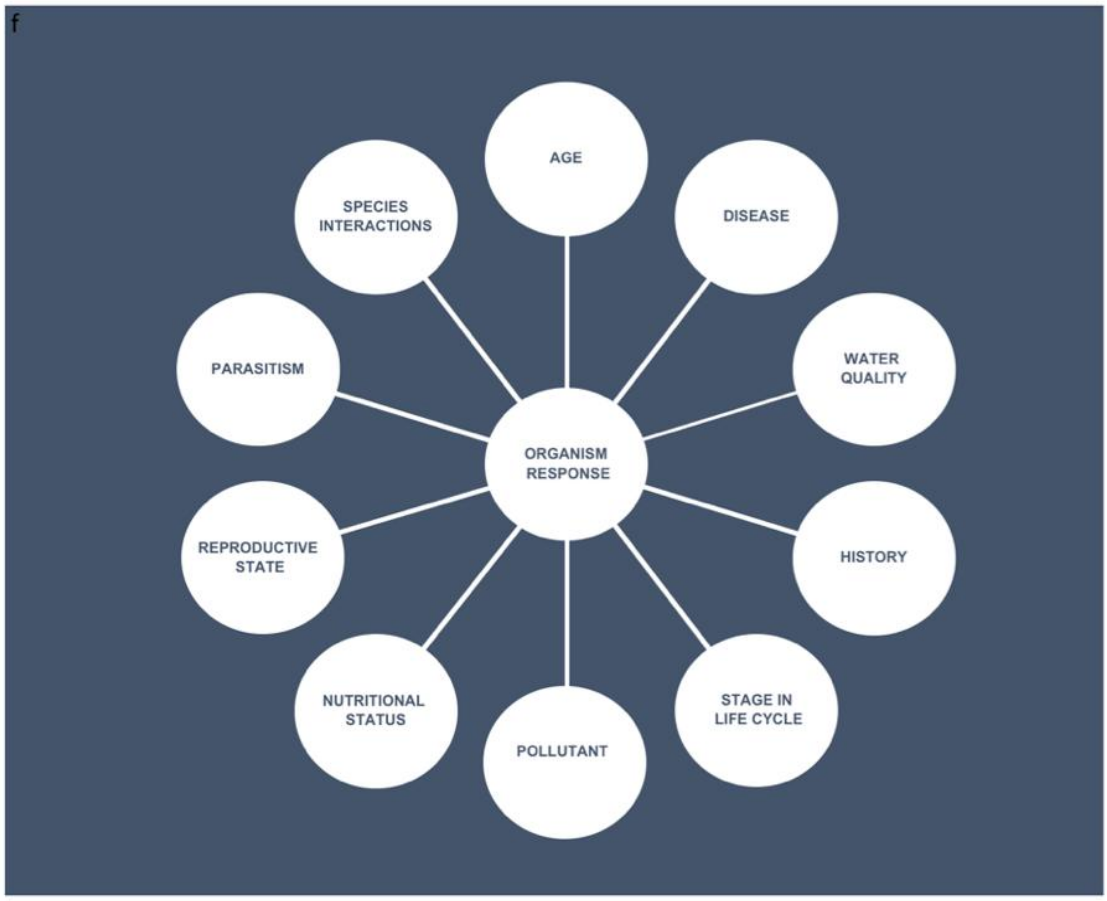

Figure 2.4: Factors affecting an organism's response during standard toxicity testing (**Source:** Bloor et al., 2010).

2.6 Summary

2.6 .1 Description of gaps in the research literature

For more than twenty years, there has been increasing interest in the occurrence and effects of pharmaceuticals in the aquatic environment primarily in Europe and North America. While their occurrence is now relatively well understood in these parts of the world to date there remains a scarcity of data from many African countries. Only three publications (Olaitan et al., 2014; Olarinmoye et al., 2016; Inam et al., 2015) reported the presence of drugs in the Nigeria aqueous environment despite being the hub for distribution of pharmaceuticals in West Africa and the largest consumer of

pharmaceuticals in Africa because of its population (198 million). The paucity of data on monitoring studies in Nigeria could be an imminent threat to the water resources of Nigeria because surface water serves as the main source of drinking water in many parts of the country (Ogunbanwo, 2011). This study will focus on evaluating the presence, spatial and temporal patterns of drugs in Africa (Nigeria) rivers and will contribute significantly to knowledge gap on occurrence of drugs for Nigeria government and the scientific community worldwide at large.

The literature review has also underscored the shortcomings in the present ecotoxicological testing of drugs where the main emphasis in ecotoxicological testing of drugs on aquatic organisms is on acute toxicity principles (Figure 2.3), at concentrations the organisms most likely cannot encounter in the aquatic environment. But pharmaceuticals are generally found in low concentrations (ngL^{-1} to μgL^{-1}) in the aquatic environment, not as a single compound but as mixtures and are continually released unabated majorly through STPs. To bridge this gap in the literature, focusing on the use of environmental relevant concentrations, prolonged low-level exposure, using sensitive species and use of mixture toxicity will improve the understanding of the effects of pharmaceutical contamination on freshwater ecosystems and will help to make conclusions about pharmaceutical toxicity much more relevant to actual environmental conditions.

2.6.2 Contribution of proposed research

It is expected that this thesis will provide novel information on the occurrence of pharmaceuticals in freshwater systems in Africa and contribute significantly to improve the understanding of the effects of prolonged low-level exposure of freshwater ecosystems to environmentally relevant concentrations of pharmaceuticals (erythromycin, diclofenac, ibuprofen and their mixtures).

CHAPTER THREE

Occurrence of pharmaceuticals in Nigerian rivers

3.0 Introduction

Pharmaceutical presence in the aquatic environment was first detected in the 1970's (Tabak and Bunch, 1970; Norpoth et al., 1973). Numerous studies had been carried out on occurrence of pharmaceuticals in the aquatic environment, but these have mostly been undertaken in Europe and North America (K'Oreje et al., 2012; Hughes et al., 2013). Fewer studies have been carried out in the developing countries of Africa, Asia, South America and the Middle East (Hughes et al., 2013; Madikizela et al., 2017). Some relatively high concentrations have, however, been found in countries such as China and India where treatment/regulation is less stringent than in the West. A similar situation exists in Africa, where high concentrations of pharmaceuticals are likely due to many regions suffering from little or no treatment of sewage before discharge to surface waters, particularly in rural areas and even in big cities. Even where sewage treatment plants (STP) exist many pharmaceuticals are not entirely removed by existing wastewater treatment methods (Gracia-Lor, 2010; Van Ginneken et al., 2017; Ortiz de Garcia et al., 2013).

Illegal discharge of raw sewage effluent into rivers by vacuum truck operators who collect sewage from residential homes may also be an issue in Africa. This activity is common in most countries in Africa where there is little or no legislation (Ogunbanwo, 2011). Even where there is legislation there is often little or no enforcement activity, leading to frequent discharges of untreated effluents into the aquatic environment (Ogunbanwo, 2011).

A particular concern arising from the pharmaceutical contamination of surface waters is where river waters are abstracted for portable uses (Lacey et al. 2008; Verlicchi et al., 2012b; Kay et al. 2017). In Western Europe, China, Canada, and the United States >30 pharmaceutical substances have been found in tap/drinking water (Tim Aur Der Beek et al., 2016). In France, traces of drugs have also been detected in bottled water (Bruchet et al., 2005) and, in many African countries, drinking water production mainly relies on surface water abstraction which may be contaminated. Surface water quality is a growing area of research in Africa, but there are significant gaps regarding the occurrence of pharmaceuticals in the aquatic environment. Scientists and their respective governments are beginning to realise the need for a cleaner environment in Africa. Evidence from industrialised nations on the occurrence of pharmaceuticals in the environment is encouraging scientists and their governments to study surface water quality more closely in Africa.

In the past six (6) years, publications on occurrence and fate of pharmaceuticals in Africa aquatic system have been on the increase, however, very limited information are available for Nigeria (only three publications to date). For example, Olarinmoye et al. (2016), using LC-MS/MS for quantification reported pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria, for the surface water, ibuprofen showed the highest concentrations up to 8.8 μg/L, while diclofenac was more abundant in sewage sludge with concentrations up to 1100 μg/kg dry weight. Olaitan et al. (2014) also reported the detection of pharmaceutical compounds in surface and groundwater samples collected from an irrigation canal and several wells in a pharmaceutical industrial area of Sango Ota, Ogun State, Nigeria. The average concentrations of the targeted pharmaceuticals such as diclofenac,

chloroquine, paracetamol and ciprofloxacin were 17 μgL^{-1}, 5 μgL^{-1}, 3 μgL^{-1} and 1 $\mu g\ L^{-1}$, respectively. Inam et al., (2015) investigated the occurrence and risks posed by emerging organic pollutants (EOPs) in Ikpa river basin freshwater ecosystem in Niger-Delta, Nigeria between April and June 2013 (medium to heavy rainfall period). Seventeen compounds were detected at the ngL^{-1} levels: seven antibiotic drugs (acetamidophenol, chloramphenicol, ciprofloxacin, erythromycin, lincomycin HCl, roxythromycin, and sulfamethoxazole), three bactericides/antimicrobial agents (sulfathiazole, triclosan and triclocarban), an antiepileptic drug (carbamazepine), an analgesic drug (diclofenac sodium), a resin precursor (bisphenol A), a sunscreen product (oxybenzone), a hormone (equilin), an insect repellent (DEET), and a stimulant (caffeine) in surface water samples from Ikpa River Basin as well as in the storm water from hospital dumpsite and municipal landfill leachate discharged into the freshwater body through run-offs. Low levels of maximum MEC were recorded for the commonly prescribed antibiotics: ciprofloxacin (2.3 ngL^{-1}), erythromycin (11.4 ngL^{-1}) and sulfamethoxazole (2.8 ngL^{-1}).

K'Oreje et al (2012) developed a new methodology involving both full-scan screening and selective target analysis to investigate the presence of 43 priority pharmaceutically active ingredients in the Nairobi River. Ten (10) human pharmaceutically active ingredients were found whose concentrations ranges from (low ngL^{-1} to high μgL^{-1}) Agunbiade and Moodley (2015) investigated the occurrence and distribution of eight acidic pharmaceuticals in South Africa and found that all were present in sediments, wastewater, and surface water samples. Wood et al. (2015) surveyed the occurrence of anti-retroviral compounds used for HIV treatment in South African surface waters and found average concentrations between 27 and 430 ngL^{-1}.

Madikizela et al. (2017) reviewed the status of pharmaceuticals in African (Kenya, South Africa and Tanzania) water bodies, finding that NSAIDs, antimicrobial and antimalarial compounds are the most common drugs in the aqueous environment and that concentrations in wastewater exceed the levels found in developed countries. K'Oreje et al. (2016) investigated the occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater in two cities in Kenya and found that antiretroviral drug-nevirapine and antibiotics were present in all the samples and more prevalent compared to Europe. K'Oreje et al. (2018) also studied the occurrence, fate and removal of pharmaceuticals, personal care products and pesticides in wastewater stabilization ponds and receiving rivers in the Nzoia basin of Kenya. Paraben concentration was up to 1 μgL^{-1}, antiretroviral and antibiotics were most prevalent measuring up to 100 μgL^{-1}, and low concentrations of pesticides was also detected. Kermia et al. (2016) investigated the presence of four (4) pharmaceutical active compounds belonging to the group of NSAIDs in the wastewater, surface water and drinking water of Algiers. The targeted compounds (ibuprofen, diclofenac, ketoprofen and naproxen) were all detected in wastewater influent/effluent with concentration ranging from 0.156 μgL^{-1} to 6.554 μgL^{-1} and surface water with concentrations of diclofenac and naproxen 0.073 μgL^{-1} and 0.228 μgL^{-1} respectively. The concentrations of ibuprofen and ketoprofen in drinking water was 0.142 μgL^{-1} and 0.111 μgL^{-1} respectively. Relic et al., (2017) studied the occurrence of two antiretroviral drugs, efavirenz and nevirapine in wastewater treatment works from Southern Gauteng, South Africa and found that efavirenz concentrations entering the WWTP ranged between 5.5 μgL^{-1} to 14 μgL^{-1} and nevirapine concentrations ranges between 0.092 μgL^{-1} and 0.473 μgL^{-1}. Ngumba et al. (2016) investigated the occurrence of three antibiotics

(sulfamethoxazole, trimethoprim and ciprofloxacin) and three antiretroviral (lamivudine, nevirapine and zidovudine) drugs in Nairobi River Basin, Kenya. All the studied compounds were detected with sulfamethoxazole having the highest detection frequency of 97.5 % and ciprofloxacin had the lowest at 60 %. Abafe et al, (2018) investigated the use of LC-MS/MS to determine thirteen antiretroviral drugs used in the treatment and management of HIV in the influents and effluents from wastewater treatment plants in KwaZulu-Natal in South Africa. He found that only three compounds were completely removed in the wastewater treatment plants.

In Nigeria, analgesics, antibiotics, antacid, antihistamines, anticonvulsants, steroids, antimalarial and antihypertensive are among the most consumed classes of compounds and are routinely purchased without a prescription (Odusanya, 2005). However, the statistics available on the usage of pharmaceuticals are not reliable because of the activities of unregistered pharmacies in some cities (e.g., Lagos) (Akande and Ologe, 2007; Oshikoya and Ojo, 2007; Nwolisa et al., 2006; Odusanya, 2005).

Here the first detailed/comprehensive study of pharmaceutical occurrence in Nigerian river is presented, covering more detected compounds than existing work in Nigeria and other African nations. The main objectives were: (i) to understand the extent to which 37 drugs belonging to different therapeutic classes are found in the river, (ii) to quantify spatial patterns of pharmaceutical contamination and, (iii) to determine seasonal dynamics of contamination.

3.1 Methods

3.1.1 Pharmaceuticals monitored

Pharmaceuticals were selected to provide data for a range of different therapeutic classes (Table 3.1.1). Many of these compounds are high-use pharmaceuticals that have

been found previously in rivers around the world (Hughes et al., 2013) thus enabling benchmarking of our new information from Lagos, Nigeria against studies undertaken worldwide.

Malaria is an endemic disease in Africa (Nigeria) and malaria drugs were initially included to be analysed in this study together with two other pharmaceuticals (diclofenac and ibuprofen) but could not be analysed because of analytical instrument and time constraints. These drugs are most likely to be present in the environmental matrix.

Table 3.1.1: Study compounds, and physico-chemical properties, monitored in the Odo Iya Alaro river, Lagos Southwest Nigeria (**Source:** www.drugbank.ca)

Therapeutic Group	Compound	LogKow	pKa	Molecular Wgt ($g\ ml^{-1}$)	Formula	Solubility ($mg\ L^{-1}$)
Analgesic & Anti-inflammatory	Codeine	1.19	8.21- 10.60	299.37	$C_{18}H_{21}NO_3$	9000
	Hydrocodone	2.16	8.23	299.37	$C_{18}H_{21}NO_3$	n/a
	Paracetamol	0.46-0.49	9.38	151.17	$C_8H_9NO_2$	14000
	Tramadol	3.01	9.41	263.38	$C_{16}H_{25}NO_2$	630
Antacid	Cimetidine	0.40	6.80	252.34	$C_{10}H_{16}N_6S$	9380
	Ranitidine	0.27	8.08	314.40	$C_{13}H_{22}N_4O_3S$	24700
Antiallergic	Loratadine	5.20	5.00	382.89	$C_{22}H_{23}ClN_2O_2$	0.011
Antibiotics	Erythromycin	3.06	8.88-8.90	733.94	$C_{37}H_{67}NO_{13}$	2000
	Sulfamethoxazole	0.89	1.60-5.70	253.28	$C_{10}H_{11}N_3O_3S$	610
	Trimethoprim	0.91	7.12	290.32	$C_{14}H_{18}N_4O_3$	400
Anticonvulsant	Carbamazepine	2.45	13.90	236.27	$C_{15}H_{12}N_2O$	17.7
	Gabapentin	-1.10	3.68-10.70	171.24	$C_9H_{17}NO_2$	4490
Antidepressant	Amitriptyline	4.92	9.40-9.76	277.41	$C_{20}H_{23}N$	9.71
	Desvenlafaxine	2.72	10.11	263.38	$C_{16}H_{25}NO_2$	1400
	Diltiazem	2.7	8.06	414.52	$C_{22}H_{26}N_2O_4S$	465
	Oxazepam	2.24	10.90	286.72	$C_{15}H_{11}ClN_2O_2$	179
	Venlafaxine	3.20	10.09	277.41	$C_{17}H_{27}NO_2$	267
Antihistamine	Diphenhydramine	3.27	8.98	255.36	$C_{17}H_{21}NO$	3060
	Fexofenadine	2.81	4.28-8.76	501.67	$C_{32}H_{39}NO_4$	0.024
	Ketotifen	3.85	8.43	309.43	$C_{19}H_{19}NOS$	15.3
	Cetirizine	1.70-3.57	3.58-7.74	388.89	$C_{21}H_{25}ClN_2O3$	65.8
Antidiabetic	Metformin	-2.64	12.40	165.63	$C_4H_{12}ClN_5$	n/a
	Sitagliptin	1.39	8.78	407.32	$C_{16}H_{15}F_6N_5O$	179.2

Antipsychotic	Diazepam	2.82	3.40	284.74	$C_{16}H_{13}CIN_2O$	50
	Temazepam	n.a.	-1.4-10.68	300.74	$C_{16}H_{13}CIN_2O_2$	164
Anti-malaria	Artemisinin	2.90	4.60	282.22	$C_{15}H_{22}O_5$	n/a
Antiarrhythmic	Lidocaine	2.26	8.01	234.34	$C_{14}H_{22}N_2O$	4100
Antiretroviral	Lamivudine	-9.54	-0.16-14.29	229.25	$C_8H_{11}N_3O_3S$	70000
Antiviral	Oseltamivir	0.95	7.70	312.41	$C_{16}H_{28}N_2O_4$	1600
Contraceptive	Norethisterone	2.97	-1.7-17.59	298.43	$C_{20}H_{26}O_2$	7.04
Beta Blocker	Atenolol	0.16	9.60	266.34	$C_{14}H_{22}N_2O_3$	13300
	Propranolol	-0.45	9.42	259.35	$C_{16}H_{21}NO_2$	61.7
SERM	Raloxifene	6.09	7.99-9.92	473.59	$C_{28}H_{27}NO_4S$	0.25
Diuretics	Triamterene	0.98	3.11-15.88	253.27	$C_{12}H_{11}N_7$	48.2
Calcium-Channel Blocker	Verapamil	3.83	8.92	454.61	$C_{27}H_{38}N_2O_4$	4.47
SSRIs	Sertraline	4.30	9.47	306.23	$C_{17}H_{17}CI_2N$	3.5
	Citalopram	1.39	9.50	324.40	$C_{20}H_{21}FN_{20}$	n/a

n.a. = Not Available
Wgt=Weight

3.1.2 Study catchment: Lagos State, Nigeria

Lagos State is a low-lying coastal region occupying 187 km of Nigeria's coastline. It is situated between latitudes 6° 22'N to 6° 42'N and longitudes 2° 42'E to 4°20'E. It is bounded in the north by Ogun state and in the east by Ondo state. It shares an international boundary of about 45 km with the Republic of Benin while the Atlantic Ocean constitutes approximately 180 km along the southern limit. The state covers an approximately 3,577 sq. km which represents 0.39 % of Nigeria's territorial land mass. Lagos drains two-thirds of South-west Nigeria and is characterized by wetlands and basin, five major upstream rivers from neighbouring states discharge into the Atlantic Ocean through the Lagos lagoon. The low-lying land and wet-lands occupy 78 % of the entire land mass of the state. 85 % of the state population resides in just 37 % of the state territorial land mass. It is the smallest state in Nigeria but has the largest population of 22 million people. It is one of the fastest growing cities in the world. 45 % of the Nigeria's skilled labour force is in Lagos. Lagos State is the commercial nerve centre of Nigeria. It harbours over 2,000 industrial complexes, 10,000 commercial ventures and 22 industrial estates. It has more than 100 both local and multinational pharmaceutical manufacturing industries located in the state. This rapid urbanisation and population increase leads to large number of pharmaceutical manufacturing industries in the state and hence large quantities of pharmaceutical wastes are generated which may leads to high level of water pollution. The state has ten Lagoons and many creeks, rivers, streams and drainage canals. The largest of all the lagoon system is the Lagos lagoon through which all others drain, and Lagos lagoon enters the Atlantic Ocean through the Lagos harbour. With this ever-increasing urban population vis-à-vis

the scarcity of dry lands, many of the natural streams had been sand filled and converted due to proliferation of urban residential and industrial establishment.

The study was conducted at Odo-Iya Alaro River (Figure 3.1.2), the Odo-Iya Alaro River forms a sub-catchment of the Ogudu river, which discharges into the Lagos lagoon. The river is 15.8 km in length and flows through Ogba, Ikeja and Maryland which have a combined population of 2.5 million. The catchment contains a sewage treatment plant (STP), two major pharmaceutical manufacturing plants and many smaller ones located in the industrial estates of Ogba and Ikeja which discharge their effluents through drainage pipes and canals into the river. Some of these canals also pass through densely populated urban areas which discharges untreated domestic waste to them. Along the river are located mechanical workshops, illegal buildings and shanty structures with domestic waste discharged untreated into the river and in places like this the river flow is slow. Raw sewage may also enter the river due to emptying of vacuum trucks which collect untreated effluent in urban areas (Ogunbanwo, 2011).

Twenty-two (22) sampling stations along the river (Figure 3.1.2) were chosen based on accessibility and the possibility of sampling both receiving waters up and downstream of the effluents discharge points (Table 3.1.2). Alausa (STP) is one of the four STPs in the whole of Lagos State with a population of 22 million people. The treatment plant aerates the wastewater influent by stirring after which it undergoes sedimentation and chlorination before the final effluent is discharged into the receiving water. The treatment plant was designed to serve a population of 255,000 but there are indications that the plant is handling far more than its installed capacity (Engr Adepoju-plant manager Alausa STP, **personal communication**, 2nd August 2017). The plant has an inflow rate of 1000 m^3 day^{-1}, hydraulic retention time (HRT) of 18 hrs and sludge

retention time (SRT) of 20 days, both domestic and municipal wastewater are being treated at Alausa (STP).

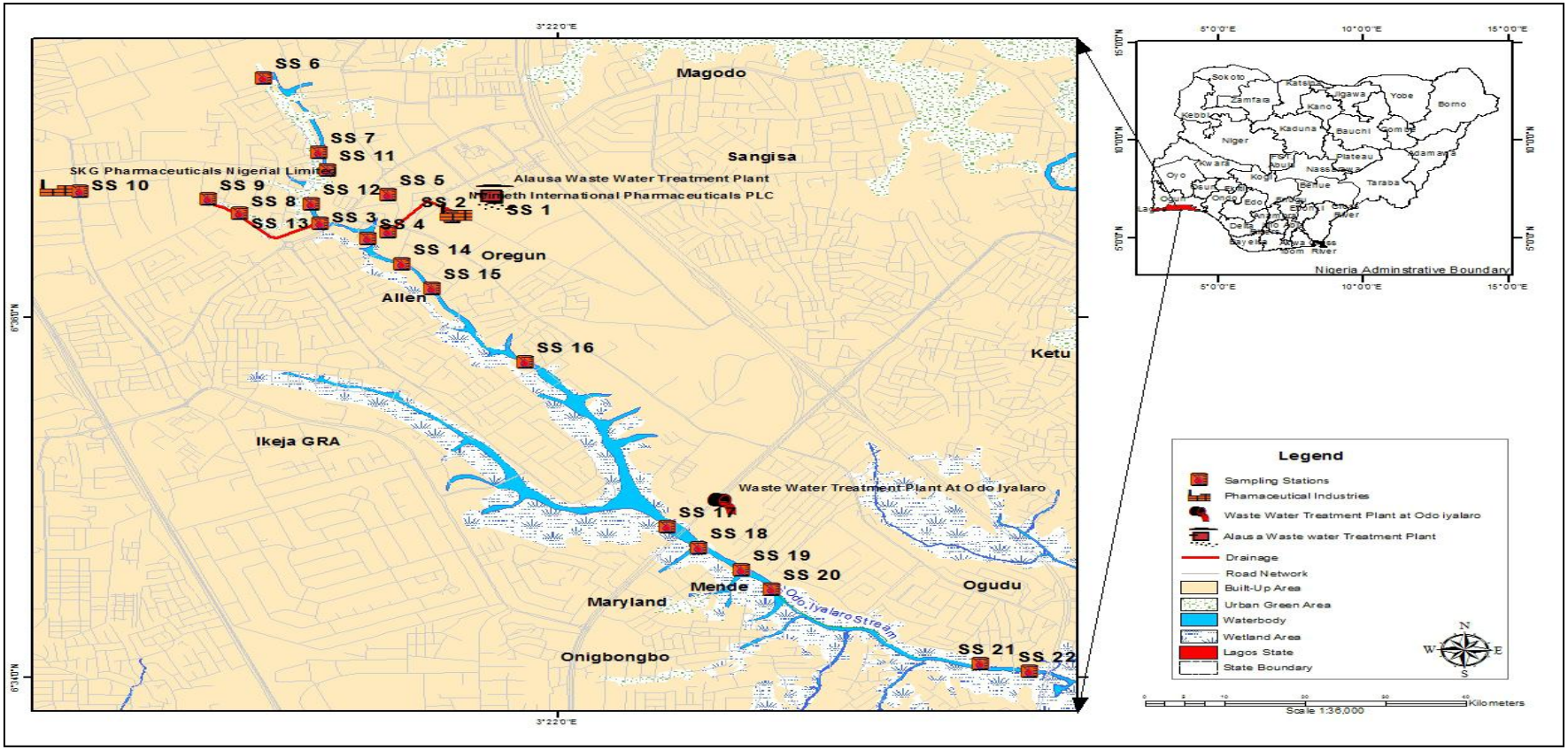

Figure 3.1.2: Map of the Odo-Iya Alaro river showing the sampling stations (n=22) in Lagos State, Southwest Nigeria.

Table 3.1.2: Sampling stations, description of the stations, site categories and Global Positioning System (GPS) locations.

Sampling station	Site Category	Description of site	Longitude	Latitude
SS1	Sewage effluents (SE)	Alausa sewage treatment plant	3˚21' 46.4"	6˚36' 38.73"
SS2	Pharmaceutical effluents (PE)	Neimeth pharmaceuticals	3˚21' 36.2"	6˚36' 33.32"
SS3	Sewage effluents (SE)	Storm water /underground discharge from Alausa STP	3˚21' 20.1"	6˚36' 29.1"
SS4	Sewage effluents (SE)	Chamber inside Oregun sewage discharge point	3˚21' 19.4"	6˚36' 27.0"
SS5	Sewage effluents (SE)	Outlet of discharge at Oregun sewage discharge point	3˚21' 18.5"	6˚36' 23.0"
SS6	River (Semi Urban)	Surulere industrial estate road	3˚20' 46.4"	6˚37' 17.1"
SS7	River (Semi Urban)	Adekunle village	3˚21' 04.9"	6˚36' 55.2"
SS8	River (Urban)	Adeniyi Jones junction 1	3˚20' 41.3"	6˚36' 39.3"
SS9	River (Urban)	Adeniyi Jones junction 2	3˚20' 41.4"	6˚36' 39.3"
SS10	Pharmaceutical effluents (PE)	SKG pharmaceuticals	3˚20' 41.25"	6˚36' 56 .3"
SS11	River (Semi Urban)	Channel along the back of Coca cola bottling company. Mechanic village 1	3˚21' 08.2"	6˚38' 38.2"
SS12	River (Semi Urban)	Channel along the back of Coca cola bottling company. Mechanic village 2	3˚21' 02.8"	6˚36' 35.2"
SS13	River (Urban)	New Alade market	3˚21' 06.6"	6˚36' 29.6"

SS14	River (Urban)	Samplers collected before joining Oregun sewage discharge point	3˚21' 17.1"	6˚36' 24.2"
SS15	Sewage effluents (SE)	Channels along Oregun discharge point	3˚21' 18.5"	6˚36' 23.0"
SS16	River (Urban)	Opebi link road	3˚21' 49.6"	6˚35' 42.6"
SS17	River (Semi Urban)	Odo Iya-Alaro (under bridge)	3˚22' 22.5"	6˚34' 48.0"
SS18	River (Urban)	End of Olatunji street, Ojota	3˚22' 32.7"	6˚34' 38.4"
SS19	River (Urban)	End of Alhaji Amoo street, Ojota	3˚22' 38.1"	6˚34' 35.4"
SS20	River (Urban)	End of Victoria, street, Ojota	3˚22' 39.2"	6˚34' 33.6"
SS21	River (Urban)	Before Ogudu bridge	3˚23' 38.1"	6˚34' 03.2"
SS22	River (Semi Urban)	Ogudu bridge before joining Lagos Lagoon	3˚23' 44.1"	6˚34' 03.0"

3.1.3 Sample collection

At each sampling station, three 50 mL water samples were collected into amber vials with Teflon® lined caps (Fisher Scientific, UK) and then homogenised into a single 150 mL composite sample of which 20 mL was taken. Sampling was undertaken on a quarterly basis to incorporate both the wet (April and July 2017) and dry seasons (October 2017 and January 2018). Sampling vials were rinsed with 100% methanol once and deionised water three times to remove potential contamination before sampling. Samples were collected at the same time of day and in the same location, checked using a Global Positioning System (GPS).

3.1.4 Sample Preparation

A 10 mL aliquot of each composite sample was filtered on site at the points of collection using the procedure of Wilde et al. (2004, with updates through 2009) through a Whatman GFF (0.7 µm pore size) glass microfiber syringe filters into a 20 mL amber glass vial with a Teflon-lined screw cap. The filtered samples were frozen immediately on site with dry ice before shipping within 24 hrs to the University of York Centre of Excellence in Mass Spectrometry, York, United Kingdom for analysis. The samples arrived in York three days after shipment and were immediately thawed and analysed. In order to reduce potential degradation during shipment, filtering was conducted in the field to remove microbial and particulate content. Other studies have shown that longer periods of storage (up to 6 months) even at 4 °C caused no appreciable change in spiked concentrations (Hughes et al., 2013). The remaining samples were stored in the dark at -12°C in the Lagos State Environmental Protection Agency (LASEPA) laboratory.

3.1.5 Analytical procedure and method validation

Quantification was achieved using HPLC-MS/MS with a Thermo Scientific TSQ Endura Mass spectrometer coupled with an UltiMate 3000 liquid chromatograph. The method employed was adapted from Furlong et al. (2014) and validated for this purpose at the University of York Centre of Excellence in Mass Spectrometry (Burns et al., 2018).

Briefly, prior to starting the quantitative analysis, 500 µL of each water sample was diluted with 495 µL of HPLC-grade water and spiked with 5 µL of a mixture of internal standards (each at a concentration of 80 μgL^{-1}) in glass autosampler vials. The 50 % dilution was done in order to clean the samples and bring analytes concentrations to within the calibrated range. Where concentration was found to still exceed the calibrated range, further dilution and reanalysis was done. A random number generator was used to randomise the order in which samples were injected onto the HPLC-MS/MS.

Analysis was conducted by direct injection of 100 µL of respective samples onto a Phenomenex Eclipse Plus C18 chromatography column using a Phenomenex C18 (ODS, Octadecyl) 4 mm x 3 mm ID guard column. Mobile phase A was HPLC-grade water with 0.01 M formic acid and 0.01 M ammonium formate while mobile phase B was 100 % HPLC-grade methanol, flow rate of 0.45 mL min^{-1} was used with a gradient starting at 10 % B which then increased to 40 % at 5 min, 60 % at 10 min, 100 % at 15 min, and remaining 100 % B until 23 min then dropping to 10 % at 23 min prior to a re-equilibration. The autosampler temperature was kept at 4°C and the HPLC column compartment at 40°C. The collision gas was argon at a pressure of 2 mTorr.

Quantification was done with a 16-point calibration using deuterated internal standards (Burns et al., 2018) ranging from 1 to 32000 ngL^{-1}. Calibration r^2-values were consistently >0.95. Analytical limits of detection were calculated as described by Burns et al. (2018) and ranged from 0.9 ngL^{-1} (carbamazepine) to 12.4 ngL^{-1} (gabapentin) (Table 3.1.5). Quality control (QC) measures were used throughout the analysis. Briefly, method blanks (n=6) were made with an identical collection procedure as the environmental samples except using HPLC-grade water. Concentrations of target pharmaceuticals were consistently below levels of analytical quantification in the method blanks. Additionally, QCs consisting of all target pharmaceuticals at a concentration of 80 ngL^{-1} were injected after every four samples followed by an instrumental blank consisting of pure HPLC-grade water. Analytical tolerance was consistently within ±15 % and the instrumental blanks did not contain detectable residues of the target analytes.

Acknowledgments

I thank the York Centre of Excellence in Mass Spectrometry situated at Environment Department, University of York, where the analytical work was conducted for this project by Professor Alistair Boxall and his postdoc student, Dr John Wilkinson, both showed great interest in my work. The York Centre of Excellence in Mass Spectrometry was created thanks to a major capital investment through Science City York, supported by Yorkshire Forward with Funds from the Northern Way Initiative, and subsequent support from EPSRC (EP/K039660/1: EP/M028127/1).

Table 3.1.5: Limits of detection (LOD) and quantification (LOQ) for selected pharmaceuticals analysed in this study (ngL^{-1}).

Pharmaceutical	LOD	LOQ
Amitriptyline	1.09	2.18
Atenolol	8.87	17.7

Carbamazepine	0.89	1.78
Cetirizine	1.87	3.74
Cimetidine	2.04	4.08
Citalopram	2.13	4.26
Codeine	2.61	7.84
Desvenlafaxine	2.15	4.3
Diazepam	1.38	2.76
Diltiazem	1.09	2.19
Diphenhydramine	1.17	2.34
Erythromycin	11.15	22.3
Fexofenadine	2.05	2.1
Gabapentin	12.39	37.16
Hydrocodone	1.02	2.04
Ketotifen	2.89	5.78
Lidocaine	1.36	2.76
Loratadine	5.03	10.06
Metformin	4.19	8.38
Noreistherone	7.25	14.51
Oseltamivir	6.67	13.33
Oxazepam	5.38	10.76
Paracetamol	7.08	14.16
Propranolol	6.49	12.98
Raloxifene	6.34	12.68
Ranitidine	6.23	12.46
Sertraline	9.14	18.28
Sitagliptin	7.06	14.11
Sulfamethoxazole	9.12	18.23
Temazepam	3.59	7.19
Tramadol	3.55	7.1
Triamterene	10.81	21.61
Trimethoprim	1.27	2.55
Venlafaxine	1.53	3.06
Verapamil	10.09	20.18

3.1.6 Data analysis

Data were organised using Excel (Microsoft, 2013) and residuals of the data were checked for normal distribution using the Shapiro-Wilk normality test and homogeneity of variance using the Bartlett test of homogeneity of variances. R (R Development Core Team, 2008) was used to analyse the data and ggplot 2 to create figures (Barplot, Box-and-Whisker). The barplot shows the distribution of the categorical variables. The box-and-whisker plots display a statistical summary of variables: median, quartiles, range and possibly extreme values (outliers). An outlier value is defined as a value that is smaller than the lower quartile (25 percentile) minus 1.5 times the interquartile range, or larger than the upper quartile (75 percentile) plus 1.5 times the interquartile range. Generalised linear model and Chi-square were used to find if there are differences between the sampling sites. Seasonal variations were analysed using one-way ANOVA where assumptions of normality and homogeneity were met followed by Tukey's post-hoc tests to determine if there is any variation in concentrations between the wet and the dry the seasons.

3.2 Results

3.2.1 Detection frequency

Out of the 37 targeted analytes 26 were detected at the Alausa STP (SE) (Figure 3.2.1A) and at the receiving river (Urban site category) (Figure 3.2.1B). 25 and 15 analytes were detected in receiving river (Semi-urban) and the pharmaceutical manufacturing effluents (PE) respectively. The ten most frequently detected compounds across the site categories were fexofenadine, carbamazepine, paracetamol, metformin, diazepam, cimetidine, codeine, sulfamethoxazole, atenolol and trimethoprim. Analytes not detected were venlafaxine (SE site category), triamterene

(Urban site category), triamterene and venlafaxine (Semi-urban site category), gabapentin, hydrocodone, raloxifene, verapamil, diltiazem, oseltamivir, propranolol, sitagliptin, temazepam, triamterene, venlafaxine and tramadol (PE site category).

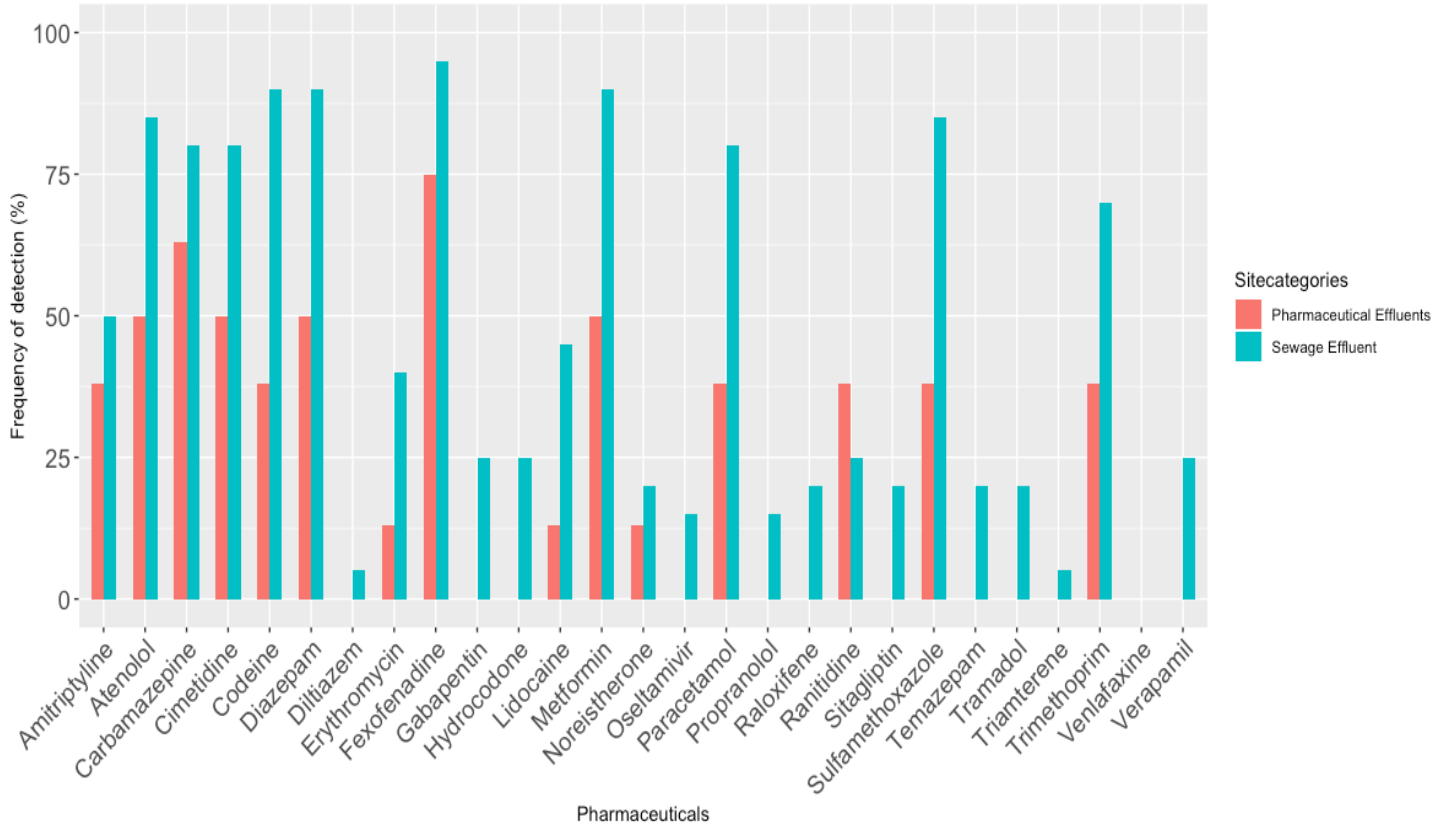

Figure 3.2.1A: Frequency of detection of pharmaceuticals in sewage effluents(SE) and pharmaceutical effluents (PE) in the Odo Iya Alaro river, Lagos, Southwest Nigeria.

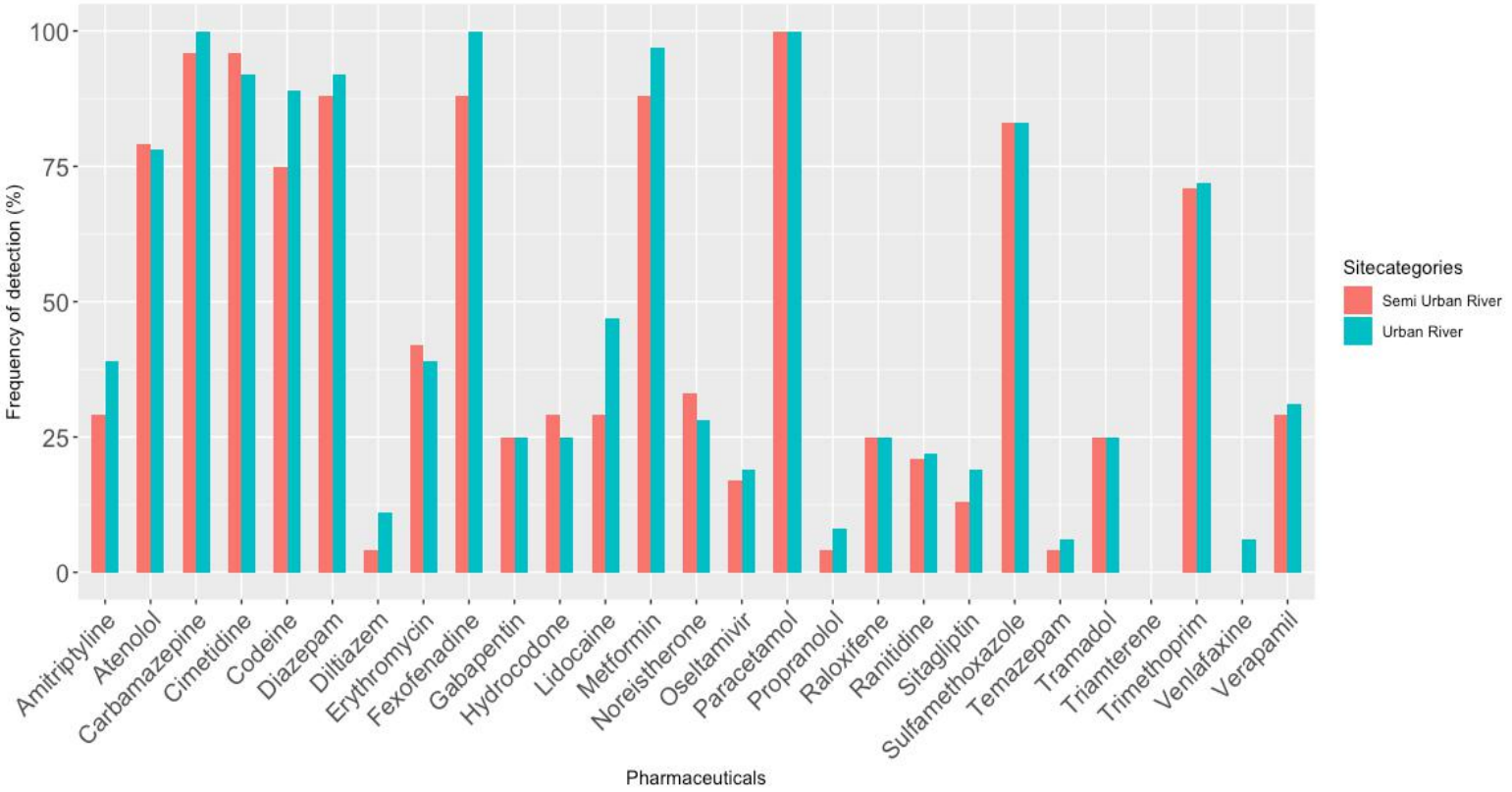

Figure 3.2. 1B: Frequency of detection of pharmaceuticals in receiving river (Urban and Semi-urban site categories) in the Odo Iya Alaro river, Lagos, Southwest Nigeria.

3.2.2 Mean and maximum concentrations

Peak concentrations were typically in the range of low micrograms per litre while mean concentrations were an order of magnitude lower (Figure 3.2.2). Antibiotic and analgesics were detected at the highest concentrations; sulfamethoxazole (129474 ngL^{-1}) had the highest maximum concentration followed by paracetamol (111374 ngL^{-1}). Over all, paracetamol had the highest mean concentration (18178 ngL^{-1}) while sulfamethoxazole had the second highest mean concentration (11160 ngL^{-1}). Cimetidine had the third highest maximum concentration of (95689 ngL^{-1}) and mean concentration (10458 ngL^{-1}). The maximum concentration of a further seven analytes (fexofenadine, carbamazepine, metformin, diazepam, atenolol, trimethoprim, and codeine) also exceeded 39000 ngL^{-1}. Mean concentrations for these substances were in the low micrograms per litre range; metformin (9690 ngL^{-1}), fexofenadine (8409 ngL^{-1}), carbamazepine (7705 ngL^{-1}), atenolol (3044 ngL^{-1}), diazepam (2551ngL^{-1}), trimethoprim (1874 ngL^{-1}), and codeine (1764 ngL^{-1}).

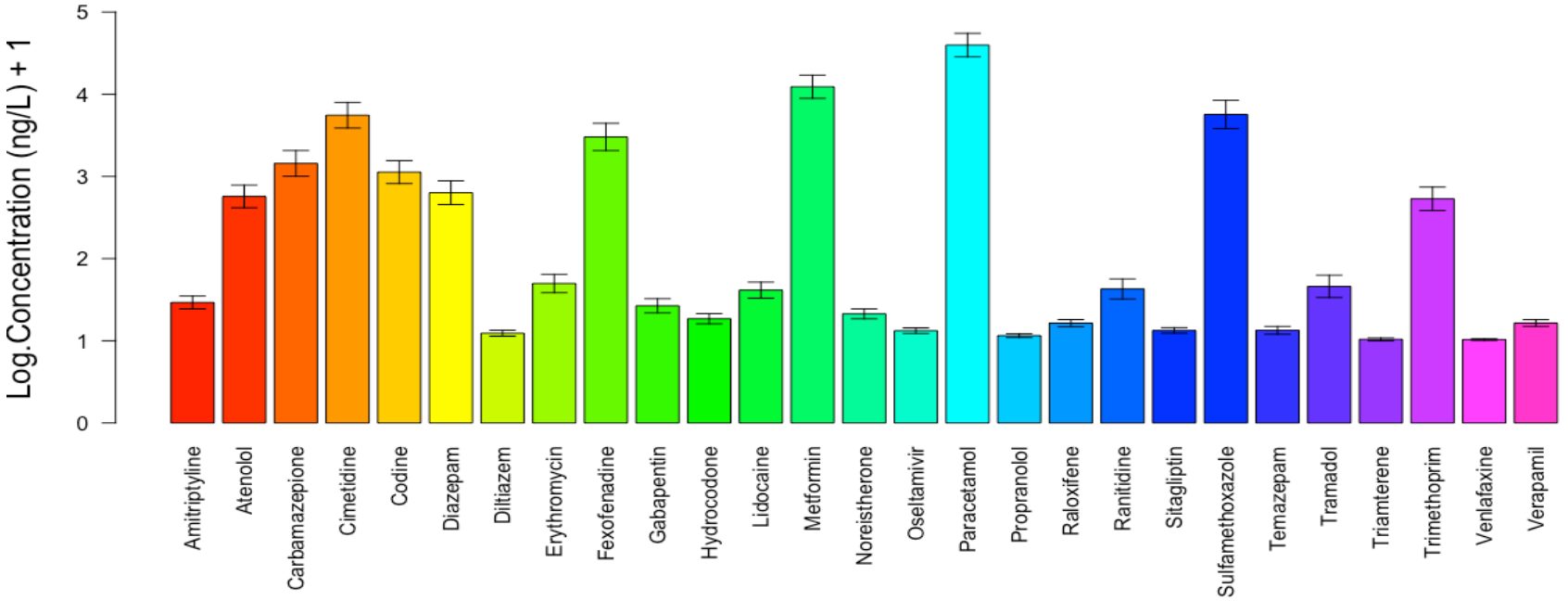

Figure 3.2.2: Mean concentrations (±SEM) of pharmaceuticals detected in the Odo Iya-Alaro river, Lagos, Southwest Nigeria.

3.2.3 Spatial distribution of pharmaceuticals in Odo Iya Alaro river

Pharmaceutical pollution was ubiquitous in the Odo Iya Alaro river with no obvious spatial patterns (Table 3.2.3). Although there are diverse sources of pharmaceuticals into the river such as STPs, pharmaceutical manufacturing facilities, urban waste collection areas and vacuum truck operators who collect sewage from residential apartments and discharged to water course without treatments. There were statistically significant differences between the different site categories (Pharmaceutical manufacturing sites (PME), Alausa STP site (SE), Semi- Urban and Urban sites) (Figure 3.2.3) (GLM: χ^2 (3) = 883.32, p <0.001).

There are variations in the number of analytes detected in each site categories. For instance, 15 pharmaceuticals were detected at the pharmaceutical manufacturing site (PME) and the list detected analyte was noreistherone with mean concentration of 2.24 ngL^{-1} and peak concentrations of 17.91 ngL^{-1}. 26 analytes were detected in Alausa STP site (SE) and Urban receiving river. The list detected was oseltamivir with mean and peak concentrations of 0.69 ngL^{-1} and 5.52 ngL^{-1} respectively for Alausa STP site (SE) and venlafaxine with mean and peak concentrations of 0.24 ngL^{-1} and 4.75 ngL^{-1} respectively was the list analytes detected in the urban site. 25 analytes were detected in semi-urban location with sitagliptin detected at mean concentration of 0.45 ngL^{-1} and maximum concentration of 4.25 ngL^{-1} respectively.

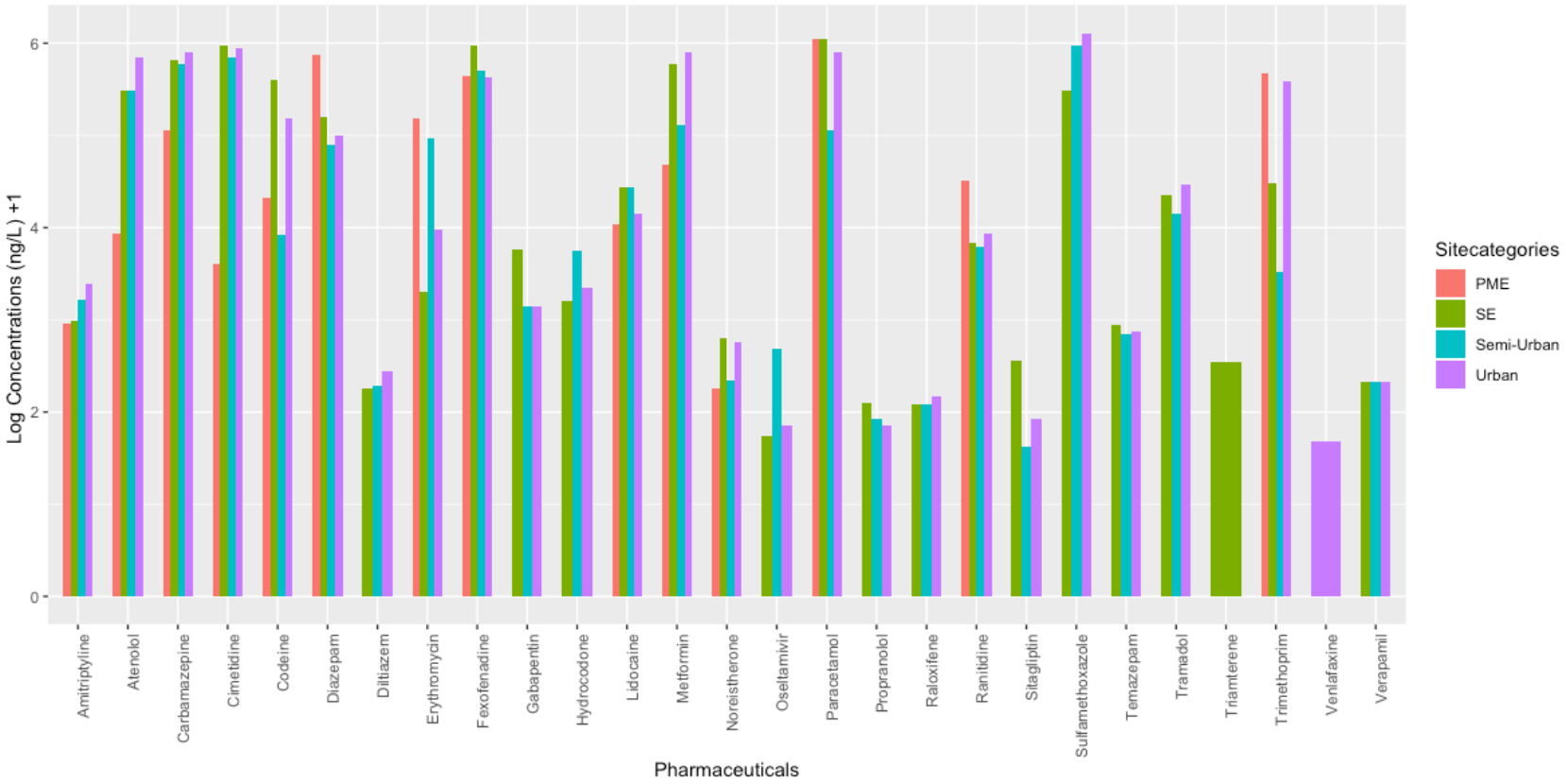

Figure 3.2.3: Log concentrations (ngL^{-1}) + 1 of pharmaceuticals detected at different locations : Pharmaceutical Manufacturing Effluents (PME), Sewage Effluents (SE) and receiving river (Semi-Urban and Urban) in the Odo Iya Alaro river, Lagos, Southwest Nigeria.

Table 3.2.3: Mean concentrations in ngL^{-1} ± 1SD (n=4) of pharmaceuticals detected in the Odo Iya- Alaro river, Lagos, Southwest Nigeria.

Compound	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13	SS14	SS15	SS16	SS17	SS18	SS19	SS20	SS21	SS22
Amitriptyline	21 ± 21	40 ± 47	2 ± 5	26 ± 46	21 ± 26	6 ± 9	1 ± 1	13 ± 27	1 ± 1	1 ± 2	2 ± 5	0.00	1 ± 2	2 ± 3	2 ± 4	37 ± 70	1 ± 1	54 ± 100	64 ± 122	28 ± 48	48 ± 96	42 ± 83
Atenolol	526 ± 459	222 ± 430	7015 ± 125	7448 ± 143	8032 ± 152	7755 ± 153	5460 ± 108	7 ± 9	40 ± 55	31 ± 43	15 ± 22	3715 ± 721	1228 ± 215	7595 ± 147	99 ± 113	99 ± 133	66 ± 79	90 ± 110	91 ± 133	183 ± 247	17233 ± 344	25 ± 29
Carbamazepine	1348 ± 184	3763 ± 525	163 ± 187	182 ± 217	1058 ± 152	638 ± 126	1291 ± 257	1784 ± 211	1890 ± 374	721 ± 144	2004 ± 399	4003 ± 799	7212 ± 143	20717 ± 410	16080 ± 320	17039 ± 340	9725 ± 194	11865 ± 234	20240 ± 398	18150 ± 349	15018 ± 174	14634 ± 292
Cimetidine	15563± 192	72 ± 145	11646 ± 217	7976 ± 151	24397± 475	3320 ± 458	1730 ± 235	34090 ± 405	3694 ± 513	117 ± 190	5104 ± 918	3611 ± 615	14298 ± 236	20760 ± 252	16967 ± 170	4155 ± 728	19221 ± 344	27392 ± 418	2010 ± 359	2235 ± 392	9298 ± 127	2436 ± 438
Codeine	6302 ± 994	2 ± 4	3651± 437	839 ± 850	11077± 189	68 ± 95	64 ± 79	810 ± 158	849 ± 129	692 ± 994	233± 395	44 ± 69	508 ± 696	2216 ± 215	1904 ± 251	1862 ± 329	141 ± 162	73 ± 121	1986 ± 177	4031 ± 737	768 ± 977	28 ± 44
Diazepam	1470 ± 291	25307 ± 35	115 ± 140	714 ± 116	4011 ± 779	337 ± 664	1095 ± 218	1807 ± 220	1156 ± 231	1084 ± 216	349 ± 665	1663 ± 329	1463 ± 289	2613 ± 493	2463 ±485	2320 ± 458	1981 ± 393	1227 ± 241	341 ± 645	531 ± 102	2653 ± 306	1435 ± 285
Erythromycin	46± 92	ND	52 ± 60	11 ± 17	50 ± 100	5 ± 11	5 ± 11	2 ± 4	ND	3777 ± 756	15 ± 30	601 ± 812	246 ± 477	184 ± 359	60 ± 73	19 ± 24	1 ± 2	10 ± 11	147 ± 286	14 ± 16	2 ± 4	2355 ± 468
Fexofenadine	11289 ± 21	15717 ± 20	8994 ± 172	23761 ± 465	10429 ± 151	3516 ± 615	3604 ± 648	7283 ± 936	6212 ± 116	7024 ± 134	5272 ± 990	3408 ± 608	3112 ± 562	3522 ± 597	7910 ± 991	11076 ± 211	12823 ± 241	7620 ± 141	7555 ± 986	5461 ± 104	8362 ± 106	11050 ± 133
Gabapentin	147 ± 294	ND	22 ± 45	31 ± 62	9 ± 18	9 ± 18	6 ± 13	5± 9	6 ± 12	ND	8 ± 16	5 ± 11	36 ± 71	33 ± 66	33 ± 66	29 ± 58	18 ± 37	17 ± 33	26 ± 53	34 ± 67	34 ± 67	35 ± 70
Hydrocodone	18 ± 34	ND	39 ± 79	2 ± 5	ND	140 ± 278	1 ± 1	55 ± 110	ND	ND	ND	1 ± 1	4 ± 8	2 ± 4	6 ± 13	4 ± 8	2 ± 3	3 ± 5	1 ± 3	8 ± 14	1 ± 2	4 ± 7
Lidocaine	13 ± 15	274 ± 548	39 ± 46	707 ± 138	16 ± 32	ND	1 ± 1	2 ± 4	2 ± 3	ND	688 ± 138	1 ± 1	8 ± 9	364 ± 696	40 ± 69	15 ± 20	12 ± 15	26 ± 39	21 ± 25	18 ± 23	25 ± 28	13 ± 25
Metformin	25726 ± 147	915 ± 166	18445 ± 214	12024 ± 111	15342 ± 302	564 ± 844	3528 ± 416	14304 ± 202	5676 ± 790	1903 ± 233	1677 ± 142	1888 ± 208	4188 ± 449	20696 ± 225	15198 ± 244	7801 ± 102	3928 ± 598	25855 ± 373	25854 ± 206	6348 ± 106	1188 ± 115	130 ± 261
Noreisterone	3 ± 7	4 ± 8	4 ± 9	7 ± 14	ND	3 ± 7	8 ± 9	2 ± 4	8 ± 9	ND	8 ± 10	3 ± 6	5 ± 10	6 ± 11	16 ± 32	3 ± 7	4 ± 7	14 ± 29	2 ± 4	4 ± 8	5 ± 10	6 ± 11
Oseltamivir	1 ± 2	ND	1 ± 2	ND	ND	6 ± 12	ND	1 ± 2	ND	ND	12 ± 24	1 ± 1	1 ± 2	1 ± 2	1 ± 3	1 ± 2	ND	1 ± 2	1 ± 3	2 ± 4	ND	2 ± 3
Paracetamol	45649 ± 552	2773 ± 548	6366 ± 513	10563 ± 106	6624 ± 132	3481 ± 129	8209 ± 315	17899 ± 459	6896 ± 543	ND	5844 ± 978	5311 ± 179	22497 ± 380	26357 ± 143	15633 ± 126	19696 ± 519	7765 ± 357	16908 ± 110	48516 ± 350	29362 ± 206	6029 ± 169	5286 ± 288
Propranolol	3 ± 6	ND	2 ± 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1 ± 2	1 ± 2	1 ± 2	2 ± 4	2 ± 4	ND	ND	ND	ND
Raloxifene	2 ± 5	ND	3 ± 6	2 ± 5	ND	3 ± 6	2 ± 4	2 ± 4	2 ± 4	ND	3 ± 6	3 ± 5	3 ± 5	3 ± 5	2 ± 4	3 ± 5	2 ± 4	2 ± 4	4 ± 8	3 ± 6	2 ± 4	3 ± 5
Ranitidine	168 ± 336	816 ± 163	110 ± 220	27 ± 54	61 ± 123	109 ± 219	74 ± 149	216± 432	26 ± 52	808 ± 151	155 ± 311	57 ± 113	182 ± 364	111 ± 221	66 ± 132	78 ± 155	49 ± 98	184 ± 368	173 ± 345	120 ± 241	ND	ND
Sitagliptin	8 ± 17	ND	8 ± 17	1 ± 2	ND	ND	1 ± 2	ND	ND	ND	ND	ND	1 ± 2	1 ± 2	1 ± 3	2 ± 3	1 ± 2	1 ± 2	1 ± 2	2 ± 4	1 ± 2	1 ± 2
Sulfamethoxazole	6469 ± 400	3632 ± 253	5899 ± 245	4739 ± 218	281 ± 533	165 ± 273	7432 ± 123	32545 ± 372	20479 ± 29	ND	18937 ± 33	13478 ± 244	13043 ± 248	33773 ± 638	8132 ± 147	8322 ± 152	26791 ± 448	3633 ± 417	13913 ± 111	3574 ± 522	19766 ± 379	517 ± 754
Temazepam	41 ± 48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	19 ± 37	ND	9 ± 13	4 ± 8	17 ± 35	ND	ND	ND	ND	ND
Tramadol	224 ± 449	ND	527 ± 105	348± 696	ND	117 ± 235	116 ± 233	341 ± 682	245 ± 490	ND	122 ± 243	119 ± 237	731 ± 146	354 ± 708	555 ± 111	452 ± 905	338 ± 676	318 ± 636	318 ± 636	339 ± 678	374 ± 747	355 ± 709
Triamterene	8 ± 17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	1165 ± 141	21479 ± 24	675 ± 773	738± 108	23 ± 37	94 ± 93	78 ± 85	89 ± 106	324 ± 345	ND	110 ± 151	82 ± 95	468 ± 610	697 ± 976	302 ± 344	144 ± 133	124 ± 133	1006 ± 137	12348 ± 180	1197 ± 174	52 ± 60	41 ± 48
Venlafaxine	ND	ND	ND	ND	ND	ND	ND	1 ± 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	1 ± 2	ND	ND	ND	ND
Verapamil	1 ± 2	ND	1 ± 2	2 ± 5	ND	1.67 ± 3.34	1 ± 2	1 ± 2	1 ± 3	ND	6 ± 10	1 ± 2	1 ± 2	7 ± 10	6 ± 10	7 ± 10	1 ± 3	1 ± 2	1 ± 3	2 ± 3	1 ± 2	2 ± 3

ND=Not detected.

3.2.4 Seasonal variations in pharmaceutical concentrations

There were statistically significant differences between the dry season, peak of dry season, the wet season and peak of the wet season ((GLM: χ^2 (3) = 8.63), $p<0.001$). More pharmaceuticals were detected in the peak of the wet season (22) than the other seasons. 17 analytes were each found in the wet and dry seasons while 16 pharmaceuticals were detected at the peak of the dry season.

Although more pharmaceuticals were detected in the peak of wet season, there was distinct variation in concentrations of many pharmaceuticals which generally higher at the peak of the dry season (concentration level) (Figure 3.2.4). Fexofenadine for example, an antihistamine, has the highest mean and median concentrations (28272 ngL^{-1} and 22318 ngL^{-1}) respectively in the peak of the dry season (Table 3.2.4) compared to all other compounds and other seasons. The mean and median concentrations of fexofenadine are more than 500 times higher than the concentration in dry, peak of the wet or wet seasons. Carbamazepine, a psychotic drug has the second highest mean concentration (25654 ngL^{-1}) in the peak of the dry season and followed closely by paracetamol (24616 ngL^{-1}) in the same season. The mean concentration of paracetamol in the peak of the dry season was almost 1.5 times higher than the peak of the wet season. The median concentration was 9228 $ngL^{-1}$for peak of dry season, 8930 ngL^{-1} for dry season, 8940 ngL^{-1} for peak of wet season and 7130 ngL^{-1} for wet season.

The mean and median concentrations of all the pharmaceuticals detected in the Odo-Iya Alaro river are extremely higher in the peak of the dry season than any other season except the following compounds that are either not detected in the peak of dry season or detected in low concentrations in other seasons: diltiazem 6 ngL^{-1} (peak of wet season), erythromycin 785 ngL^{-1}(dry season), gabapentin 97 ngL^{-1} (peak of the wet

season), lidocaine 269 ngL^{-1} (dry season), noreistherone 18 ngL^{-1} (peak of wet season), oseltamivir 6 ngL^{-1} (peak of wet season), propranolol 2 ngL^{-1}(peak of wet season)(Table 3.2.4). Raloxifene was only detected in the peak of the wet season at a mean concentration of 9 ngL^{-1}. Other compounds detected at low mean concentrations are ranitidine (dry season and wet season), sitagliptin (peak of wet season), temazepam (not detected in peak of wet season only), tramadol (detected only in peak of wet season), triamterene (detected only in wet season), venlafaxine (detected only in peak of wet season) and verapamil (detected both in wet and peak of wet seasons).

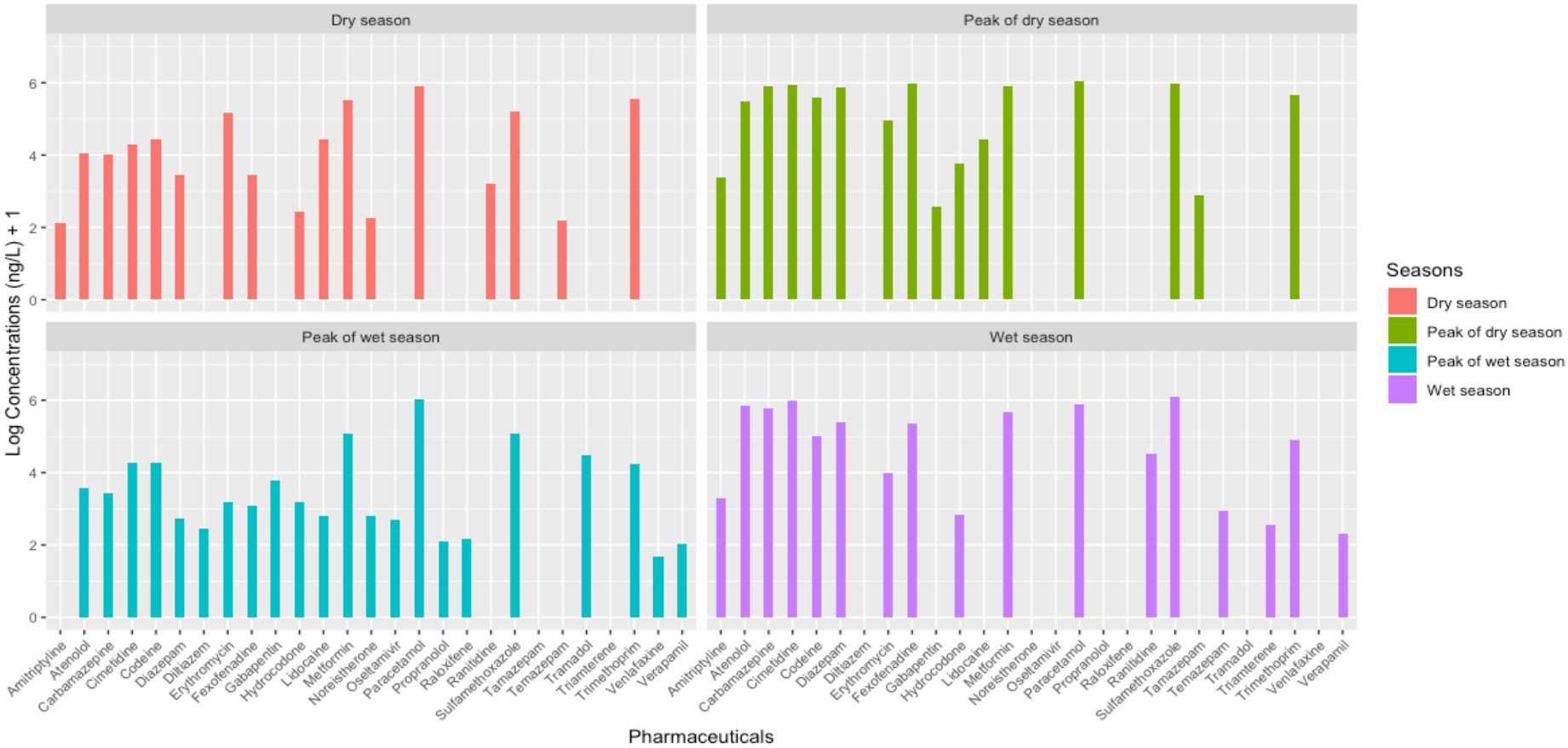

Figure 3.2. 4: Barplots displaying log concentrations (ngL^{-1})+1 of seasonal variation of pharmaceuticals detected at Odo Iya Alaro river, Lagos, Southwest Nigeria.

Table 3.2.4: Summary results (ngL^{-1}) for the seasonal variations in concentrations during the quarterly monitoring campaign at the Odo-Iya Alaro river. The concentration range, median and mean concentrations for dry and peak of dry seasons, wet and peak of wet seasons are reported.

Pharmaceuticals	**Peak of dry season**			**Dry season**			**Peak of wet season**			**Wet season**		
	Range	Median	Mean	Range	Median	Mean	Range	Median	Mean	Range	Median	Mean
Amitriptyline	n.d. - 248	0	42	n.d. - 13	0	1	n.d.	0	0	0.50 - 193	9	32
Atenolol	n.d. - 30768	0	8473	n.d. - 1077	40	115	n.d. - 371	41	63	n.d. - 68869	292	3527
Carbamazepine	n.d. - 82196	7749	25654	n.d. - 1016	32	128	n.d. - 272	39	58	1.78 - 58418	322	4984
Cimetidine	n.d. - 88681	10388	22739	4.08 - 2001	247	469	n.d. - 1895	353	468	n.d. - 95690	8547	18160
Codeine	n.d. - 39381	106	4505	n.d. - 2756	250	564	n.d. - 1872	58	150	n.d. - 9977	1261	1839
Diazepam	n.d. - 75031	4727	8292	n.d. - 275	8	34	n.d. - 55	8	10	6.39 - 25923	45	1871
Diltiazem	n.d.	0	0	n.d.	0	0	n.d. - 28	0	6	n.d.	0	0
Erythromycin	n.d. - 9373	0	505	n.d. -15110	22	785	n.d. - 149	6	19	n.d. - 962	0	74
Fexofenadine	11180 - 93448	22318	28272	12.85 - 286	26	47	n.d. - 119	5	11	757 - 22238	1553	5308
Gabapentin	n.d. - 37	0	2	n.d.	0	0	n.d. - 590	82	97	n.d.	0	0
Hydrocodone	n.d. - 559	0	35	n.d. - 28	0	1	n.d. - 160	5	13	n.d. - 70	0	3
Lidocaine	n.d. - 2751	0	125	n.d. - 2779	30	269	n.d. - 63	18	22	n.d.	0	0
Metformin	n.d. - 80967	15165	22525	n.d.-32917	842	4658	n.d.-12378	549	1574	n.d.- 49325	3138	10003
Noreistherone	n.d.	0	0	n.d. - 18	0	3	n.d. - 63	15	18	n.d.	0	0
Oseltamivir	n.d.	0	0	n.d.	0	0	n.d. - 48	3	6	n.d.	0	0
Paracetamol	n.d. - 111374	9228	24616	n.d.-78731	8930	18173	n.d.-105028	8940	15120	n.d. - 75415	7130	14804
Propranolol	n.d.	0	0	n.d.	0	0	n.d. - 13	0	2	n.d.	0	0
Raloxifene	n.d.	0	0	n.d.	0	0	n.d. - 15	10	9	n.d.	0	0
Ranitidine	n.d.	0	0	n.d. - 164	0	7	n.d.	0	0	n.d. - 3265	441	646

Sitagliptin	n.d.	0	0	n.d.	0	0	n.d. - 35	4	6	n.d.	0	0
Sulfamethoxazole	n.d. - 93359	5599	22204	n.d.-16022	718	2224	n.d. - 12396	983	1752	n.d.-129475	5741	18460
Temazepam	n.d. - 76	0	8	n.d. - 15	0	1	n.d.	0	0	n.d. - 89	0	7
Tramadol	n.d.	0	0	n.d.	0	0	n.d. - 2924	1312	1144	n.d.	0	0
Triamterene	n.d.	0	0	n.d.	0	0	n.d.	0	0	n.d. - 35	0	2
Trimethoprim	n.d. - 47025	0	3907	n.d.-37241	190	2176	n.d. - 1784	95	175	n.d. - 7820	437	1240
Venlafaxine	n.d.	0	0	n.d.	0	0	n.d. - 5	0	0	n.d.	0	0
Verapamil	n.d.	0	0	n.d.	0	0	n.d. - 11	5	5	n.d. - 21	0	4

n.d. = Not Detected

3.3 Discussion

Pharmaceuticals are biologically active and pseudo-persistent, in the environment due to the continual input of wastewater effluent to rivers (Kay et al., 2017; Yamamoto et al., 2009). They, therefore, potentially pose a toxicological risk to non-target organisms (Boxall et al., 2002; Huang et al., 2012). The results presented in this work provide new information about the presence of pharmaceuticals in a Nigerian river, including frequency of occurrence, concentration ranges, spatial and temporal patterns and seasonal distribution. This work contributes significantly to the knowledge of pharmaceuticals in African rivers, which has wider relevance to developing countries worldwide.

3.3.1 Frequency of detection

The detection of 26 pharmaceuticals in the Odo Iya Alaro river has helped to confirm the presence of these substances in Nigerian watercourses including some that have not previously been observed in African rivers more widely. Pseudo persistence was observed, presumably due to continuous discharge of effluents to the river, similar to that found in other studies around the world (Burns et al., 2018; Hughes et al., 2013; Kay et al., 2017). Many of the substances found are the same as those in these other studies and certain substances are clearly used in great quantities around the world, including, for instance, fexofenadine, cimetidine, paracetamol, the sulphonamides and carbamazepine. Similarly, some substances appear to enter the aquatic environment in much lower amounts globally, e.g. propranolol. Furthermore, frequency of occurrence is higher in the receiving water than the STP and the pharmaceutical manufacturing effluents.

3.3.2 Mean and maximum concentrations

Overall, the mean (18178.27 ngL^{-1}) and maximum (129474.92 ngL^{-1}) concentrations in this study were 2-3 orders of magnitude higher than previously reported for Europe and the US (Tim Aus der Beek et al., 2016; Burns et al., 2018; Hughes et al., 2013; Madikizela et al., 2017; Verlicchi et al. 2012) but similar or an order of magnitude higher than those measured in China (Fatta-Kassinos et al., 2011) and India (Balakrishna et al., 2017). Some compounds found at particularly high concentrations were sulfamethoxazole and paracetamol which were prevalent at all seasons and locations in the catchment throughout the year. This indicates very frequent release of pharmaceuticals to rivers, a suggestion supported by other works (Andreozzi et al., 2003; Tim Aus Der Beek et al., 2016; Matongo et al., 2015a & 2015b; Ternes, 1998; Vieno et al., 2007). In South Africa, however, Agunbiade and Moodley (2014) reported surface water concentrations ranging from 500 to 30000 ngL^{-1} showing that concentrations may be lower in more developed regions of Africa. Concentrations in Kenya (Ngumba et al. 2017) were of the same order as the ones measured in this study and may be attributed to a range of factors including over-the-counter sales, differences in health issues, poorer removal efficiencies at STPs, unregulated discharges by pharmaceutical manufacturing companies, illegal disposal of sewage by vacuum trucks and climatic conditions. Without further study it is currently not possible to disentangle the range of factors potentially influencing pharmaceutical pollution of rivers.

3.3.3 Spatial distribution of pharmaceuticals

There were no specific spatial trends observed in this work and concentrations were high throughout the catchment revealing that there are potentially many contributing sites. Studies in Europe and the US have found that STP are the major source of pharmaceutical pollution (Hughes et al., 2013) but in the developing world it seems that there are a greater range of sources contributing to loads in rivers. These may include STPs, pharmaceutical manufacturing plants, urban waste collection areas and disposal of effluent by vacuum trucks. Similarly, pharmaceutical production facilities in Hyderabad, India have been found to be a key source in this developing country (Balakrishna et al., 2017: Fick et al., 2009; Larsson et al., 2007).

3.3.4 Seasonal variations in pharmaceutical concentrations

A number of studies have previously proposed a range of reasons for variation on concentrations of pharmaceuticals in river across the year, including seasonal usage and changes in environmental conditions (e.g. temperature and river flow) (Kolpin et al., 2014; Tewari et al., 2013). Typically, concentrations are highest during low flow conditions when sewage effluent makes up a greater proportion of river flow. As for spatial patterns though, seasonal trends in the data were complex with some compounds being found at extremely high concentrations in the peak of the dry season and conversely, some compounds such as atenolol, carbamazepine, cimetidine, codeine, diazepam, fexofenadine, metformin, paracetamol, sulfamethoxazole and trimethoprim are equally high during the wet period (Table3.2.4). Seasonal usage is unlikely to explain this as many compounds would be used equally over the year to treat persistent illnesses, e.g. carbamazepine and metformin. It may be that the multiple sources of pharmaceuticals in the catchment results in this complex picture with some that are

associated with continuous effluent discharges (e.g. from STPs and manufacturing facilities) being diluted in the wet season but other sources (e.g. urban waste sites) which see pollutants mobilised in periods of rainfall.

Table 3.4.1: Comparison of pharmaceutical concentrations measured in Nigeria (this study), Africa and globally (global data are taken from Hughes et al., 2013 and Der Beek et al., (2016)).

Pharmaceuticals	Max Conc in THIS STUDY (ng/L)	Max Conc in Africa (ng/L)	Max Conc worldwide (ng/L)	Median Conc in THIS STUDY (ng/L)	Median Conc in Africa (ng/L)	Median Conc worldwide (ng/L)
Amitriptyline	248	NA	<19[e]	11	NA	<19[e]
Atenolol	68869	39000[p]	859[a]	48	NA	39[a]
Carbamazepine	82196	<1[g], 735[h], 1240[o]	12000[a], 8050[b]	88	NA	174[a]
Cimetidine	95690	NA	1000[a]	560	NA	97[a]
Codeine	39381	NA	1000[a]	153	NA	49[a]
Diazepam	75031	NA	34[a]	42	NA	9[a]
Diltiazem	28	NA	146[a]	2	NA	13[a]
Erythromycin	15110	11[g], 240[j], 1000[l]	90000[a], 5i	1	NA	51[a]
Fexofenadine	93448	NA	1144[f]	522	NA	253[f]
Gabapentin	590	NA	7780[a]	5	NA	103[a]
Hydrocodone	559	NA	92[f]	1	NA	22[f]
Lidocaine	2779	NA	40[f]	4	NA	11.8[f]
Metformin	80967	NA	47[d]	1877	NA	NA
Noreistherone	63	NA	<19[e]	1	NA	<19[e]
Oseltamivir	48	NA	9[f]	5	NA	<19[e]
Paracetamol	111374	5500[o], 16000[p]	15700[a], 23000[b]	8525	NA	148[a]

Propranolol	13	NA	590[a]	2	NA	18[a]
Raloxifene	15	NA	7[f]	10	NA	NA
Ranitidine	3265	NA	570[a]	2	NA	27[a]
Sitagliptin	36	NA	121[e]	5	NA	37[e]
Sulfamethoxazole	129475	3[g], 4090[k], 6010[j], 13800[m], 38900[k], 23350[n]	11920[a], 29000[b]	1482	NA	83[a]
Temazepam	89	NA	39[c]	4	NA	17[e]
Tramadol	2924	NA	8000[a]	8	NA	802[a]
Triamterene	345	NA	NA	5	NA	NA
Trimethoprim	47025	160[k], 2650[m], 400[l], 6950[k], 9480[n]	4000[a], 13600[b]	91	NA	53[a]
Venlafaxine	45	NA	4[f]	4	NA	<19[f]

a = Hughes et al., 2013, b = Der Beek et al., 2016, c = Jerker et al., 2017, d = Niemuth et al., 2015, e = Burns et al., 2017, f = Burns et al., 2018
g = Inam et al., 2015 (Nigeria), h = Li et al., 2014, i = Kim et al., 2007, j = Matongo et al., 2015b, k = K'Oreje et al., 2016, l = Olarinmoye et al., 2016 (Nigeria), m = Ngumba et al., 2016, n = K'Oreje et al., 2012, o = K'Oreje et al., 2018, p = Agunbiade and Moodley, 2014.
NA = not available

3.4 Conclusion

This is the most detailed study to date of pharmaceuticals in African rivers and has highlighted their occurrence at high concentrations. Concentrations in Nigerian rivers appear to be several orders of magnitude higher than those reported for Europe and the US and, in some cases, even higher than the few existing values produced for other developing countries (e.g. Africa, China and India). Spatial and temporal patterns were complex and probably affected by a greater range of sources contributing to pharmaceutical loads than in many existing studies. This pose a particular issue for understanding and managing pharmaceutical pollution in African rivers. The scenario presented here has a strong likelihood of being replicated in other major African cities as well as megacities in other developing nations globally, where pharmaceuticals are available over the counter and where wastewater discharges to rivers proceed untreated. A key implication for the global research agenda on pharmaceutical effects in surface waters (e.g. ecotoxicological effects, antibiotic resistance) is that studies of pharmaceuticals in the environment should focus more on developing countries where contamination of water is likely much more significant.

CHAPTER FOUR

Effects of pharmaceuticals on the freshwater shrimp, Gammarus pulex

4.0 Introduction

Pharmaceutical compounds such as NSAIDs, antibiotics, anticonvulsive drugs, cancer drugs, lipid regulators, psychiatric drugs and recreational drugs have been detected in a range of water bodies, including surface waters, freshwaters, marine waters and ground water (Jones et al., 2004; Kasprzyk-Hordern et al., 2008; Hughes et al., 2013; Orn et al., 2016).

There has been concern about the biological effects of these drugs in the aquatic environment since the early 70's and Environmental Quality Standards (EQS) have been in use after the US Water Quality Act of 1965 and the US Clean Water Act of 1977 (Conolly et al., 2017). In the EU the origin of EQS is driven by the Water Frame Directive (WFD 2000; Crane and Babut 2007) which establishes a legal framework to protect and restore clean water across Europe and ensure its long-term, sustainable use. Since then standard risk assessment methods (acute toxicity) became the most favoured testing method used in assessing toxicity of chemicals to aquatic organisms.

This method involves an LC_{50} test- the toxicant concentration at which 50% of the assayed organisms die within a predetermined time, usually 48 or 96 h. Although this method is very useful there are limitations given the fact that pharmaceuticals are generally found in low concentrations (ngL^{-1} to μgL^{-1}) in the aquatic environment (Fent et al. 2006; Behera et al., 2011; Hughes et al., 2013; Jiang et al., 2014; Miller et al., 2015; Orn et al., 2016). Potential risks exposure to low concentrations of pharmaceuticals could be classified into ecotoxicological effects (acute toxicity and chronic toxicity, carcinogenicity and genotoxicity); pharmacological effects

(interference of the hormone and immune system) and development of resistant micro-organisms (Sayadi et al., 2010)

In recent times, questions have been asked about the use of acute data within pharmaceutical risk assessment and the European Union (EU) regulatory authorities have embraced the use of chronic testing methods (Ferrari et al., 2005; EMEA, 2006). Acute toxicity testing procedure serves as a guide for setting limits for concentrations of toxicants entering the aquatic environment except if acute effects happened at lower concentrations. It also serves as the first line of action in a series of procedures to set concentration levels for sublethal effects of chemicals on organisms. With acute toxicity testing, large amount of reproducible data can be generated within a short period of time. Acute effects data show that, generally, an effect concentration of over 1 mgL^{-1} is required to induce mortality in aquatic organisms (Crane et al., 2006; Fent et al., 2006; Orn et al., 2016). However, these short-term toxicity assays (acute toxicity) with pharmaceuticals may not be a suitable means of defining the ecological risk of these compounds. The subtle effects of pharmaceuticals to non-target organisms can best be assessed by long-term toxicity assays. Chronic toxic effects occur at concentrations orders of magnitude lower compared with acute exposures in an organism exposed to the same drug. This further demonstrates that long-term exposure to environmental realistic concentration is likely to be of greater concern for aquatic organisms.

Laboratory investigation of the impact of new chemicals on the aquatic habitat has mostly been based on fish and a few aquatic macroinvertebrate animals such as *Daphnia magna*, *Ceriodaphnia dubia* and *Hyalella azteca*. Macroinvertebrates represent a variety of trophic levels while fish represent only one trophic level. Among benthic macroinvertebrate animals, *Daphnia magna* has been used more than any other.

They are standard animals used in aquatic ecotoxicology tests for new chemicals (OECD, 2004) including antibiotics, antidepressants, and non-steroidal anti-inflammatory drugs (Santos et al., 2010; Hughes et al., 2013). However, the relevance of *D. magna* has been questioned because of its absence in many running waters where pharmaceutical contamination is most likely to be an issue (Hughes et al., 2013).

Gammarus pulex is a freshwater macroinvertebrate amphipod, universally distributed in rivers, streams and ponds. *G. pulex* is easy to sample using kick sampling methods and often very abundant. It is a benthic dwelling detritivore, pollution sensitive and used in biomonitoring studies. *Gammarus* are useful indicator species due to their requirement of oxygen rich water. *G. pulex* plays a prominent role in the freshwater food chain, serving as source of food for fish, birds and other invertebrates (Friberg et al., 1994; Maltby et al., 2002; Miller et al., 2015). *G. pulex* had been used in the past to assess the adverse effects of contaminants such as metals and leachates (Maltby and Naylor, 1990; Sundelin and Eriksson, 1998; Gross et al., 2001; Forbes and Cold, 2004; Schirling et al., 2005; De Lange et al., 2006, 2009; Bloor, 2010; Chaumot et al., 2015; Escher et al., 2017) but rarely used for pharmaceuticals (Meredith-Williams et al 2012; Ashauer et al., 2012).

G. pulex was chosen as a test animal because they play an important role in the food chain and therefore their loss or reduced abundance has the potential for widespread ramifications through the aquatic ecosystem. They are also bio indicators of stream health, generally abundant, easy to sample and most likely to be affected by pollution because they have little mobility. They play an important role in the decomposition of coarse particulate organic matter, are an important prey for many fish and non-piscean predators (McNeil et al., 1999), they are generally classified as an omnivorous shredder (Maltby et al., 1990). *G. pulex* has been successfully used in a variety of toxicity tests,

including feeding activity (Huang et al., 2012), precopula separation (Pascoe et al., 1994), scope for growth (Maltby et al., 1990), in situ tests (Crane and Maltby, 1991), and behaviour (Gerhardt et al., 1994).

The current work investigated the ecological effects of prolong low-level exposure of *G. pulex* (water column dweller) to erythromycin, diclofenac, ibuprofen and their mixtures at environmentally relevant concentrations on growth, feeding and mortality with the aim of broadening knowledge about the potential risk of such contaminant to aquatic ecosystems.

4.1 Aims, objectives and hypothesis

4.1.1 Aim and objectives

The general aim of this chapter is to seek to improve the understanding of the effects of prolonged low-level exposure of *G. pulex* to pharmaceutical contamination. Response variables included growth, feeding and mortality. Specific objectives were;

1. To assess the effects of prolonged low-level exposure to environmentally relevant concentrations of erythromycin, diclofenac and ibuprofen on growth, feeding, and mortality of the freshwater macro-invertebrate crustacean, *Gammarus pulex.*
2. To examine the effects of mixtures of the above pharmaceuticals on *G. pulex* relative to individual compounds.

4.1.2 Hypotheses

(H1): That prolonged low-level exposure to environmentally relevant concentrations would have a direct lethal effect on *G. pulex*.

(H2): That extended exposure to environmentally relevant concentrations will cause significant reductions in sub-lethal endpoints.

(H3): That the effects of mixtures will be more pronounced than compounds acting singly.

4.2 Single compound and mixture experiments with G. pulex

4.2.1 Materials and methods

4.2.1.1 Study compounds

The study compounds (erythromycin, diclofenac and ibuprofen) were chosen based on their high prescription rates, volumes and availability of a reliable analytical method. They are among the 25 most prescribed drugs in the UK and because of their widespread occurrence in rivers worldwide (Hughes et al., 2013). Calculations of the ratio of predicted environmental concentration (PEC) and predicted no effect concentration (PNEC) has shown that the ratios for these drugs exceeded one. A risk quotient (RQ) ≥1 indicates the potential for impacts on aquatic organisms (Jones et al., 2002). Hence, the basis for their selection. (Table 4.2.1.1).

Table 4.2 1.1: Physico-chemical properties of the study compounds

Compound	CAS number	Purity (%)	Molecular weight (g/mol)	Molecular formula	Physico-chemical properties and risk quotients
Erythromycin	114-07-08	>99	733.93	$C_{37}H_{67}NO_{13}$	Solubility (mgL^{-1}) =1.44, pKa = 8.9, log Kow = 2.48, Excretion rate = 5% parent, RQmin = 0.01, RQmax = 1.25
Diclofenac	15307-79-6	>98	296.148	$C_{14}H_{10}Cl_2NNaO_2$	Solubility (mgL^{-1}) =2430, pKa = 4.0, log Kow = 4.02, Excretion rate = 15% parent, <1% conjugate, RQmin = 0.01, RQmax = 1.13
Ibuprofen	15687-27-1	98	206.29	$C_{13}H_{18}O_2$	Solubility(mgL^{-1}) =21.00, pKa = 4.91, log Kow = 3.79, Excretion rate = 1% parent, RQmin = 0.55, RQmax = 4.20

pKa = dissociation constant; log Kow = octanol: water partition coefficient; RQ data from: (Jones et al., 2002; Thompson, 2006; Yamamoto et al., 2009)

4.2.1.1.1 Behavioural effects of the selected pharmaceuticals

4.2.1.1.1.1 Diclofenac

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) dispensed either topically, through injection or orally. Globally, it is obtainable in prescribed and non-prescribed human drug (Gonzalez-Pleiter et al., 2013) though the oral form is a prescription only medication in the UK (Gov.uk, 2015). It is also commonly used as a veterinary analgesic (Blanco et al., 2016). Diclofenac is a sodium or potassium salt of 2-(2-(2,6-dichlorophenylamino) phenyl) acetic acid that is used to treat a wide range of inflammatory disorders such as muscle strain and arthritis, but also may be used to treat chronic pain associated with cancer (Zhang et al., 2008). Its widespread use in treating farm animals, particularly cattle, has led to the near extinction of three species of

vultures in the Indian sub-continent resulting in its veterinary licence being withdrawn there in 2006 (Prakash et al., 2012). However, with the exception of old-world vultures, diclofenac has a well-known safety profile and is one of the most popular NSAIDs available with an estimated worldwide annual consumption (as a human and veterinary pharmaceutical drug) of over 1000 tons per year (Gonzalez-Pleiter et al., 2013). After ingestion, diclofenac is partially biotransformed to its hydroxylated metabolites (e.g., 4′-hydroxy (OH) and 5–OH diclofenac) and is excreted via urine and faeces, before reaching wastewater treatment works (WwTW) and thereafter potentially the ecosystem. Depending on the route of administration, between 10 – 50% of diclofenac and its metabolites can be eliminated from the body, in as little as two hours after application (Heberer & Feldmann, 2005; Wishart et al., 2006).

The proportion of diclofenac and its metabolites that are eliminated through the faeces and urine are typically 35% and 65% respectively (Zhang et al., 2008). Once the diclofenac reaches STPs, its removal rate is notably variable, mostly due to the variation in the treatment regime used (that is, less removal through activated sludge than filter beds). Rates of between 0 and 100% have been found under different regimes (Zhang et al., 2008; Beltrán et al., 2009). Furthermore, rates vary according to the time of year: Wiest et al. (2016), found that an average of 55% was removed in summer, dropping to 1% in winter - due to differences in photolysis and oxidation. Generally, however, typical removal values seem to lie between 21 – 40% (Paxéus, 2004; Zhang et al., 2008). Considering its frequency of use, its high excretion rates and potentially low removal at STPs, it is perhaps unsurprising that, globally, diclofenac is one of the most frequently detected pharmaceuticals in surface waters (Schwaiger et al., 2004; Zhang et al., 2008; Loos et al., 2009; Schmidt et al., 2011; Gonzalez-Rey & Bebianno, 2014). Indeed, a recent report found that after testing the final effluent of

160 sewage treatment works in the UK, diclofenac was one of several drugs present in concentrations apparently high enough to potentially affect ecosystems (Boxall et al., 2014). Several studies have found diclofenac in surface waters and the aquatic environment. Such is the concern of its prevalence and potential environmental effects that it was due to be included in the of Priority Substances list (Gonzalez-Rey & Bebianno, 2014) but currently it remains included in the EC's 'Watch List' of compounds that needs more data to support further prioritisation, along with 17-α-ethinylestradiol, and 17-β-estradiol (Pusceddu et al., 2017). Diclofenac is relatively water soluble (2.37 mgL^{-1} at 25 °C) (Gonzalez-Rey & Bebianno, 2014) and does not tend to adsorb to organic matter (Johnson et al., 2007), therefore, it tends to remain in the aquatic phase once in the environment (Ericson et al., 2010). It is bio accumulative (Schwaiger et al., 2004; Ericson et al., 2010), fairly persistent in the environment (Bendz et al., 2005), and has been reported as progressing through the aquatic food chain to top-predators (Owens, 2015). In terms of potential toxicity, diclofenac, like other NSAIDs, decreases the biosynthesis of prostaglandins from the phospholipid arachidonic acid by non-selectively inhibiting the cyclooxygenase (COX)-1 and -2 isoforms (Fent et al., 2006; Schmidt et al., 2011). COX enzymes are found in all vertebrates and some invertebrates, such as *Gammarus* species (Varvas et al., 2009). In reducing prostaglandins, NSAIDs diminish the cellular response to injury and trauma and lessen pain and the inflammatory response (Boxall, 2012). However, prostaglandins are also involved in other critical physiological functions such as reproduction, osmoregulation and immune defence (Rowley et al., 2005) which may be similarly diminished by the action of NSAIDs (Fent et al., 2006; Zhang et al., 2012). In addition, and unlike other NSAIDs, diclofenac has been shown to inhibit the

proliferation of progenitor cells and cause cell death (Ericson et al., 2010) which may contribute to it being regarded as the most acutely toxic NSAID (Santos et al., 2010).

4.2.1.1.1.2 Ibuprofen

Ibuprofen, like diclofenac, is an NSAID. Its production and consumption is prodigious being one of the few drugs in Europe that are consumed in amounts in excess of 100 tonnes annually (Bound & Voulvoulis, 2005). In common with other NSAIDs, ibuprofen reduces the inflammatory response by inhibiting COX-1 and COX-2 (Van Hecken et al., 2000) which, in turn, diminishes the formation of prostaglandins involved in the processes such as reproduction, immune system and ion transport in both vertebrates and invertebrates (Rowley et al., 2005). After oral administration, the absorption of ibuprofen is generally rapid and complete (Davies & Skjodt, 2000). Ibuprofen is highly bound to plasma proteins, specifically to albumin (>90%) and only 1- 8% is typically excreted (Ternes, 1998). Furthermore, treatment in STPs appears to eliminate the vast majority of ibuprofen and its metabolites (hydroxy-ibuprofen and carboxy-ibuprofen) with degradation rates (75 - >95%) exceeding most other drugs, including other NSAIDs (Buser et al., 1999). Therefore, its comparative abundance in surface waters is evidence to the considerable quantities consumed. In the study of EU rivers, Loos et al. (2010) found it in higher concentrations than any other drug except caffeine, and Boleda et al. (2014) identifies it as the most common pharmaceutical found in Spanish, European, and North-American finished drinking water (with maximum concentrations of 54, 28 and 1,320 ngL^{-1} respectively). Once in the aquatic environment, there is some disparity about the persistency of ibuprofen; Ericson et al. (2010) reported that it is considered to be fairly persistent, and it has been found

throughout lotic food chains (Owens, 2015). In contrast, Buser et al. (1999) found that it is less persistent than other pharmaceuticals. Loos et al. (2010) found that around 20% was degraded after 3 weeks and Tixier et al. (2003) estimated its half-life to be 32 days in the field. In any case, ibuprofen appears to be amongst the least toxic of the NSAIDs, with LC_{50} values for fish and invertebrates several orders of magnitude higher than the greatest environmental concentrations (Kim et al., 2009). On the other hand, a 2014 report by UK Water Industry Research found that in most of 160 sewage treatment works studied, ibuprofen was one of several drugs present in the final effluent in concentrations apparently high enough to potentially affect ecosystems (Boxall et al., 2014). Discrepancies like this are not easily resolved when the effects of all NSAIDs on non-target organisms, notably invertebrates, are not well understood (Fent et al., 2006; Wiklund et al., 2011).

4.2.1.1.1.3 Erythromycin

Erythromycin and other macrolide antibiotics inhibit protein synthesis by binding to the 23S rRNA molecule (in the 50S subunit) of the bacterial ribosome blocking the exit of the growing peptide chain. of sensitive microorganisms. (Humans do not have 50S ribosomal subunits, but have ribosomes composed of 40S and 60S subunits). Certain resistant microorganisms with mutational changes in components of this subunit of the ribosome fail to bind the drug. The association between erythromycin and the ribosome is reversible and takes place only when the 50S subunit is free from tRNA molecules bearing nascent peptide chains. Gram-positive bacteria accumulate about 100 times more erythromycin than do gram-negative microorganisms. The non-ionized from of the drug is considerably more permeable to cells.

4.2.1.2 Materials

Erythromycin, diclofenac and ibuprofen (Table 4.2.1.1 & Figure 4.2.1.1) were purchased from Sigma-Aldrich, (Dorset, UK). High performance liquid chromatography (HPLC) grade methanol was purchased from Fischer Scientific (Loughborough, UK). Ultra-pure water was obtained from a Sartorius Purite Select HP160/BP/IT water purification system with a specific resistance of 18.2 MΩcm. Chemical stock solutions for each compound were prepared in methanol on a weight basis in 100 ml of 100 % methanol and stored at −20 °C, and the working solutions were diluted aliquots of the stock solutions (100 mgL^{-1} = 10 mg/100 ml). Glassware and vessels were disinfected then pre-rinsed with 100 % methanol and ultra-pure water twice and left to dry in the fume cupboard prior to the experiments.

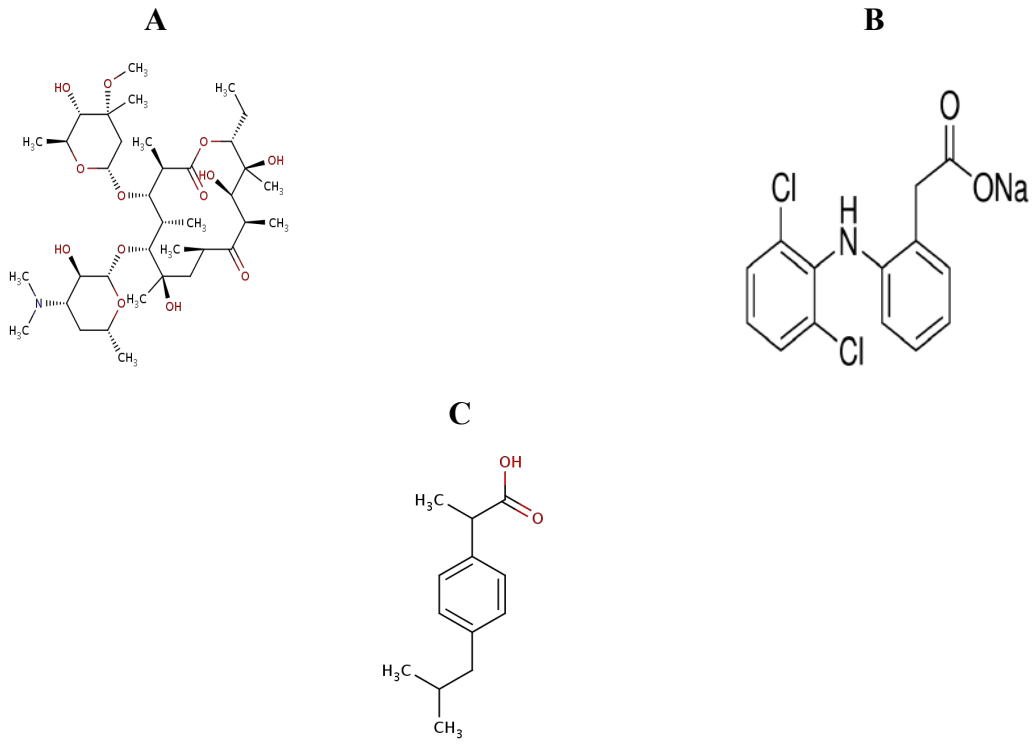

Figure 4.2.1.1: Chemical structures of erythromycin (**A**), diclofenac (**B**) and ibuprofen (**C**) from left to right respectively. Image from (Sigma-Aldrich).

4.2.1.3 Preparation of solutions

Environmentally relevant concentrations of each of the compounds ERY, DIC, IBU and their mixtures were used in these experiments (UK mean measured environmental concentration [LT] and UK maximum measured environmental concentration [HT] and medium concentration [MT] was the average of the LT and HT). These treatment concentrations were chosen as an indicator of likely exposures based on published data for UK rivers (Bound and Voulvoulis, 2006; Hughes et al., 2013) and an indicator of worst-case exposure scenario based on maximum concentrations in UK rivers.

One hundred mgL-1 solutions (100 mgL-1 = 10 mg/100 ml) of each of the compounds (ERY, DIC and IBU) were prepared by dissolving each separately in methanol (HPLC grade) to make the stock solutions. 1 mL was measured from each stock solution and

each dissolved in 100 mL of solvent to make the intermediate solution for each experiment. The desired experimental concentrations for each experiment were achieved through a series of dilutions.

For the mixture experiment environmental concentrations of each of the compounds were measured from the intermediate solutions, mixed together and dissolved in 250 mL of solvent to form the working solution. All solutions were stored at −20 °C in the dark for optimum stability and to avoid photodegradation.

The working solutions of LT, MT and HT were poured on transparent silica glass beads and allowed to evaporate to dryness in the fume cupboard in order to avoid methanol toxicity, then the dried extracts were reconstituted/resuspended with 10 mL of pond water and washed into the beakers before *G. pulex* were introduced. Before the transparent silica glass beads were reused, they were washed with ultra clean water, ashed in the furnace at 550º C and allow to cool in the fume cupboard to prevent toxicity in any form to the test animals. Separate beads were used for the different treatments and controls to prevent contamination.

4.2.1.4 Test animals: origin and maintenance

Gammarus pulex used for the experiments were collected in ponds at Bramham estate, Leeds, West Yorkshire. This site was chosen because it was located upstream of any STP effluent inputs, hence reducing the possibility for pollution by the compounds being investigated. Invertebrates were sampled with a net from 1.5 to 4 m depth. *Gammarus* individuals were hand selected from other organisms and detritus and then brought to the laboratory in cool boxes (5° C). Amphipods of approximately the same size averaging 21.84 ± 3.06 mg, 21.47 ± 2.45 mg, 22.47 ± 3.16 mg and 21.52 ± 0.99 mg were used for erythromycin, diclofenac, ibuprofen and their mixture experiments

respectively. Individuals were sexed by placing pre-copular pairs on a dry filter paper and allowing them to disentangle from each other and kept in incubators at 12° C with a diurnal light rhythm of 16 h: 8 h (day-night) and allowed to acclimatise in aerated pond water before the exposure experiments started.

4.2.1.5 Preparation of leaf material for feeding of test animals

Alnus glutinosa (Alder leaves) were collected from Bramham Estate near the ponds and oven dried at 60° C for 24 hrs. The leaves were conditioned in a nutrient medium (Brown et al., 2006) in an aerated bucket at room temperature for 10 days together with alder leaves previously exposed in the ponds in which the test animals were collected. This was to establish a natural microbial community consisting of fungi and bacteria. This conditioning process increases the nutritive value of leaf material for shredders, such as gammarids (Bärlocher, 1985), and simulates the environmentally relevant processes. *G. pulex* were fed with 0.1 g of the conditioned/standardised alder leaves (*Alnus glutinosa*).

4.2.1.6 Exposure media

Water from Bramham Park ponds (where the animals were sourced) was used for this experiment. The physico chemical parameters at the point of collection of the culture media were dissolved oxygen (DO): 12.3 mgL^{-1}, water temperature: 17.2º C, electrical conductivity (EC): 662 µS cm^{-1} and pH: 7.5.

The pH, DO, water temperature and EC were measured weekly with a HACH HQ40d multimeter and the instruments were rinsed with deionised water before every reading taken.

4.2.1.7 Experimental design

For each of the experiments (ERY, DIC, IBU and their mixtures), there were three treatments (LT, MT and HT), negative and solvent controls with 15 replicates of each treatment and 15 replicates of each control. Test concentrations were selected to mimic environmental detection levels reported for UK rivers in the literature (Table 4.2.1.7). The negative control contained no treatment and the solvent control contained 0.1 mI/L of methanol.

Table 4.2.1.7: Concentrations of the test compounds (environmental detection levels reported for UK). **Sources:** (Hughes et al., 2013; Bound and Voulvoulis, 2006).

Compound	**Low Concentration (ngL^{-1})**	**Medium Concentration (ngL^{-1})**	**High Concentration (ngL^{-1})**
Erythromycin	159.7	768.8	1377.8
Diclofenac	202.2	1596.5	2990.7
Ibuprofen	420.8	2629.6	4838.4

For the mixture experiments, there were two treatments, low treatment (LT) and high treatment (HT) and a solvent control. The low and high treatments were mixtures of ERY, DIC and IBU concentrations in the single compound experiments. Only two treatments and control could be established in the mixture experiments due to an inability to obtain sufficient test animals.

The experiments were carried out in clear glass SS jars (500 ml) kept in incubators (Figure 4.2.1.7) at a temperature of 12° C and 16:8 h light: dark regime. The animals were illuminated with a fluorescent light (with a specification for freshwater invertebrates), to simulate on a small scale the macroinvertebrates' natural climatic

condition. The glow mimicked the thermal warmth and daytime illumination obtained from the sun radiation.

Each glass jar contained one *G. pulex* with 300 ml of pond water, which was assigned and arranged randomly in the experimental chambers using a random integer generator. Individuals were weighed at the start of the experiment and subsequently every week with a Sartorius Quintex 224-1s balance.

For each of the experiments (ERY, DIC and IBU), seventy-five (75) male *G. pulex* were assigned at random among the five experimental groups and forty-five (45) male *G. pulex* for the mixture experiment. Exposures were static-renewal with 100 % water replacement every week with fresh concentrations of the pharmaceuticals. The experiments were each run for four (4) weeks. Growth was measured weekly by deducting the initial mass of each *G. pulex* from the mass each week. Mortality was determined at the end of the experiments by counting the surviving animals and calculating percentage mortality. Remaining alder leaves (feed material) at the end of the experiments were oven dried, weighed and combusted to determine the feeding rate (ash free dry mass).

Figure 4.2.1.7: One of the experimental set-ups in the incubators showing glass jars with one *G. pulex* in each jar exposed to experimental media (Negative control (NCTR), Solvent control (STCR), Low treatment (LT), Medium treatment (MT) & High treatment (HT)).

4.3 Data analysis

Data were organised using Excel (Microsoft, 2013) and residuals of the data were checked for normal distribution using the Shapiro-Wilk normality test and homogeneity of variance using the Bartlett test of homogeneity of variances. R (R Development Core

Team, 2008) was used to analyse the data and create figures (Box-and-Whisker). The box-and-whisker plots display a statistical summary of variables: median, quartiles, range and possibly extreme values (outliers). An outlier value is defined as a value that is smaller than the lower quartile (25 percentile) minus 1.5 times the interquartile range, or larger than the upper quartile (75 percentile) plus 1.5 times the interquartile range. Changes in *gammarus pulex* mass, physicochemical parameters and mass of feed materials (*Alnus glutinosa*) from week 1 to week 4 were tested using generalised linear model and Chi-square. Mortality was analysed using one-way ANOVA where assumptions of normality and homogeneity were met followed by Tukey's post-hoc tests to identify and compare the treatment means with the respective controls.

4.4 Results

4.4.1 Erythromycin experiments

4.4.1.1 Initial test conditions

When the experiment was initiated (day 0) the average mass of *G. pulex* (both treatments and controls) was 21.47 ± 2.45 mg with no statistically significant difference (ANOVA: $F_{4,\ 70} = 0.09$, $p = 0.99$) between treatment and control groups. The mean dissolved oxygen (DO) was 9.30 ± 0.01 mgL^{-1} and p=0.57, pH was 8.5 ± 0.03 and p=0.43, water temp was 14.10 ± 0.19° C and p=0.18, mean electrical conductivity (EC) was 598.09 ± 4.64 $\mu Scm^{-1}$and p=0.004. There were no statistically significant differences in the water chemistry across replicates except for EC. However, 5 μScm^{-1} is small and not ecologically relevant even if statistically different and unlikely to affect the fitness of *G. pulex*. The solvent control used in the experiment was also tested for different responses of the physiological measurements compared to the negative

control. No statistically significant difference was found between control treatments with and without solvent.

4.4.1.2 Growth

The growth of *G. pulex* was significantly reduced relative to controls at 159.70 ngL^{-1}, 768.75 ngL^{-1} and 1377.80 ngL^{-1} concentrations of erythromycin over 4 weeks (Figure 4.4.1.2). When the residual of the data was analysed, there were statistically significant differences between the treatment groups and the control for growth (GLM: χ^2 (4) = 662.04, $p < 0.001$). At the end of the experimental period *G. pulex* increased in mean mass in the control groups (NCTR: 23.35 ± 2.99 mg & SCTR: 23.09 ± 3.96 mg) but there was decreased mass in the treatment groups (LT: 20.29 ± 2.54 mg, MT: 20.03 ± 2.85 mg and HT: 19.97 ± 2.62 mg). However, mean mass decrease was more pronounced in the high dose treatment than the other treatments.

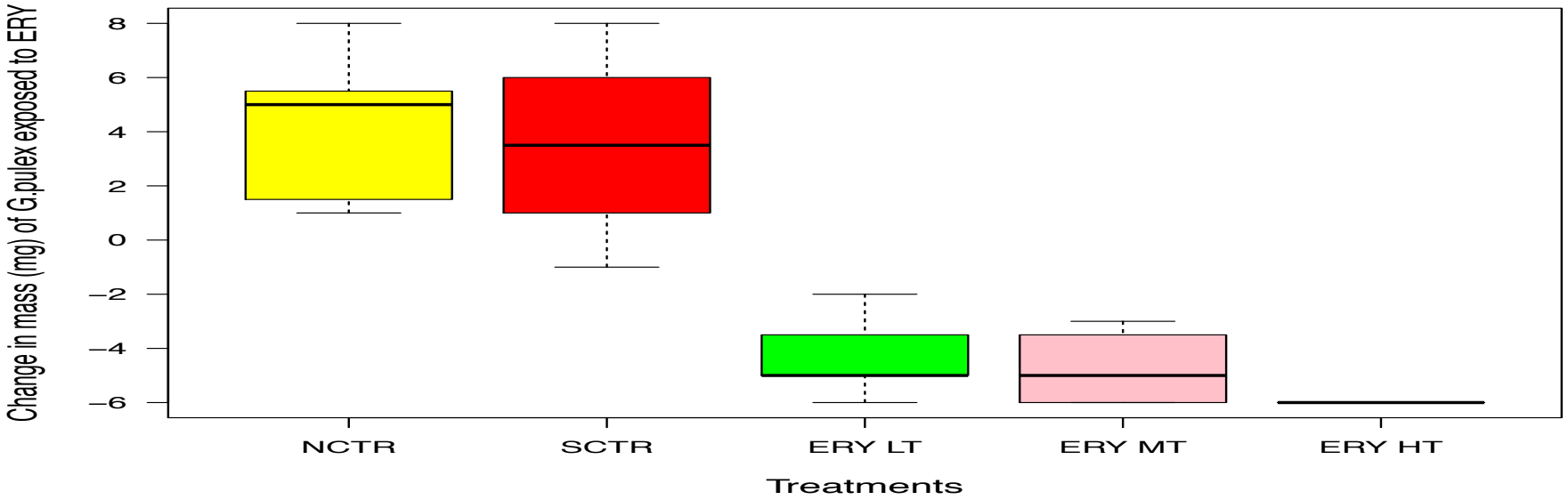

Figure 4.4.1.2. **Boxplots** displaying change in mass of *G. pulex* exposed to environmental relevant concentrations of erythromycin after a 4 week static renewal experiments. Negative control (NCTR), solvent c trol (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents the 3nd quartiles (75th percentile), top whisker represents the 4rd quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers. Sample size at the end of the experiment (n): NCTR=15; SCTR=14; LT=7; MT=4 and HT=2.

4.4.1.3 Feeding

There were statistically significant differences in the mass of feed materials consumed between controls and treatments (GLM: χ^2 (4) = 0.17691, p-value<0.001) with the mass loss of Alnus glutinosa litter by the control group being higher than in the treatment groups. Even between the treatments group, the feed materials loss was dose dependant and significantly influenced by erythromycin (Figure 4.4.1.3).

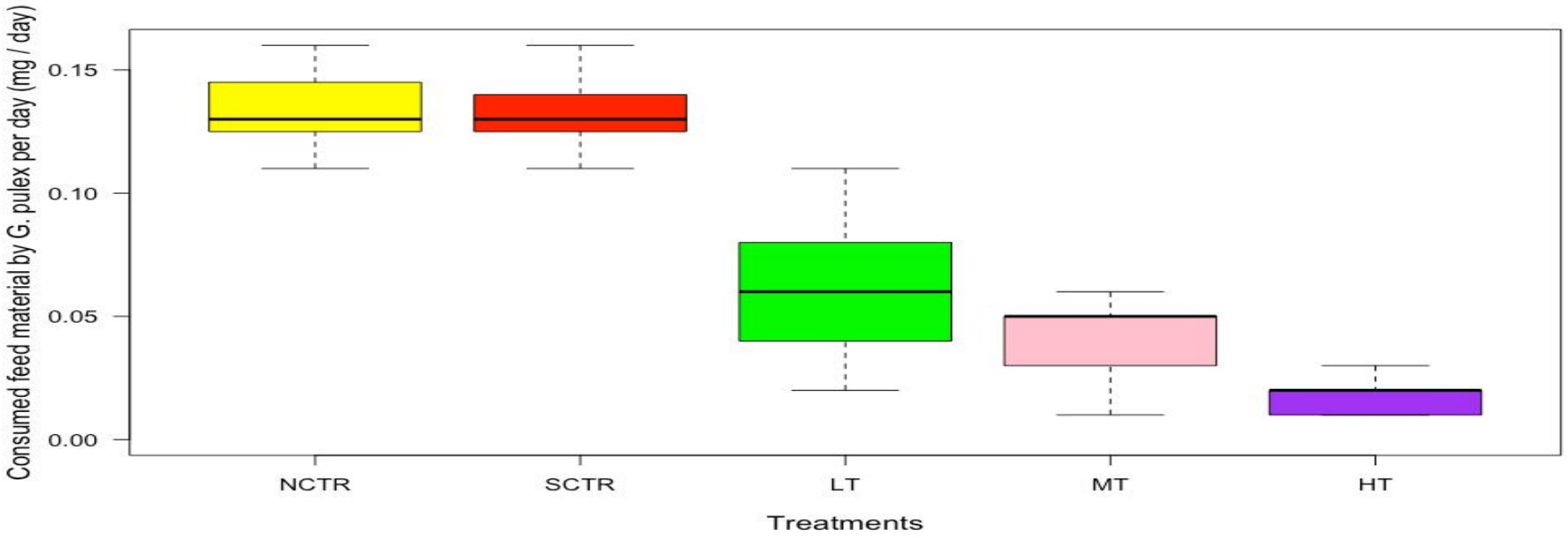

Figure 4.4 1.3. Boxplots displaying consumed feed materials by *G. pulex* exposed to environmental relevant concentrations of erythromycin after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There was no outlier.

4.4.1.4 Mortality

In the first week of the experiment there was no mortality recorded in all the treatments (LT, MT, HT) and the controls (NCTR, SCTR). Mortality commenced in the second week with 4, 5 and 5 *G. pulex* dying in the LT, MT, HT respectively but none in the controls. For both medium and high treatments, more than 50 % mortality had taken place before the fourth week. In the fourth and final week of the exposure 53 %, 73 % and 86 % mortality were recorded for LT, MT, HT respectively. In the control group, total mortality was one individual (7 %) and this was recorded in the third week in the SCTR (Figure 4.4.1.4). Statistically significant differences (GLM: χ^2 (4) = 6665.9, p = 0.02) were thus, found in % cumulative mortality between the high, medium and low-dose treatments and the control groups.

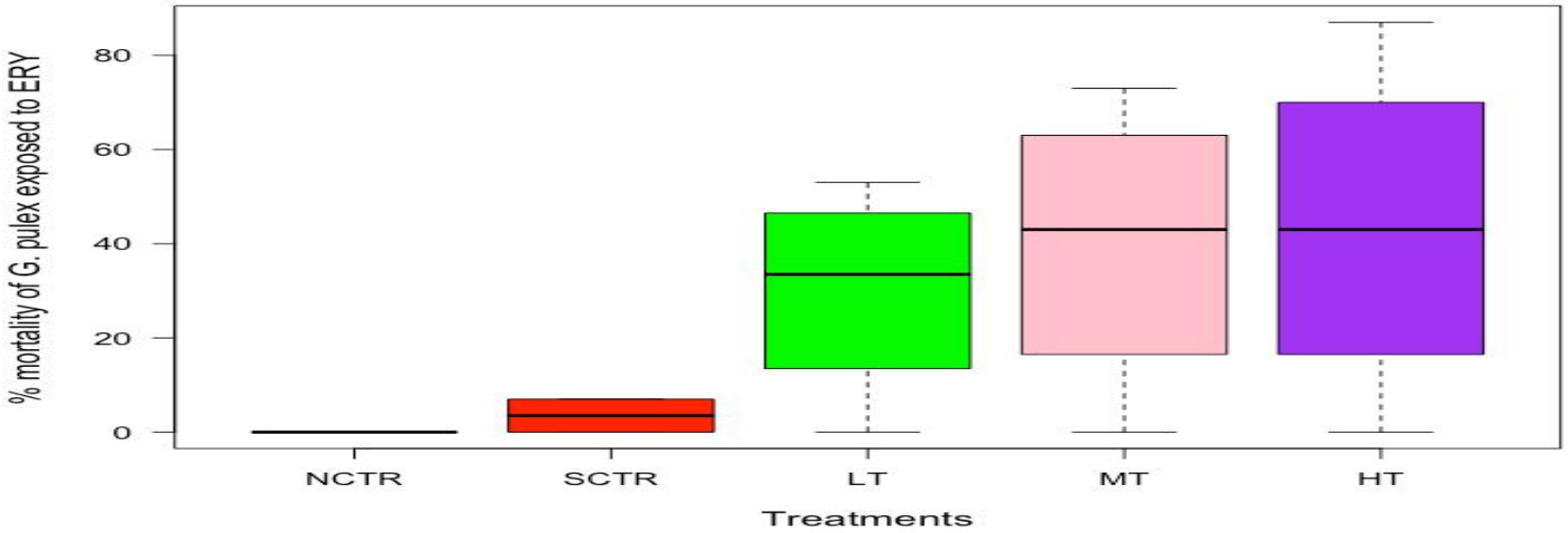

Figure 4.4.1.4. Boxplots displaying % mortality of G. pulex exposed to environmental relevant concentrations of erythromycin after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

4.5 Diclofenac experiments

4.5.1 Growth

When the experiment was initiated (day 0) the mean mass of *G. pulex* was 21.84 ± 3.06 mg and no statistically significant difference (ANOVA: $F_{4, 70} = 0.42$, $p = 0.79$) was recorded between treatment and control groups. pH fluctuated between 8.39 – 8.57 throughout the exposure period (p=0.09), electrical conductivity was within 470.3-568.2 µs cm^{-1} (p=0.22), and mean water temperature was 10.8° C (p=0.95). Dissolved oxygen was maintained between 9.45 mgL^{-1} - 10.07 mgL^{-1} throughout the duration of the experiments (p=0.45).

In the experimental period *G. pulex* final mass in the control groups was NCTR: 24.33 ± 4.05 mg and SCTR: 23.33 ± 4.70 mg and the final masses in the treatment groups were LT: 21.63 ± 3.81 mg, MT: 18.17 ± 1.47 mg and HT: 14.00 ± 2.83 mg. However, mass decrease (LT: -0.75± 2.38 mg, MT: -1.17± 0.75 mg, HT: -7.5± 0.71 mg) was more pronounced in the high dose treatment than the other treatments. At the end of the experiment statistically significant differences were found between the treatment and control groups (ANOVA: $F_{4, 41} = 9.75$, $p<0.001$) (Figure 4.5.1).

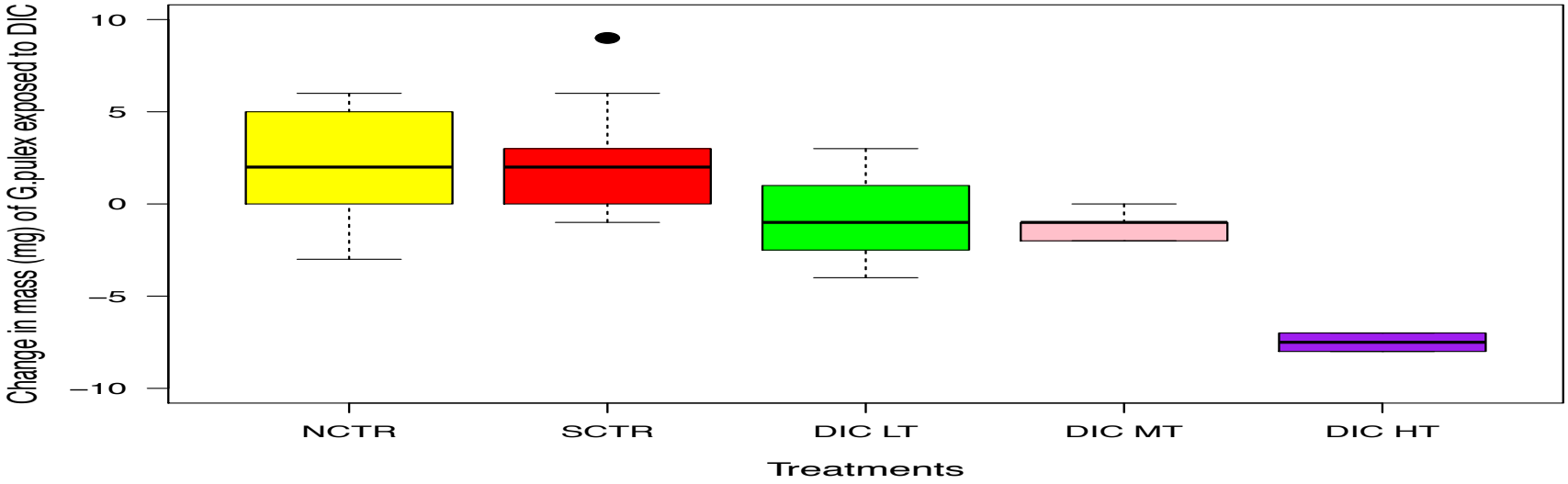

Figure 4.5.1 Boxplots displaying change in mass of *G. pulex* exposed to environmental relevant concentrations of diclofenac after 4 a week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There was outlier. Sample size at the end of the experiment (n): NCTR=15; SCTR=15; LT=8; MT=6 and HT=2.

4.5.2 Feeding

There were statistically significant differences in the mass of feed materials consumed between controls and treatments (ANOVA: $F_{4,70} = 42.19$, $p < 0.001$). The feeding rates in the controls were higher than the treatments. Even between the treatments group, the amount of feed materials consumed was dose dependant and significantly influenced by DIC (Figure 4.5.2).

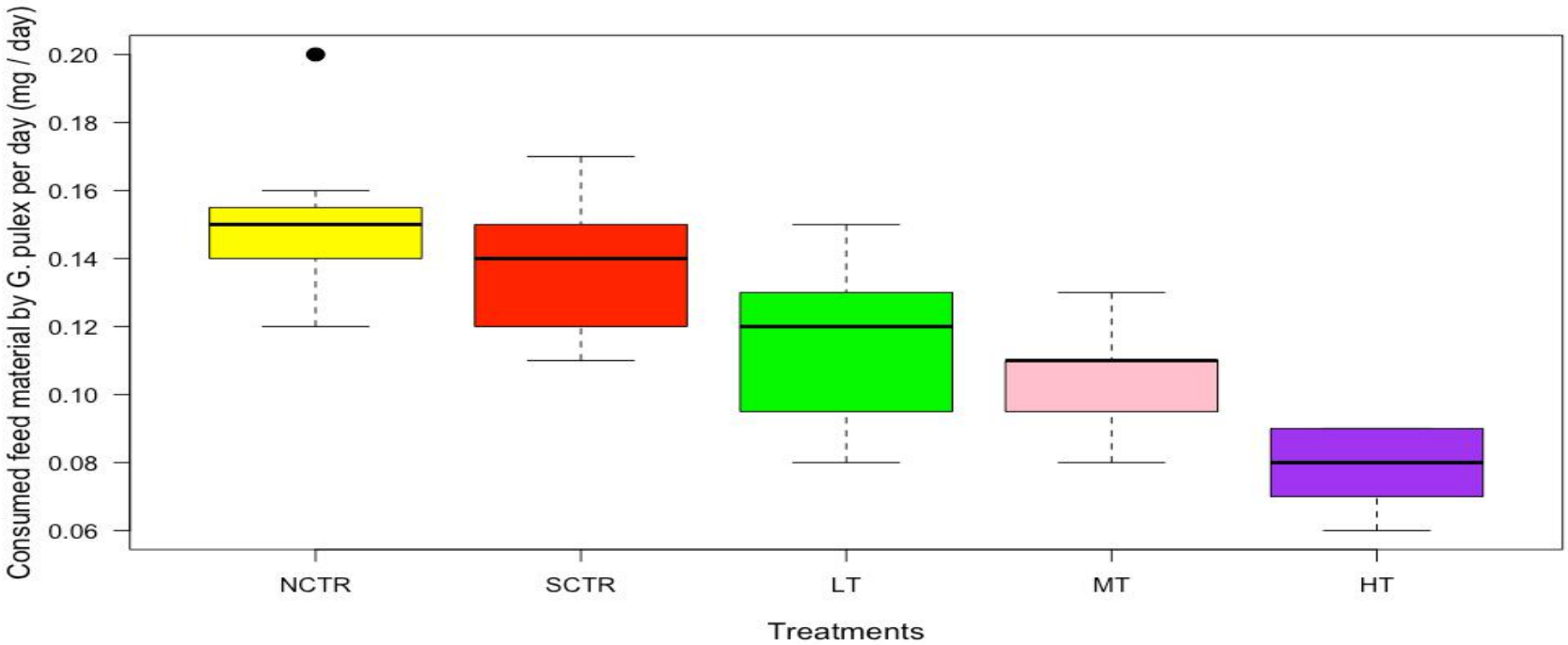

Figure 4.5.2 Boxplots displaying change in feed materials of *G. pulex* exposed to environmental relevant concentrations of diclofenac after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There was outlier.

4.5.3 Mortality

In the first week of the experiment there was no mortality recorded in all the treatments (LT, MT, HT) and the controls (NCTR, SCTR). The treatments started showing a considerable increase in mortality from week two compared to the controls (Figure 4.5.3). Mean mortality was more than 80 % in the high treatment in the 3rd week of the experiment, 60 % in the medium treatment, more than 40 % in the low treatment while in the controls there was no mortality. There were statistically significant differences between the treatments and controls (GLM: χ^2 (4) = 5502.3, $p < 0.05$).

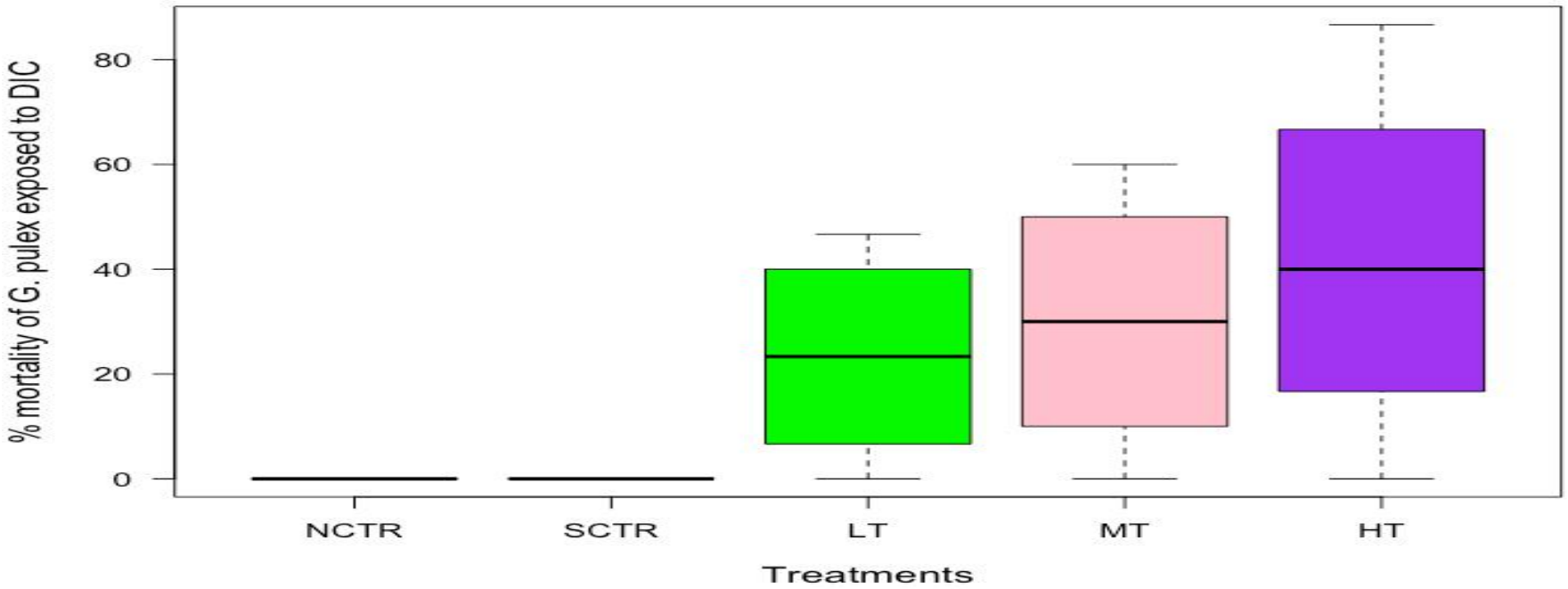

Figure 4.5.3 Boxplots displaying % mortality of *G. pulex* exposed to environmental relevant concentrations of diclofenac after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

4.6 Ibuprofen experiments

4.6.1 Growth

When the experiment was initiated (day 0) the cumulative mean mass of *G. pulex* (across treatments and controls) was 22.47 ± 3.16 mg and no statistically significant difference (ANOVA: $F_{4, 70}$ =0.14, p = 0.97) was recorded between treatment and the control groups. The cumulative mean dissolved oxygen (DO) was 9.61 ± 0.07 mg/L, pH was 8.69 ± 0.04, water temp was 11.89 ± 0.32°C and mean electrical conductivity (EC) was 694.28 ± 64.34 µS/cm. There were no statistically significant differences in the water chemistry.

When the change in mass was analysed there was no statistically significant difference between the treatment and the control groups (ANOVA: $F_{4, 65}$ = 2.10, p =0.09) (Figure 4.6.1).

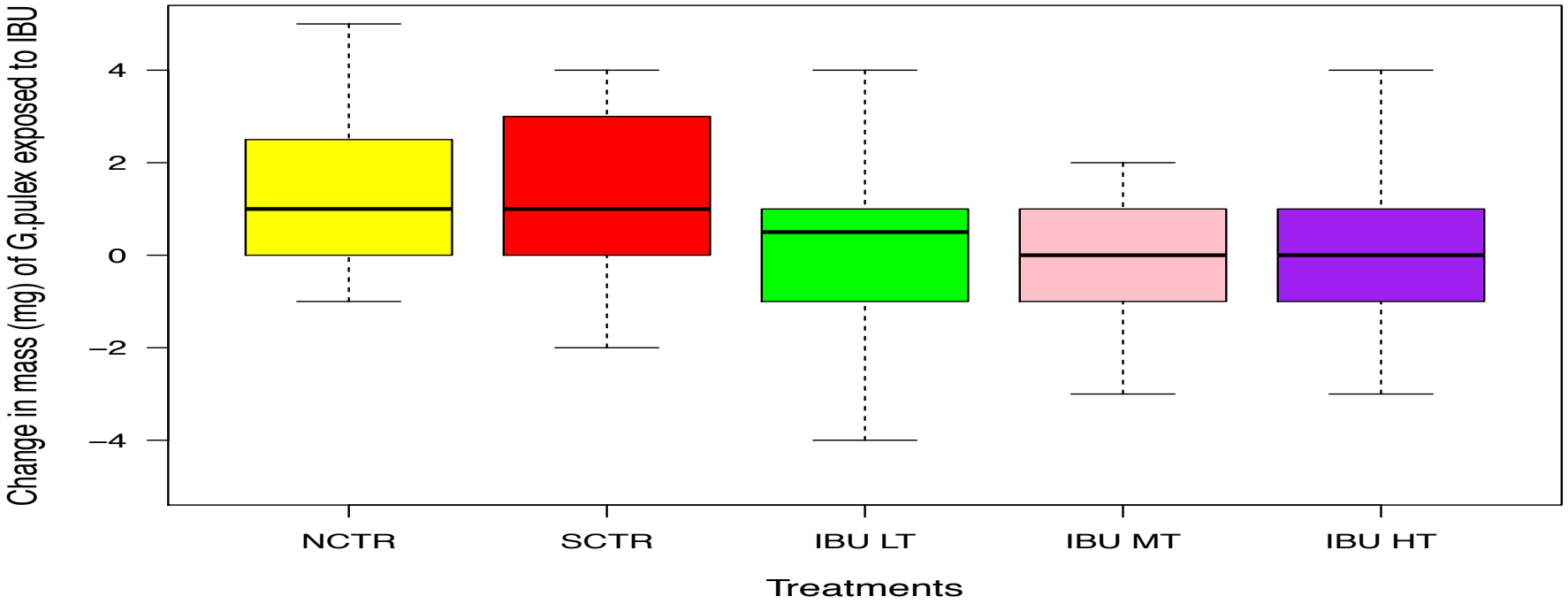

Figure 4.6.1: Boxplots displaying change in mass of *G. pulex* exposed to environmental relevant concentrations of Ibuprofen after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT), The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There were no outliers. Sample size at the end of the experiment (n): NCTR=15; SCTR =15; LT=14; MT=13 and HT=13.

4.6.2 Feeding

The change in feed materials (*Alnus glutinosa*) per *G. pulex* per feeding day were NCTR = 0.030 ± 0.003 SD, SCTR = 0.029 ± 0.007 SD, LT = 0.028 ± 0.003 SD, MT = 0.027 ± 0.004 SD and HT = 0.026 ± 0.008 SD (Figure 4.6.2). There were no statistically significant differences in feeding between controls and treatments (GLM: χ^2 (4) = 0.00013, p =0.356).

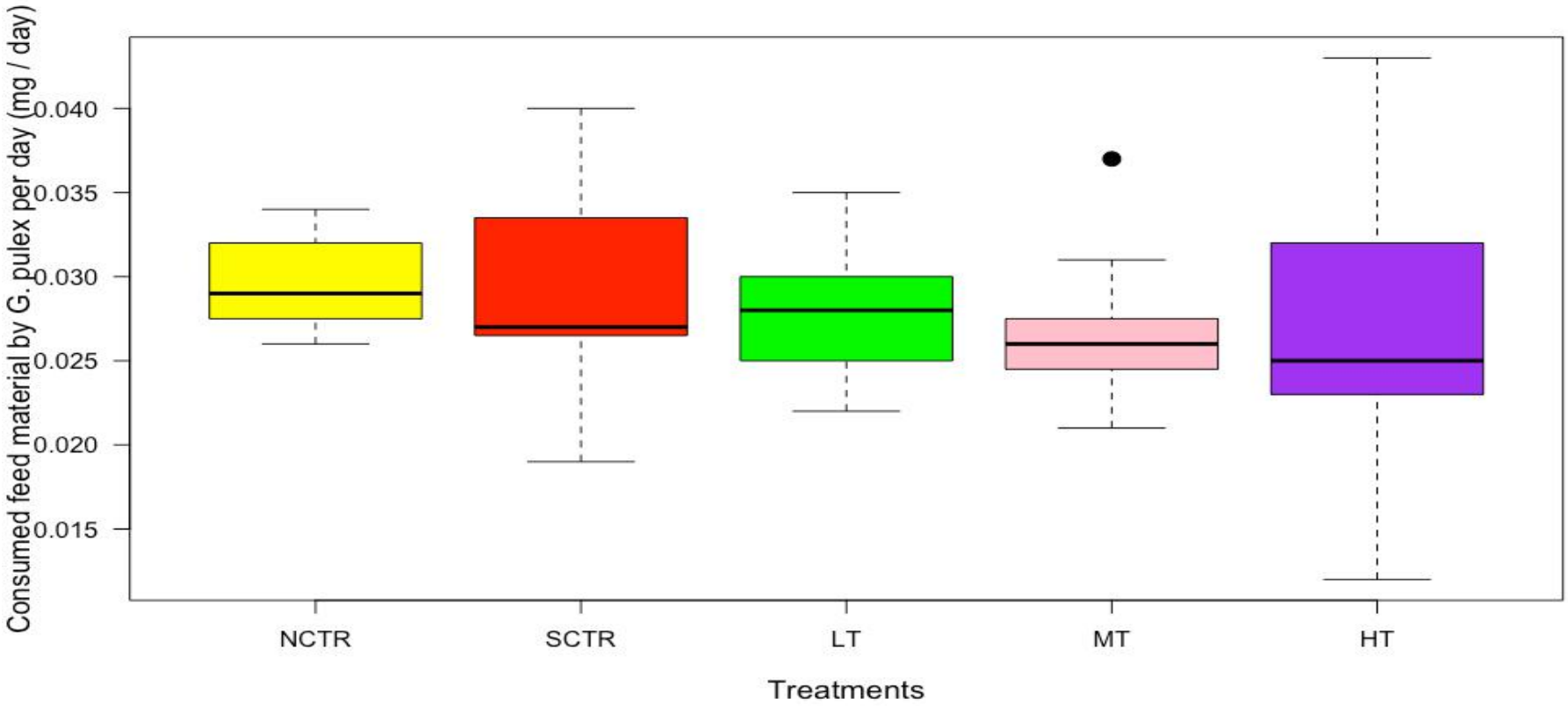

Figure 4.6.2: Boxplots displaying change in feed materials of *G. pulex* exposed to environmental relevant concentrations of Ibuprofen after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There was outlier.

4.6.3 Mortality

In the first week of the experiment there were no deaths recorded in all the treatments (LT, MT, HT) and the controls (NCTR, SCTR). Mortality commenced in the second and third week for HT with two *G. pulex* recorded for both weeks, while in the fourth week, one and two mortalities were recorded for LT and MT respectively. No mortality was recorded for the control group (Figure 4.6.3). There were no statistically significant differences (GLM: χ^2 (4) = 211.12, p = 0.53) between treatment and control groups at the end of the study.

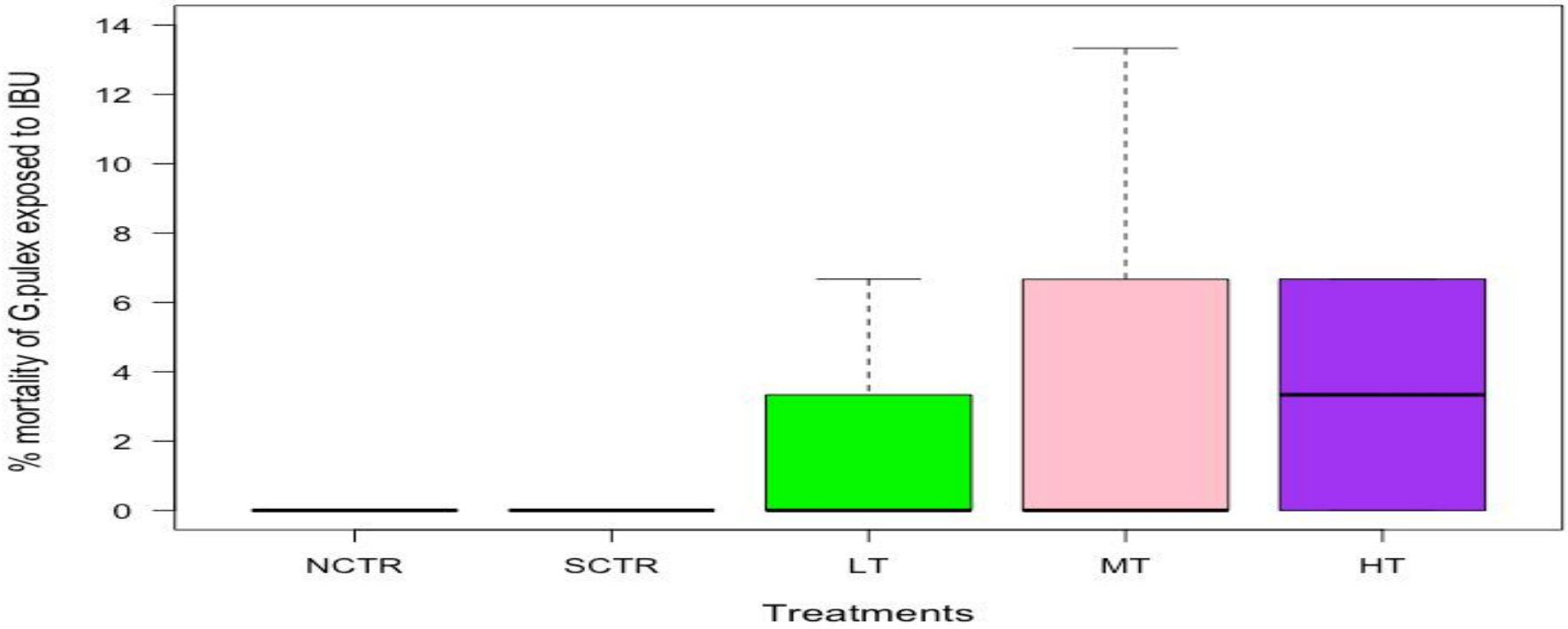

Figure 4.6.3 Boxplots displaying % mortalit y of *G. pulex* exposed to environmental relevant concentrations of Ibuprofen after a 4 week static renewal experiments. Negative control (NCTR), solvent control (SCTR), low treatment (LT), medium treatment (MT) and high treatment (HT). The dark horizontal line inside the box represents the median(50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There were no outliers.

4.7 Multiple mixture (DIC+ERY+IBU) experiments

4.7.1 Initial test conditions

The cumulative initial mean mass of *G. pulex* was 21.52 mg ± (0.99SD) at the start of the experiment. There was no statistically significant difference in *G. pulex* mass at the start of the experiments between treatments and controls (p=0.95). Dissolved oxygen was maintained consistently at or close to saturation (≥9.5 mgL^{-1}) throughout the exposure period even though there are slight fluctuations between the treatments, but they are not statistically significant (F=1.40, DF=4,70, p=0.24). pH fluctuated between 8.47 and 8.56 throughout the exposure and no statistically significant difference was observed (F= 0.96, DF=4,70, p=0.43). The temperature varied between 11.2° C and 12.5° C and there was no statistically significant difference between the treatment and control groups (F=1.90, DF=4, 70, p=0.12). The EC was ≥590 μs cm^{-1} throughout the experiment.

4.7.2 Growth

When the change in mass was analysed there were statistically significant differences between the treatment and control groups ($F_{2, 31} = 7.44$, $p < 0.01$) (Figure 4.7.1).

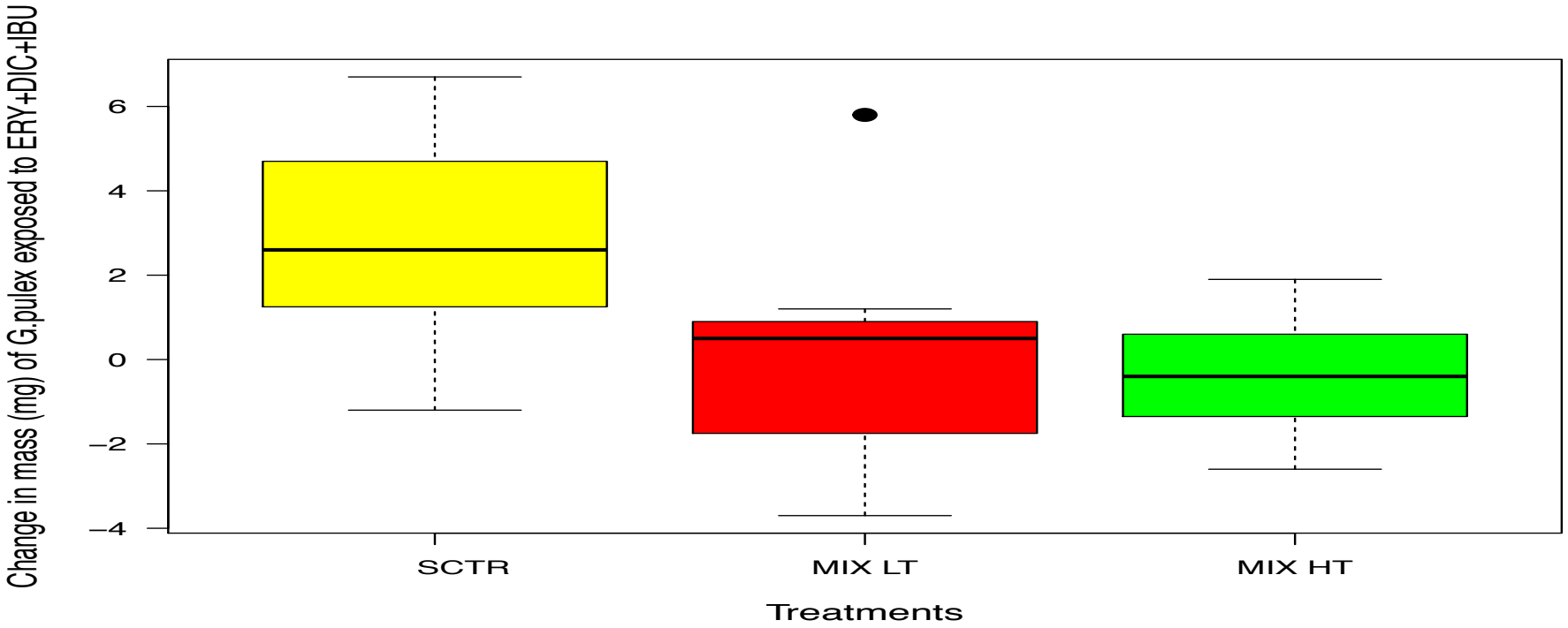

Figure 4.7.1: Boxplots displaying change in mass of *G. pulex* exposed to environmental relevant concentrations of ERY, DIC & IBU after a 4 week static renewal experiments. Solvent control (SCTR), low treatment (LT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were outliers. Sample size at the end of the experiment (n): SCTR=15; LT=11 and HT=8.

4.7.3 Feeding

The mean change in feed materials (*Alnus glutinosa*) when *G. pulex* was exposed to mixtures of ERY, DIC and IBU was SCTR = 0.027 ± 0.005 SD; LT = 0.024 ± 0.003 SD) and HT = 0.019 ± 0.005 SD (Figure 4.7.2). There were statistically significant differences in feed materials between controls and treatments ($F_{2, 42} = 12.68$, $p < 0.001$).

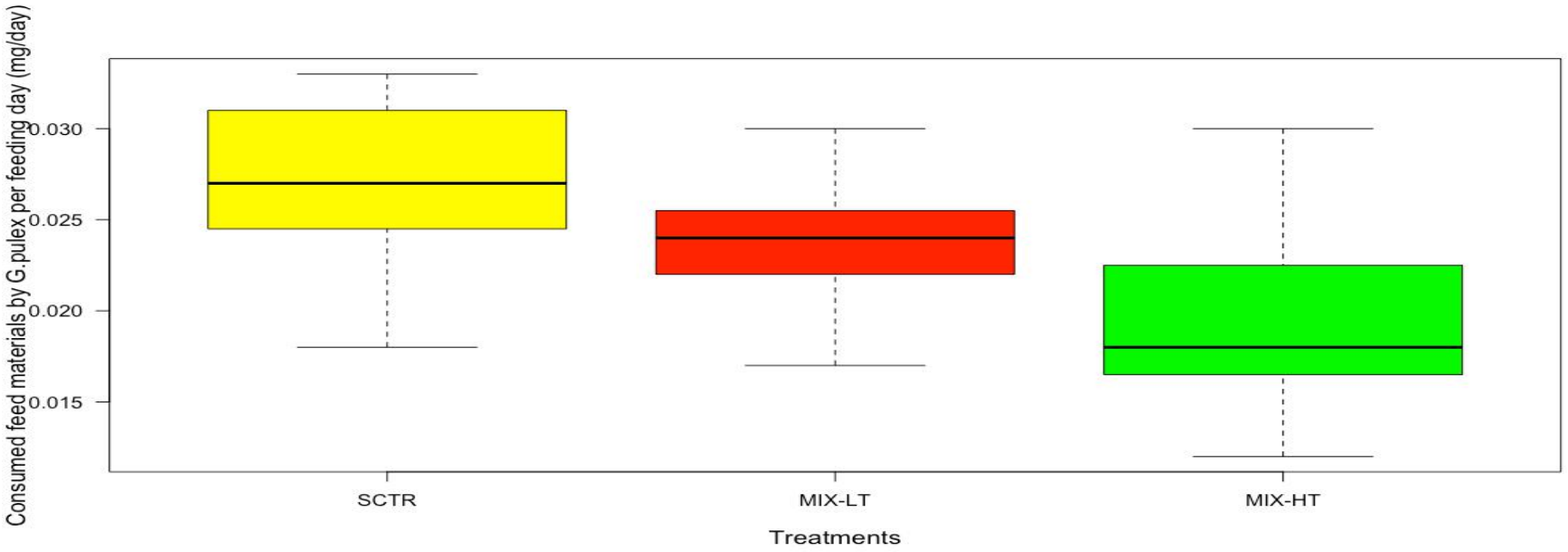

Figure 4.7.2. Boxplots displaying change in feed materials of *G. pulex* exposed to environmental relevant concentrations of (ERY, DIC & IBU) after a 4-week static renewal experiments. Solvent control (SCTR), low treatment (LT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

4.7.4 Mortality

There were no statistically significant differences (GLM: χ^2 (2) = 274.12, p = 0.57) between treatment and control groups at the end of the study. In terms of mortality there were one and two mortalities in LT and HT respectively and none in the solvent control throughout the duration of the exposure (Figure 4.7.3).

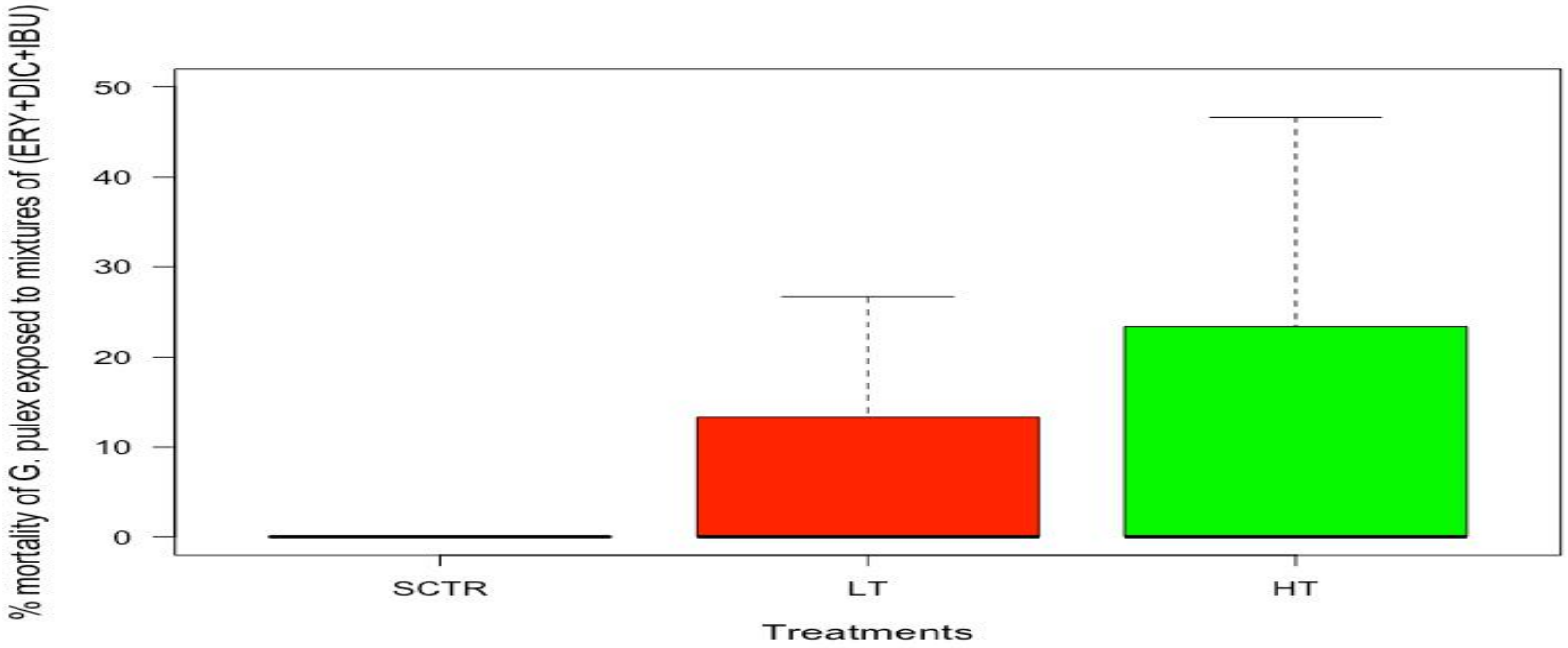

Figure 4.7.3 Boxplots displaying % mortality of *G. pulex* exposed to environmental relevant concentrations of (ERY, DIC & IBU)after a 4 week static renewal experiments. Solvent control (SCTR), low treatment (LT) and high treatment (HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outlier.

4.8 General Discussion

The objective of this study was to investigate the ecological implications of the extended low-level exposure of erythromycin, diclofenac, ibuprofen and their mixtures at environmentally relevant concentrations on feeding, growth and mortality of *G. pulex.* Generally, there were significant effects on growth, feeding and mortality when *G. pulex* was exposed to erythromycin and diclofenac. However, when exposed to ibuprofen, there was no effect on all the endpoints. The mixtures of erythromycin, diclofenac and ibuprofen significantly affected the growth and feeding of *G. pulex* but no substantial increase in the mortality was observed. The water quality parameters such as pH, DO, EC and water temperature were all within the acceptable tolerance range for *G. pulex*. However, there were a few fluctuations in the magnitude of the differences, but they were very small and unlikely to affect the health of *G. pulex* (Graca et al., 1993b; Hughes et al., 2013).

G. pulex had long been suggested as an additional crustacean to *Daphnia* for toxicity testing, studies with cultured animals of various sizes and ages over the years have demonstrated the sensitivity of *G. pulex* (McCahon and Pascoe, 1988b). This investigation affirms the suitability of *G. pulex* as a test animal for aquatic toxicity testing. It is easy to culture, maintain and handle both in the field and laboratory.

4.8.1 Effects of erythromycin on growth, feeding and mortality of G. pulex

The results from this study reveal that erythromycin negatively affected growth, causing decreased body mass, reduced feeding and increased mortality of *G. pulex* in a dose-dependent manner. The effects were more pronounced in the highest concentration (1377.80 ngL^{-1}) and were detected after an exposure period of four weeks. This observation is consistent with studies by Gracia-Lor et al., (2012) and Liu et al. (2017) in which *G. fasciatus* was exposed to cimetidine at concentrations relevant to the environment. They observed that the growth and biomass of *G. fasciatus* was significantly reduced, albeit for a different pharmaceutical.

Literature is sparse in terms of growth and feeding rate in *G. pulex* exposed to pharmaceuticals but the effects of erythromycin on growth and feeding of other aquatic organisms at environmental relevant concentrations similar to this study has been investigated. For example, Liu et al. (2014) investigated the exposure of *Carassius auratus* (Goldfish) to environmental concentrations of erythromycin and found significant behavioural and biochemical disturbance. Yang et al., (2017) investigated the effect of erythromycin exposure on the growth of *Microcystis flosaquae* and found that the growth was inhibited at concentrations above 10 μgL^{-1} but that at low concentrations (0.001, 0.01, 0.1 and 1.0 μgL^{-1}) erythromycin promoted growth. Gracia-Lor et al., (2012) also reported that erythromycin stimulated the growth of *Skeletonema costatum* (algae) at a low concentration of 0.5 mgL^{-1}, 1.05 mgL^{-1} and 2.05 mgL^{-1} and inhibited growth at concentrations > 2.0 mgL^{-1}. These findings showed that erythromycin may have two-fold effects (inhibition and enhancement) on the growth of *M. flosaquae*. Erythromycin may possibly be causing Hormesis. The principle of Hormesis is that when an organism is exposed to low concentration of a chemical

stressor that normally can cause lethality when administered at high concentrations, a positive effect is observed, such as increased growth rate, enhanced well-being, and tolerance of contaminants (Calabrese, 2003; Rodrigues et al., 2016). Several studies have reported that environmental contaminants can cause Hormesis on bacteria, animals and higher plants (Crane et al., 2006; Calabrese., 2005; Cedergreen et al., 2007; Hunt et al., 2010; Deneshvar et al., 2010; Rodrigues et al., 2016).

In a similar study by Gonzalez-Pleiter et al. (2013), in which the effects of five antibiotics including erythromycin on two representatives of aquatic organisms (*cyanobacterium Anabaena* and green alga *Pseudokirchneriella subcapitata*) was investigated, erythromycin was highly toxic and prevented growth.

The inhibition of growth when exposed to erythromycin may possibly be due to *G. pulex* been stressed by enzymes involved in behavioural, biochemical and physiological reactions possibly because the concentration of erythromycin exceeded the tolerance limit of *G. pulex* before the animals enter the state of zero or negative growth.

Animal behaviour and physiology are increasingly being used as a sensitive means of measuring sub-lethal exposure to toxic contaminants. In the literature, nutrition is commonly reported to be impacted by contaminants (Graca et al., 1994; Karthikeyan and Meyer, 2006). In this study, exposure to erythromycin caused reduced feeding rate in *G. pulex*, which was significant after four weeks of exposure at the three treatment concentrations of erythromycin (0.16 μgL^{-1}, 0.77 μgL^{-1} and 1.38 μgL^{-1}). In a similar study, Moore and Farrar (1996) reported that growth rates in *G. pulex* decrease significantly with reduced food rations.

Feeding is an essential component of an organism's fitness and ecologically relevant for all activities of *G. pulex*; feeding is required for energy and energy is needed for growth. Thus, any contaminant, such as erythromycin, that interferes with this activity

is likely to reduce the fitness of organisms and could lead to ecological death and distortion of the balance in the ecosystem (Scott and Sloman, 2004). The alteration of feeding activity was found in another aquatic organism: after 72 h of exposure, the activity of *Gammarus lawrencianus* was reduced by erythromycin exposure at a concentration of 62 μgL^{-1} (Xu et al., 2007; Liu et al., 2017).

In a similar report but with a trace metal compound, Felten et al. (2008) exposed *G. pulex* to cadmium; exposure to the metal reduced the feeding rate of *G. pulex* by 30 % at 7.5 $\mu gCdL^{-1}$ and by 36 % at 15 $\mu gCdL^{-1}$ after only 168 hours of exposure compared to the control animals. In a series of field studies, the feeding rate of *G. pulex* was demonstrated to be a sensitive indicator of water quality (Crane and Maltby, 1991). In another study by Alonso et al. (2009) cadmium reduced the feeding activity of *G. pulex* at environmental concentrations of erythromycin. Again *G. pulex* demonstrated sufficient sensitivity which confirms the suitability and reliability to provide adequate data which may be useful in environmental risk assessment (Hughes et al., 2013). A similar result was also reported for other crustaceans exposed to different insecticides, such as fenitrothion and deltamethrin, which strongly inhibit their feeding activities (Oliveira et al., 2015). For some psychotherapeutic drugs, however, the enhanced feeding activity was obtained in crustacean exposed to sertraline (Zhao et al., 2015). These contaminants have high potential to directly alter behaviour since they are neurotoxins.

Recent studies also indicated that erythromycin concentrations as low as 1 μgL^{-1} negatively affected aquatic bacteria production in Wascana Creek as well as the composition and structure of attached microbial biofilm communities (Waiser., 2017). Also, a dosage of 1 mgL^{-1} erythromycin (far greater than those in this study), inhibits

Synechocystis sp. and *Lemna minor* growth by 70 and 20 %, respectively. This observation was consistent with the findings of Cleuvers (2003, 2004) who reported that the sensitivity of *Daphnia lemna* and *Desmodesmus* algal growth inhibition were in the same range.

Mortality was more pronounced in the high treatment than the other treatments. The highest concentration for the treatment was 1.378 μgL^{-1}, which is far below the acute toxicity testing concentration for other aquatic invertebrates (Hughes et al., 2013). There was very sparse literature reporting mortality of *G. pulex* exposed to pharmaceuticals, even though sub-lethal effects have been receiving attention in recent years. De Lange et al. (2006) investigated the effects of fluoxetine at relevant concentrations on *G. pulex* and found that there was a significant reduction in movement. In another study by De Lange et al. (2009), *G. pulex* were exposed to fluoxetine at environmentally relevant concentrations and the ventilation of the amphipod increased. Other studies investigated the effects of trace elements (Cadmium) on mortality of *G. pulex* and mortality was significantly higher than in controls.

Finally, this study generally demonstrated the toxicity of erythromycin and that chronic exposure to erythromycin can lead to its interference in the behavioural and physiological functions of amphipods, highlighting concern about this pharmaceutical in the aquatic environment (Mehinto et al., 2010).

4.8.2 Effects of diclofenac on growth, feeding and mortality of G. pulex

Despite being regarded as one of the most toxic of NSAIDs (Santos et al., 2010) and of particular environmental concern (Fent et al., 2006), diclofenac is not widely studied in

ecotoxicology so there are few studies' results with which to contrast this investigation. This study showed that growth and feeding behaviour of *G. pulex* were affected when exposed to environmentally relevant concentrations (202.20 ngL^{-1}, 1596.45 ngL^{-1} and 2990.70 ngL^{-1}) of diclofenac. There was growth retardation, test species were not feeding, and mortality increased in a dose-dependent manner.

Most studies done on the ecotoxicological effects of diclofenac have studied fish. For example, in a study conducted by Hong et al. (2013), in which male medaka fish were exposed to 1 μgL^{-1} concentration of diclofenac, a high induction of CYP1A expression was found in three different tissues tested, i.e. gills, intestine and liver. The effect was induced up to 5.7-fold, 18.4-fold and 9.3-fold in gills, intestine and liver respectively. Schwaiger et al., (2004) and Triebskorn et al. (2004) evaluated the toxic effect of diclofenac on rainbow trout at concentrations of 1μgL^{-1} to 500 μgL^{-1} (i.e. 1, 5, 20, 100, and 500 μgL^{-1} for 28 days. The threshold level of 5 μgL^{-1} leading to histopathological organ lesions after 28 days of exposure is about 102-fold lower than that reported to induce sublethal effects in invertebrates (*Daphnia magna*, 21 d reproduction test). Exposed fish showed changes in the gills and kidneys and bioaccumulation of diclofenac was also observed in fish by Schwaiger et al., (2004). In another study by Hoeger et al. (2005), there was a reduction in the number of erythrocytes in the blood on exposure of brown trout to diclofenac at environmentally relevant concentrations.

The results obtained in this study are similar to effects on aquatic invertebrates reported by previous studies. For example, in a study conducted by De Lange et al. (2006) there was a decrease in the feeding behaviour of *G. pulex* when exposed to low concentrations (1-100 ngL^{-1}) of diclofenac. In the same study, the feeding rate of *G. pulex* decreased by 45 % when compared to the controls when exposed to 10 ngL^{-1} of

carbamazepine for one and half hours. This study was also in agreement with Gabet-Giraud et al., (2010) in which *Carcinus maenas* (green crab) was exposed to environmentally relevant concentrations of diclofenac; it significantly reduced feeding and ultrastructural change was observed in the gill lamellae of the exposed crustacean. Also, when fingerlings of rainbow trout were exposed to 0.0071 mgL^{-1} of diclofenac there was modification in fish behaviour and abnormal swimming behaviour was reported (Orvos et al., 2002; Oliveira et al., 2015). Observations from this study are in agreement with some previous studies of aquatic organisms in which Quinn et al. (2014) demonstrated that exposure to carbamazepine at 50 mgL^{-1} for 96 hr significantly reduced feeding activity in *Hydra attenuata* and exposure to 10 mgL^{-1} diclofenac for 96 h significantly reduces the time for prey ingestion.

However, this result is not in agreement with that of Richard et al. (2015), who reported that food intake increased in Wistar rats (though a vertebrate animal) exposed to 2.5 mg kg^{-1} of diclofenac for 10 days. The observed difference in effects between *G. pulex* and Wistar rat exposed to diclofenac may be due to dose and species differences. However, the decrease in feeding by *G. pulex* exposed to environmentally relevant concentrations of diclofenac may have broad effects on growth, reproduction and population success. Decrease in feed intake and growth in *G. pulex* are interrelated (Schmidt et al., 2011) and the reduction in feeding activity of *G. pulex* could links the concurrent decrease in growth rate. De Lange et al. (2009) hypothesize that reduced feeding in *G. pulex* exposed to carbamazepine or diclofenac may interfere with growth.

We may equally hypothesize that the observed decline in action by diclofenac can inhibit *G. pulex* behaviour, for example, avoidance of predator (locomotion) and feeding behaviour. Reduced feeding will unavoidably cause reduced intake of energy, which can have far-reaching consequences on growth and reproduction. Change in

predator avoidance behaviour will distort the predator–prey balance in the ecosystem. This may have a short-term positive impact for the predator (i.e. increased prey consumption), however, long-term adverse effects may be observed when the prey source is overexploited. This observed reduction in activity of *G. pulex* in response to low levels of diclofenac is in accordance to its pharmacological purpose in humans (Fent et al., 2006).

Although the mechanism of diclofenac toxicity is not fully understood there is some evidence from previous work by Mastrangelo et al. (2014) that showed diclofenac hindered serotonin-induced reproducible levels in pig ureter. Inhibition of feeding behaviour by diclofenac may be related to the functions of serotonin (5-HT) as a neurotransmitter and also coordinate behaviours including feeding activities (Furuhagen et al., 2014; Baker et al., 2013). Nephrotoxicity of diclofenac is thought to be mainly due to the inhibition of prostaglandin synthesis and subsequent changes in prostaglandin regulated mechanisms, such as vessel tone, vascular permeability and ion regulation (Sanchez et al., 2002). Previous studies were able to demonstrate that diclofenac can inhibit cyclooxygenase activity and accordingly, synthesis of prostaglandin E2 in brown trout head, kidney, macrophages in vitro, thus demonstrating the same mode of action as reported for mammalian species. The effects of non-steroidal anti-inflammatory drugs on prostaglandin synthesis in humans are known to result in hyperkalaemia and hyponatraemia. Gracia-Lor et al., (2012) suppose that oxidative damage and subsequent necrosis and possibly apoptotic cell death also play an important role in diclofenac-induced nephrotoxicity. In mammalians, prostaglandins are known to be principal regulators of blood circulation and ion concentrations in kidney and gills in fish. It is feasible to assume that in aquatic invertebrates' prostaglandins may also display similar mechanistic roles and biological mechanisms.

Consequently, *G. pulex* exposed chronically to low levels of diclofenac could suffer adverse effects associated with the inhibition of COX and PGE2 synthesis. Diclofenac is a small molecule, with a log Kow of 0.7 (sodium-diclofenac) and low lipophilicity and may therefore easily pass through cell membranes. These results showed that exposure of *G. pulex* to environmentally relevant concentrations of diclofenac have significantly affected feeding activity, impacted growth and increased mortality, suggesting that prolonged exposure, use of sensitive points (behavioural signs) and use of susceptible test species (*G. pulex*) are more useful for assessing sublethal impacts of contaminants and are sensitive indicators of toxicity in benthic macroinvertebrates animals. Hence, these tools are useful in the aquatic environmental risk assessment of drugs.

4.8.3 Effects of ibuprofen on growth, feeding and mortality of G. pulex

Ibuprofen is a non-steroidal anti-inflammatory drug that has been shown to significantly affect the growth of several bacterial and fungal species. But the situation with invertebrates is less certain as there have been conflicting reports about the effects of ibuprofen on invertebrates.

In this study, the exposure of *G. pulex* to environmentally realistic concentrations of ibuprofen had no statistically significant effects on feeding, growth and mortality even though the feeding rate in the control groups was higher than the treatment groups. At the same time, the leaf consumption of these organisms was not substantially affected relative to the control (~10% reduction relative to the control). This suggests an increased palatability of the leaf material conditioned with bacteria and fungi, hence healthier test organisms may want to feed more (Bundschuh et al. 2009). Hence, more

increase in mass of *G. pulex* in the control groups than the treatments groups was noticed. The results indicate that the exposure of *G. pulex* to environmentally realistic concentrations of ibuprofen are inconsequential i.e. would most likely be of minor importance. However, a definite conclusion might not be reached about the risks of environmentally relevant ibuprofen concentrations before potential effects on multi-generational exposure have been assessed, which is beyond the scope of this study. Furthermore, no significant differences were observed in mortality between the treatments and controls. Although a minimal increase in mass of the treated *G. pulex* was noticed but this was not significant and indicates that the concentration thresholds at which ibuprofen could potentially cause toxicity effects were not reached. Hence, the internal concentration of ibuprofen in *G. pulex* is not enough to effect a significant change within the 4 weeks exposure, however, this may have effects later on in offspring of *G. pulex* or in a multigenerational experiment.

In a similar experiment conducted on *Hydra vulgaris*, a freshwater invertebrate (Pascoe *et al.,* 2003), no negative effects of pharmaceuticals (ibuprofen, paracetamol, acetylsalicylic acid, amoxicillin, bendroflumethiazide, furosemide, atenolol, diazepam, digoxin, and amlodipine) on survival, feeding, and bud formation were found at concentrations up to 1000 μgL^{-1}. Cleuvers (2003) exposed daphnia, chlorophyte, and macrophyte to environmentally relevant concentrations of major pharmaceuticals and concluded that acute effect stemming from single substances in the aquatic environment are very unlikely. Recent findings have also shown that population effects of ibuprofen in *D. magna* were reversible, consistent with the known action of ibuprofen on eicosanoid synthesis in mammals.

Similarly, when duckweed was exposed to 1 μgL^{-1} of the ibuprofen this resulted in no negative effect on growth compared to the other treatments. In a behavioural experiment conducted by De Lange et al. (2006) on *G. pulex* using multispecies freshwater biomonitoring (MFB), the exposure to ibuprofen resulted in decreased activity of *G. pulex* from 65% in the control to 45% at concentrations of 1 and 10 ngL^{-1} but this difference was not significant. In other studies, ibuprofen has been shown to inhibit the growth of *Synechocystis* and *Lemna*, the effect however turned into a growth stimulation after the second day of freshly added ibuprofen (Pomati *et al.,* 2014).

There are other animals and compounds that behave in a similar way to ibuprofen, for example Henschel *et al.* (1997) reported high EC_{50} values for fish embryos for several related pharmaceuticals such as salicylic acid (37 000 μgL^{-1}) and clofibric acid (86 000 μgL^{-1}). These levels are far more than 1000-fold greater than the highest concentration used in the present study which was only 4.84 μgL^{-1}. Other human drugs such as valpromide, methylhexanoic acid, pentenoic acid, and diethylacetic acid were also found to behave similarly to ibuprofen, i.e. weak inhibition or no effect on zebrafish development (Graca et al., 1994). Similar findings were reported by Hallare et al. (2004) who reported that environmentally relevant concentrations of ibuprofen do not cause detrimental effects on the early life stages of zebrafish, if they were exposed via the water only. The same study also reported that no differences were observed in either mortality or incidence of malformations between the treated and control embryos. Another study that agrees with this report was by Love A., (2016) where no significant effects was observed in feeding activity of *G. pulex* exposed to environmentally realistic concentrations of ibuprofen over a three-week period.

Similar work supports these results; a study on killifish (*Oryzias latipes*) revealed that, although reproduction was delayed following a 6-week chronic exposure to µgL^{-1} levels of ibuprofen, total reproduction of killifish did not differ between treatments (Flippin *et al.,* 2007). Egg abortion and reduced PGR has also been reported previously in *D. magna* exposed chronically to a metabolite (o-hydroxyhippuric at 10 mgl^{-1}) of the NSAID acetylsalicylic acid (Marques *et al.,* 2004b); although the parent compound had no impact at the same concentration (Marques *et al.,* 2004a).

The mode of action of ibuprofen in humans is the inhibition of prostaglandin biosynthesis. Prostaglandins are capable of causing contractions or atony of muscles in different organs (Cleuvers, 2004). Some publications indicate the presence of prostaglandins in other vertebrates and invertebrates such as crustaceans (Bundy, 1985). A possible explanation of the observed reduced activity of *G. pulex* may be that ibuprofen interferes with the normal pattern of muscle contractions in *G. pulex*.

Ibuprofen is an instable chemical, degraded in aquatic environments with a $DT_{50} < 1$ day (Richardson and Bowron, 1985). Pomati *et al*. (2014) suggested that ibuprofen metabolites are nontoxic for the aquatic organisms tested (*Synechocystis* and *Lemna*) and they may also have growth stimulating properties. Hence, ibuprofen exposed *G. pulex* did not demonstrate significantly increased mortality, feeding and growth.

4.8.4 Effects of mixtures of erythromycin, diclofenac and ibuprofen on growth, feeding and mortality of G. pulex

This study examined the effects of environmentally realistic concentrations of mixtures of ERY, DIC and IBU on *G. pulex* over an extended period of time. Studies examining the toxicity of simple or complex pharmaceutical mixtures are relatively sparse

although there has been much increased attention over recent years (Brain *et al.*, 2004a; Brain *et al.*, 2004b; Brain *et al.*, 2005).

In this study, the exposure to low levels of a complex mixture of ERY, DIC and IBU for a period of 4 weeks indicated statistically significant trend of reduced growth and feeding in *G. pulex.* Looking at the box plots in this study, there are indication that exposed *G. pulex* may have been feeding at a reduced rate compared to the controls and this was statistically significant. Although feeding and growth of *G. pulex* is dependent on size and gender (Willoughby & Sutcliffe, 1976; Sutcliffe *et al.*, 1981, Hughes *et al*, 2013) the animals were standardised by these factors before the experiments and extreme care was taken to prevent stress and injury to the animals especially during precopula separation and weekly measurement.

The results suggest that the compounds interact to produce enhanced effects during this study and therefore hypothesis $\boldsymbol{H_3}$ was accepted. This is in agreement with previous works which has shown that the effects of some pharmaceuticals, when combined, can elicit even greater effects than when present alone (Cleuvers, 2003; Cleuvers, 2004). However, the enhanced interaction demonstrated here is probably due to the fundamentally different target receptors between antibiotics and NSAIDs with erythromycin targeting prokaryotic cells and diclofenac and ibuprofen targeting cyclooxygenase-COX-1 and COX-2.

Furthermore, some studies have shown that pharmaceuticals with different target receptors can interact and that these interactions vary with exposure (Pomati *et al.*, 2006). Also, some pharmaceuticals demonstrate non-polar narcosis which is not dependent upon the presence of specific receptors and as such remains a concern when considering the exposure to complex mixtures in freshwater environments (Cleuvers,

2003). The behaviour of each substance in a multi-component mixture may vary, depending on the composition, concentration and the bioassay applied to evaluate the effects. Furthermore, the duration and frequency of exposure could alter the toxicological effects of pharmaceutical mixtures. Combinations of compounds can have unfavourable joint outcomes that may be synergistic, antagonistic or additive. The three major characteristics of their effects are: (a) the toxicity of mixtures can be higher than the effects of their individual components (Cleuvers, 2003; Cleuvers, 2004: Han *et al*., 2006), (b) a mixture can have considerable toxicity effects even if all components are present in low concentrations that do not induce toxic effects singly (Backhaus et al., 2008) or, (c) a mixture of chemical compounds can have lower effects, e.g. enzyme induction than the effect of the single compounds (Li et al., 2011).

Clearly, the issue of pharmaceutical mixtures is highly complex and there are many unanswered questions. For instance, Dietrich et al. (2010), in a multigenerational study of single and multiple mixtures of 4 different pharmaceuticals on *D. magna*, concluded that "Comparing the influence of the drug mixture with the impact of the single compounds CBZ, DIC, EE2 and MET, it seems that the pharmaceutical mixture did not provoke stronger effects on the exposed daphnids than the single drugs". This is in disagreement with Cleuvers (2003, 2004) who detected an enhanced toxicity of pharmaceuticals toward daphnids when applied as a mixture. In addition, this result was supported by another study by Schnell et al. (2009) who showed that the toxic effects of a mixture of pharmaceuticals and personal care products from different therapeutic classes on liver cells of rainbow trout (*Oncorhynchus mykiss*) were greater than predicted due to synergistic effects of the substances. Flaherty and Dodson (2005) showed that the toxicity of drug mixtures is unpredictable, and complex compared to

effects of single pharmaceuticals. Therefore, it is very difficult to assess the risk of pharmaceuticals on non-target organisms in natural aquatic systems, where animals are permanently exposed to complex drug mixtures. However, mixture toxicity studies like the one presented here and elsewhere are one of the steps in improving understanding of pharmaceuticals in the environment.

In this study, the exposure to low levels of the complex mixture of ERY, DIC and IBU for four weeks indicated decrease in the growth of *G. pulex* when data was analysed statistically. From the current study, it could be deduced that *G. pulex* feeding rate diminished when compared to the controls and this was statistically significant. The results suggest that there was interaction between the drugs and hence, there was increased impacts. Some other work had demonstrated that pharmaceuticals with various target receptors can combine and that these synergies differ with exposure (Pomati et al., 2006). These results provide a potential explanation for the interaction of pharmaceutical mixtures that the elevated pharmaceutical bioaccumulation in an organism could be related to drug–drug interactions resulting from another pharmaceutical inhibition of CYP activity (Franzellitti et al., 2015). An in vivo exposure for *Carassius auratus* showed that 89.78 μgL^{-1} and 20.2 μgL^{-1} of ketoconazole (KCZ) in the water caused an almost 80% and 36% decrease in CYP enzyme activity, respectively (Huang et al., 2012; Yang et al., 2017), which may support the suggestion by Franzellitti et al. (2015).

4.9 Conclusions

The results of this study showed that the toxicity of drug mixtures is unpredictable, and complex compared to effects of single pharmaceuticals. Also, this study confirms the suitability of *G. pulex* as an ecotoxicological test species that is both amenable to

laboratory culture and sufficiently sensitive to provide reliable quantification of environmental risk. Studies examining the effects of pharmaceuticals on *G. pulex* are relatively sparse. The body of research on *G. pulex* response to stressors suggest it is an ecologically sensitive indicator species for a wide range of aquatic pollutants, including pharmaceuticals. The results presented here lend support to its use for detecting effects of pharmaceuticals with effective mortality evident at concentrations well below those reported for the much more widely used test species, *D. magna*. The lack of samples testing nominal concentrations in the exposure matrix means it was not possible to fully establish a dose-response relationship for mortality at specific concentrations of pharmaceuticals. However, the experimental design allowed comparisons between exposures to high, medium and low levels of each pharmaceutical and their comparisons against control conditions. Given the important role that *G. pulex* plays in the processing of organic matter, increased mortality may have serious secondary implications for leaf litter decomposition and nutrient cycling in freshwater ecosystems (Macneil *et al.*, 1997), in addition to implications for its predators and the wider food web.

CHAPTER FIVE

Effects of pharmaceuticals on the freshwater isopod, *Asellus aquaticus*

5.0 Introduction

Pharmaceuticals are consumed all over the world including the poorest countries on the planet because it increases life span, sustainability of lives, increases human productivity and mass production of food and livestock to sustain ever-growing human population. As a result, in the last few decades, global manufacturing of pharmaceuticals had increased geometrically (Borgmann et al., 2007). However, the presence of these drugs in the aquatic environment may elicit unintended biological response on non-target organisms among other responses, physiological changes, such as feeding, growth, mobility and behavioural changes (Orn et al., 2016; Jobling and Sumpter, 1993; Rand 1985 Boyd et al., 2003) are most vulnerable/important endpoints for assessing the effects of pharmaceuticals on aquatic organisms (Orn et al., 2016).

Over the years, invertebrates have been found useful as model animals for investigating the toxicity of compounds in the environmental (Daughton and Ruhoy, 2009b; Plahuta et al., 2017; Relic et al., 2017; Gasperi et al., 2014). Macro invertebrates has been used regularly in past for measuring the toxicity of chemicals because they are sensitive to toxic compounds and environmentally significant (Hutchinson and Pickford, 2002; Okuda et al., 2008). They are simple to handle, easy to rear, varieties of animal species to choose from and have short life span, hence, they are suitable for toxicity testing of water.

The test animal-*Asellus aquaticus*, a freshwater isopod, was chosen because they play a significant part in freshwater environment; they are leaf shredders and transfer and store metabolic energy within the ecosystems (Van Hecken et al., 2000; Graca et al.,

1993). They also serve as food for both fish and invertebrate predators (Rask & Hiisivuori, 1985; McCahon et al., 1990; De Jong et al., 2010; Bundschuh et al., 2012). *Asellus aquaticus* has a life cycle of one year and has been used as a test species in toxicity testing experiments both in the laboratory and the field (Rask & Hiisivuori., 1985; Migliore & De Giudici., 1990; Bloor M., 2010; Ebele et al., 2017). They serve as an indicator of the health of stream, can be found in large number and breed in captivity and very slow in movement in water. Unlike *G. pulex* that is a water column dweller *A. aquaticus* are sediment-dwellers and constantly in contact with contaminants both in the water column and sediments (McCahon et al., 1990). They are seen as a robust organism, tolerant to fluctuations of pH value, dissolved oxygen concentrations and other physico-chemical parameters (Van-Hattumetal., 1989). They are considered to be relatively tolerant to pollution (Backhaus & Karlsson, 2011; Maltby, 1995; Bloor & Banks, 2005; Bloor., 2010), but can be sensitive to trace metals (Migliore & De Giudici., 1990). They play a prominent role in transfer of contaminants in the aquatic food chain (Peeters et al., 2000; MacNeil et al., 2002; Orn et al., 2016). Their small size and robust nature make them ideally suited for application in toxicity tests and eliminating them will disrupt the balance in the ecosystem (Bundschuh et al., 2012; Rask & Hiisivuori., 1985). Hence, they are of great importance for the sustainability and balancing in the ecosystem. Very few studies have investigated effects of pharmaceuticals on *A. aquaticus* in the aquatic environment; in the past three decades the majority of work done using this model organism focused on metal pollution. For example, mercury, cadmium and copper were found by Ort and Siegrist, (2009) to be toxic to *A. aquaticus*. Long-term effects of metals on *A. aquaticus* mortality was investigated by Van Ginneken et al. (2017) and found that lethal concentrations were lower than nominal and effective concentrations. Plahuta et al. (2014) investigated the

effects of exposure of *A. aquaticus* to selected organic pollutants and found that there were significant effects on the mortality rate. In a similar experiment by De Nicola Giudici et al., (1988, the effects of chronic exposure to 5 μgL^{-1} cadmium and copper on *A. aquaticus* were investigated and it was found that the juvenile body growth was stimulated by cadmium and depressed by copper. Other studies in which *A. aquaticus* were exposed to metal toxicity were Migliore et al. (1990); Rainbow & Black. (2005); Qiu et al. (2005); Grosell et al. (2002, 2006); Pestana et al. (2007); De Jonge et al. (2010); Bundschuh et al. (2012).

The current work investigated the ecological effects of prolong low-level exposure of *A. aquaticus* (bottom/sediment dweller) to erythromycin, diclofenac, ibuprofen and their mixtures at environmentally relevant concentrations on growth, feeding and mortality with the aim of broadening knowledge about the potential risk of such contaminants to aquatic ecosystems.

5.1 Aims, objectives and hypothesis

5.1.1 Aim and objectives

The general aim is to seek to improve the understanding of the effects of prolong low-level exposure of freshwater ecosystems (*Asellus aquaticus*-bottom dweller) to pharmaceutical contamination. Response variables included growth, feeding and mortality. Specific objectives were;

1. To assess the effects of prolonged low-level exposure to environmentally relevant concentrations of erythromycin, diclofenac and ibuprofen on growth, feeding, and mortality of freshwater macro-invertebrate isopod, *Asellus aquaticus*.

2. To examine the effects of mixtures of the above pharmaceuticals on *A. aquaticus* relative to individual compounds.

5.1.2 Hypotheses

(H1): That prolonged low-level exposure to environmentally relevant concentrations will have a direct lethal effect on *A. aquaticus.*

(H2): That extended exposure to environmentally relevant concentrations will cause significant reductions in sub-lethal endpoints (e.g. growth and feeding).

(H3): That the effects of mixtures will be more pronounced than compounds acting singly.

5.2 Single compound and mixture experiments with Asellus aquaticus

5.2.1 Materials and methods

Please refer to Chapter Four, section 4.2.1

5.2.1.1 Study compounds

Please refer to Chapter Four, section 4.2.1.1

5.2.1.2 Materials

Please refer to Chapter Four, section 4.2.1.2

5.2.1.3 Preparation of solutions

Please refer to Chapter Four, section 4.2.1.3

5.2.1.4 Test animals: origin and maintenance

Asellus aquaticus used for the experiments were sourced from Blades Biological Ltd, Cowden, Edenbridge, Kent, United Kingdom. Isopods of approximately the same size

averaging 21.80 ± 1.31 mg, 21.84 ± 1.46 mg, 22 ± 1.38 mg and 22.29 ± 1.31 mg were used for erythromycin, diclofenac, ibuprofen and their mixture experiments respectively. They were sexed and kept in incubators at 12° C with a diurnal light rhythm of 16 h:8 h (day-night) and allowed to acclimatise for ten days in aerated pond water before the exposure experiments started. Sexing is achieved by placing the pre-copular pairs on a dry filter paper and allowed them to disentangle from each other.

5.2.1.5 Preparation of leaf for feeding of test animals

Please refer to Chapter Four, sections 4.2.1.5

5.2.1.6 Exposure media

Please refer to Chapter Four, sections 4.2.1.6

5.2.1.7 Experimental design

For each of the experiments (ERY, DIC, IBU and their mixtures), there were two treatments (LT and HT) and solvent controls with 15 replicates of each treatment and 15 replicates of the control. Test concentrations were selected to mimic environmental detection levels reported for UK rivers in the literature. The low treatments (LT) were UK mean measured environmental concentrations of 159.7 ngL^{-1} (ERY), 202.2 ngL^{-1} (DIC), 420.8 ngL^{-1}(IBU) and the high treatments were 1377.8 ngL^{-1}(ERY), 2990.7 ngL^{-1}(DIC) and 4838.4 ngL^{-1}(IBU) respectively (Hughes et al., 2013, Bound and Voulvoulis, 2006) and the solvent control contained 0.1 mI L^{-1} of methanol.

For the mixture experiments, the low and high treatments were mixtures of ERY, DIC and IBU concentrations in the single compound experiments. Only two treatments and

control could be established in this set of experiments due to inability to obtain sufficient standardised test animals from Blades Biological Ltd.

The experiments were carried out in clear glass SS jar (500 mL) kept in incubators (Figure 5.2.1.7) at a temperature of 12° C and 16:8 h light: dark regime. The animals were illuminated with a fluorescent light (with a specification for freshwater invertebrates), to simulate on a small scale the macroinvertebrates' natural climatic condition. The glow mimicked the thermal warmth and daytime illumination obtained from the sun radiation.

Each glass jar contained one *A. aquaticus* with 300 ml of pond water, which was assigned and arranged randomly in the experimental chambers using a random integer generator. Individuals were weighed individually at the start of the experiment and subsequently every week with a Sartorius Quintex 224-1s balance.

The working solutions of LT and HT were poured on transparent silica glass beads and allowed to evaporate to dryness in the fume cupboard in other to avoid methanol toxicity, then the dried extracts were reconstituted/resuspended with 10 ml of pond water and washed into the beakers before *A. aquaticus* were introduced.

Figure 5.2.1.7: One of the experimental set-ups in the incubators showing the arrangement of the jars with one *A. aquaticus* in each jar exposed to experimental media (Solvent control (STCR), Low treatment (LT) & High treatment (HT)).

Before the transparent silica glass beads were reused, they were washed with ultra-clean water, ashed in the furnace at 550º C and allow to cool in the fume cupboard to prevent toxicity in any form to the test animals.

For each of the experiments, forty-five (45) *A. aquaticus* were used, making a total of one hundred and eighty (180) *A. aquaticus* used for (ERY, DIC, IBU and mixtures) experiments. Exposures were static-renewal with 100% water replacement every week with fresh concentrations of the pharmaceuticals and the experiments were each run for

4 (four) weeks. Growth was measured weekly by deducting the initial mass of each *A. aquaticus* from the mass each week. Mortality was determined at the end of the experiments by counting the surviving animals and calculating percentage mortality. Remaining alder leaves (feed material) at the end of the experiments were oven dried, weighed and combusted to determine the feeding rate (ash free dry mass).

5.3 Data analyses

Please refer to Chapter Four, sections 4.3

5.4 Results

5.4.1 Erythromycin experiments

5.4.1.1 Initial test conditions

When the experiment was initiated (day 0) the average mass of *A. aquaticus* (both treatments and controls) was 21.80 ± 1.31 mg with no statistically significant difference (ANOVA: $F_{2, 42} = 0.26$, $p = 0.77$) between treatment group and control.

5.4.1.2 Growth

There was a statistically significant difference in mass between the treatment groups and the control (ANOVA: $F_{2, 34} = 166.2$, $p < 0.001$). There was a consistent increase in the mass of *A. aquaticus* in the control from week one to week four. The mean change in mass at the end of the experiments was SCTR 2.28 mg ± 1.0 SD, ERY-LT -3.28 mg ± 0.97 SD and ERY-HT -4.42 mg ± 1.03 SD (Figure 5.4.1.2).

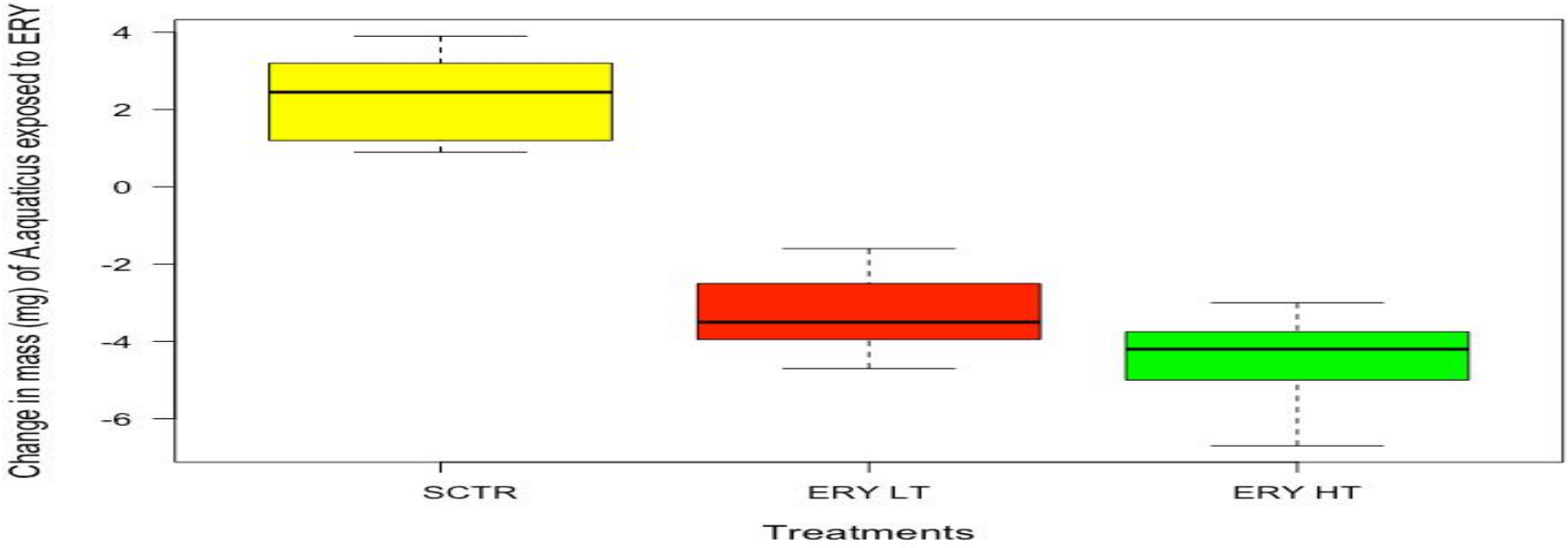

Figure 5.4.1.2: Boxplots displaying change in mass o *A. aquaticus* exposed to environmental relevant concentrations of erythromycin after a 4-week static renewal experiments. Solvent control (SCTR), low treatment (ERY-LT) and high treatment (ERY-HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents the 3rd quartiles (75th percentile), top whisker represents the 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

5.4.1.3 Feeding

There were statistically significant differences in the mass of feed materials between the control and treatments (ANOVA: $F_{2, 42} = 199.6$, $p < 0.001$). The residuals of the data were normally distributed (Shapiro-Wilk normality test: $p = 0.2914$ and Bartlett test of homogeneity of variances $p = 0.2855$). The mass loss of *Alnus glutinosa* litter in the control was higher than those in the treatment groups i. e. feeding rate in the control was higher than the treatments. Even between the treatment groups, the feed material loss was dose dependant (Figure 5.4.1.3). Thus, erythromycin had deleterious effects on both feeding behaviour and growth of *A. aquaticus.*

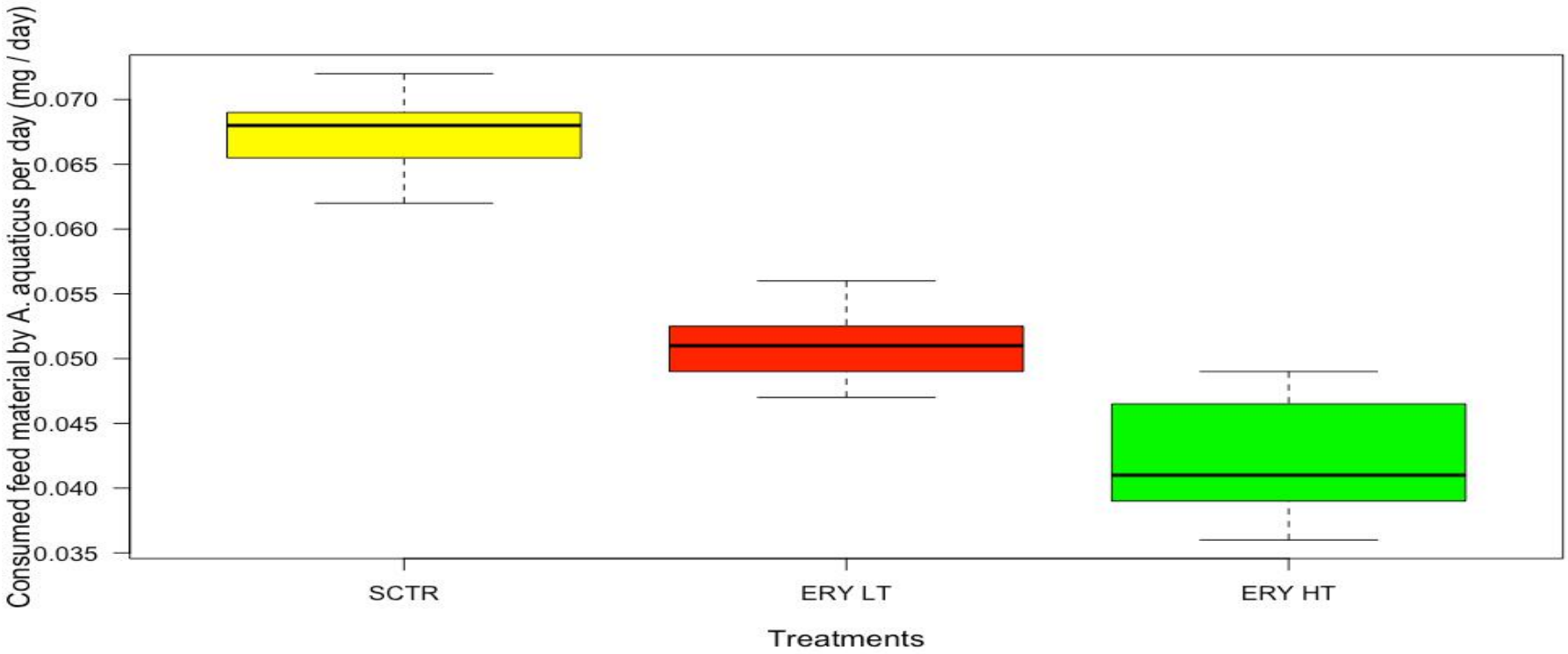

Figure 5.4.1.3: Boxplots displaying consumed feed materials by A. aquaticus exposed to environmental relevant concentrations of erythromycin after a 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (ERY-LT) and high treatment (ERY-HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

5.4.1.4 Mortality

There were no statistically significant differences between the treatments and the control (ANOVA: $F_{2, 9} = 0.55$, $p = 0.59$). Four mortalities occurred in ERY-HT, three mortalities in the ERY-LT and one mortality in the control (SCTR), (Figure 5.4.1.4).

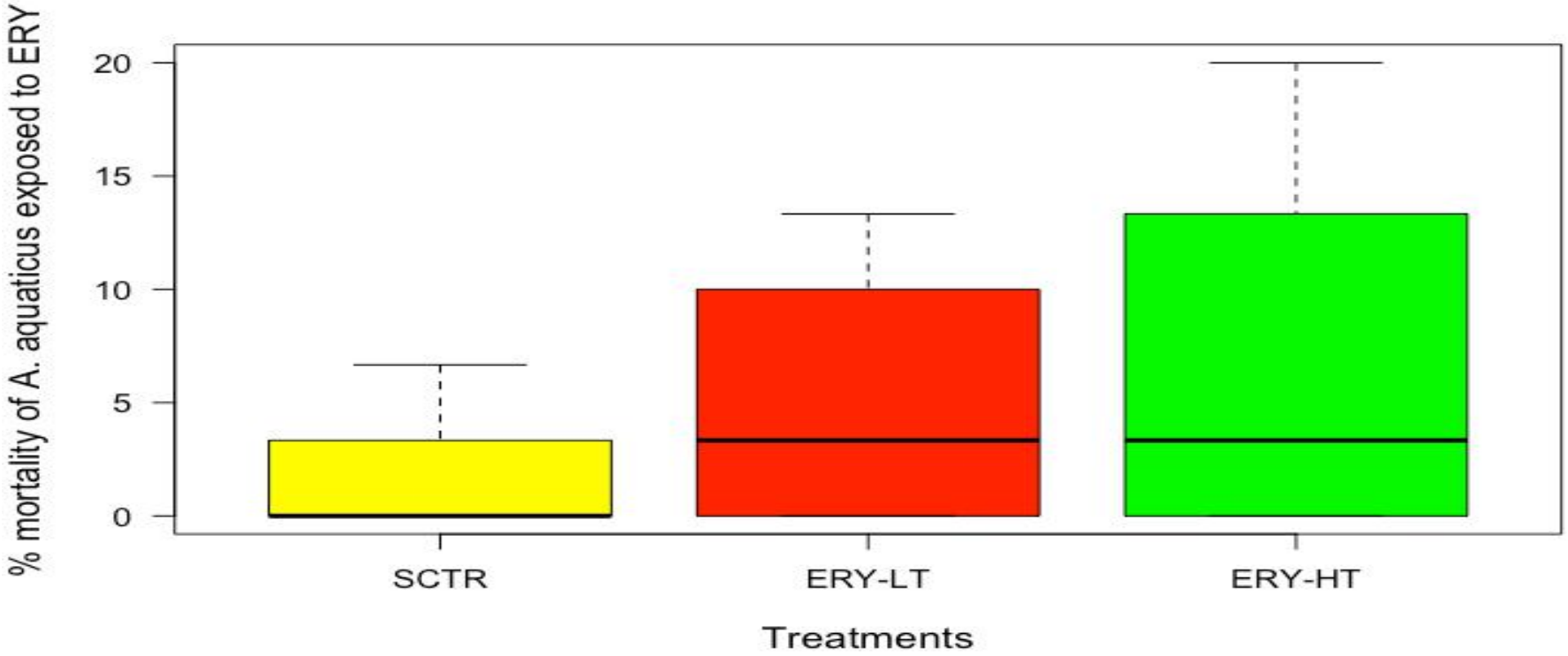

Figure 5.4.1.4: Boxplots displaying % mortality of *A. aquaticus* exposed to environmental relevant concentrations of erythromycin after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (ERY-LT) and high treatment (ERY-HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

5.5 Diclofenac experiments

5.5.1 Initial test conditions

When the experiment was initiated (day 0) the average mass of *A. aquaticus* in the solvent control (SCTR) was 21.6 mg ± 1.35 SD, low treatment was 22.20 mg ± 1.47 SD and high treatment 21.73 mg ± 1.58 SD in diclofenac experiments. There was no statistically significant difference (ANOVA: $F_{2, 42} = 0.69$, $p = 0.58$) between treatment groups and the control.

5.5.2 Growth

When the residual of the data was analysed, there was statistically significant differences between the treatment groups and the control (ANOVA: $F_{2, 35} = 88.01$, $p < 0.001$). There was a consistent increase in the mass of *A. aquaticus* in the control from week one to week four. The mean changes in mass were SCTR 2.28 mg ± 1.0 SD, DIC-LT -1.60 mg ± 1.01 SD and DIC-HT -2.66 mg ± 1.02 SD (Figure 5.5.2).

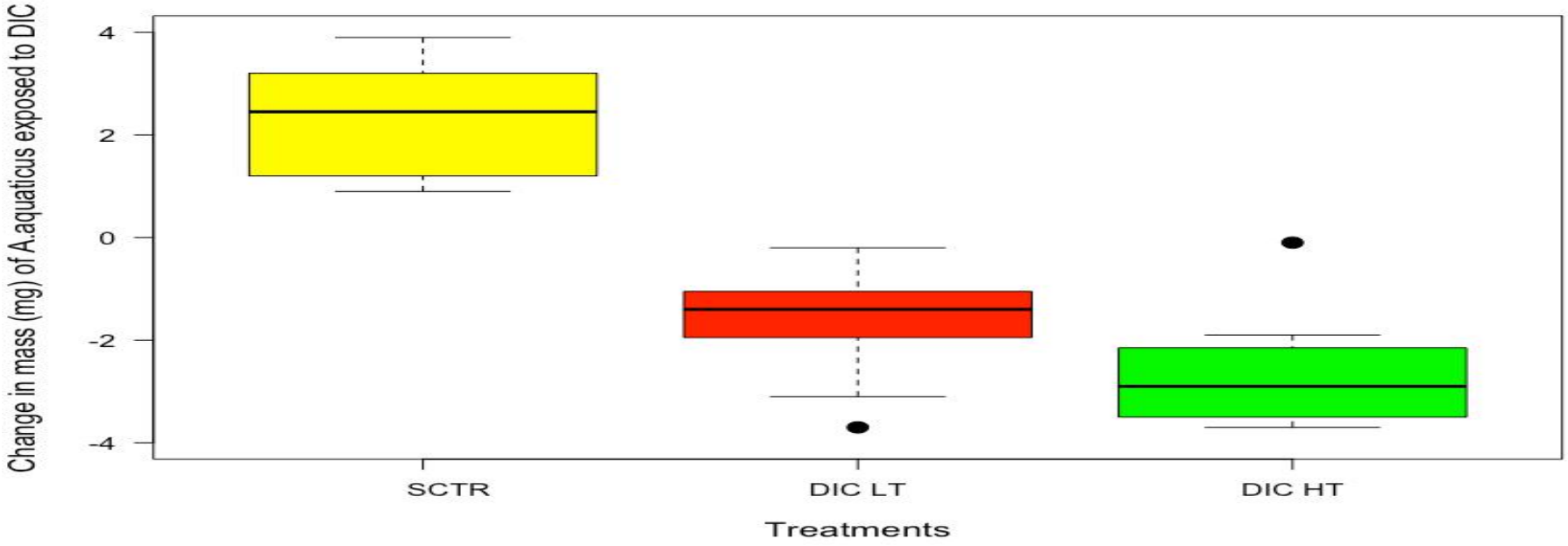

Figure 5.5.2: Boxplots displaying change in mass of *A. aquaticus* exposed to environmental relevant concentrations of diclofenac after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (DIC-LT) and high treatment (DIC-HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were outliers.

5.5.3 Feeding

There were statistically significant differences in the mass of feed materials between the control and treatments (GLM: χ^2 (2) = 77.38, $p < 0.001$). The mass loss of *Alnus glutinosa* litter by the control was higher than those in the treatment group i. e. feeding rate in the control were higher than the treatments. Even between the treatment groups, the feed materials loss was dose dependant and significantly influenced (Figure 5.5.3).

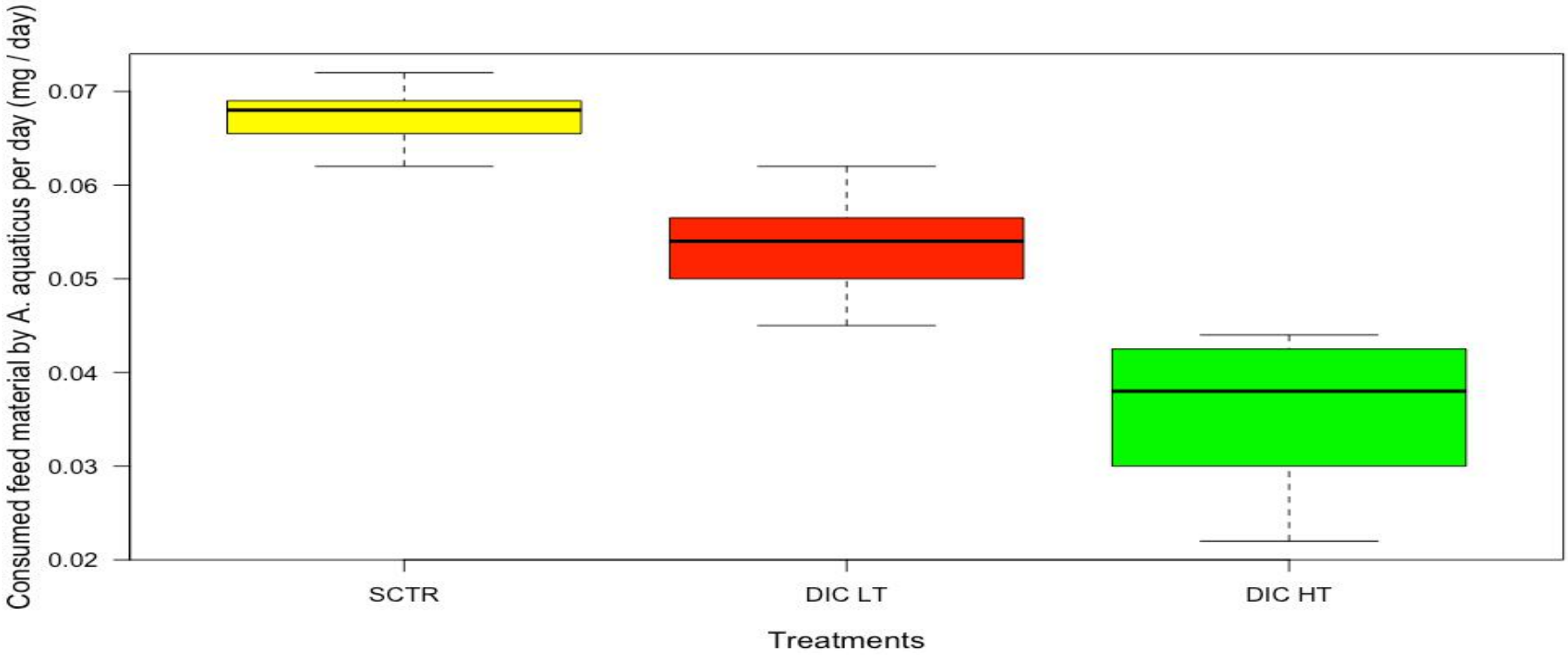

Figure 5.5.3: Boxplots displaying consumed feed materials by *A. aquaticus* exposed to environmental relevant concentrations of diclofenac after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (DIC-LT) and high treatment (DIC-HT). The dark horizontal line inside the box represents the median (50th percentile), top of the coloured box represents 3rd quartiles (75th percentile), top whisker represents 4th quartiles (90th percentile), bottom of the coloured box represents the 2nd quartiles (25th percentile) and the vertical lines represents the 1st quartiles (10th percentile). There were no outliers.

5.5.4 Mortality

There was one mortality in the SCTR during the third week of the experiments. In the DIC-LT there were three deaths, one in week three and a further two in week four, as there were in the DIC-HT. There was no statistically significant difference in mortality between treatments and control groups (GLM: χ^2 (2) = 29.62, p = 0.75, Figure 5.5.4).

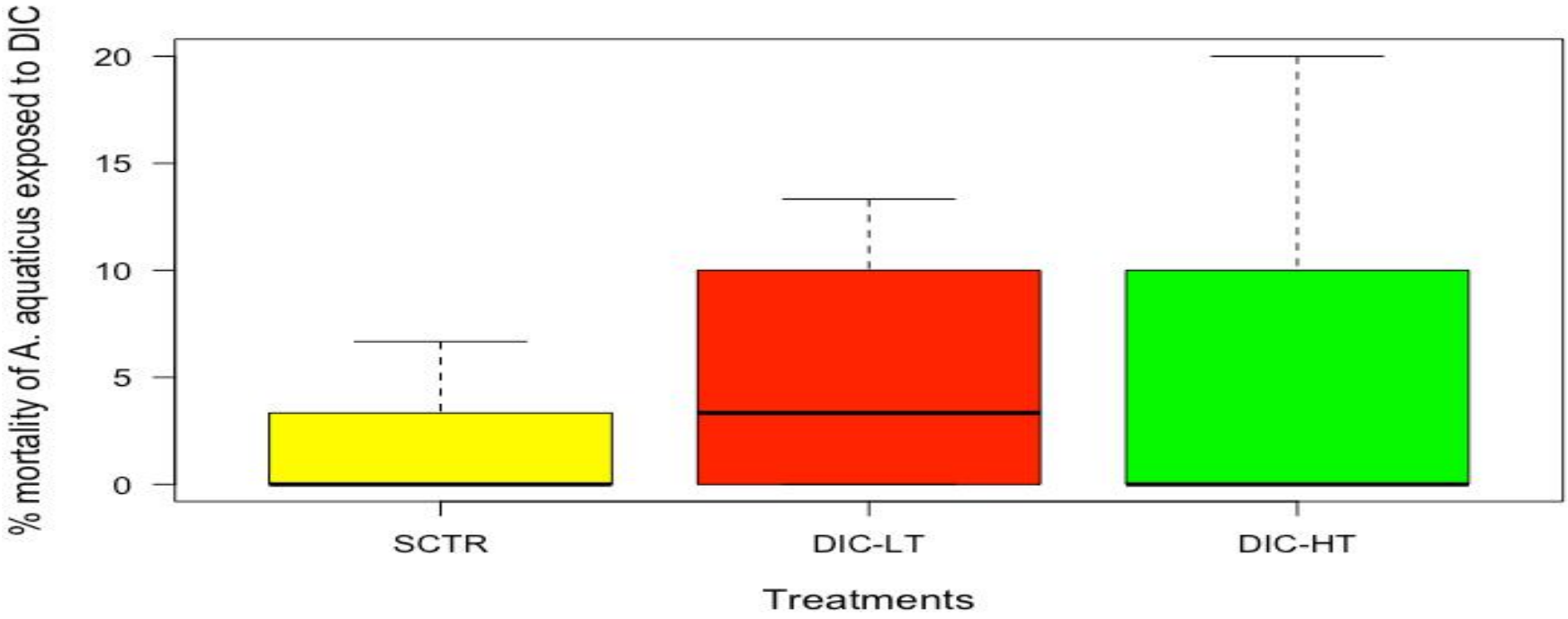

Figure 5.5.4: Boxplots displaying % mortality of *A. aquaticus* exposed to environmental relevant concentrations of diclofenac after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (DIC-LT) and high treatment (DIC- HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There were no outliers.

5.6 Ibuprofen experiments

5.6.1 Initial test conditions

When the experiment was initiated (day 0) the average mass of *A. aquaticus* in the solvent control (SCTR) was 21.6 mg ± 1.35 SD, low treatment was 22.47 mg ± 1.41 SD and high treatment 21.93 mg ± 1.33 SD in ibuprofen experiments. There was no statistically significant difference (ANOVA: $F_{2, 42} = 1.54$, $p = 0.23$) recorded between treatment groups and the control.

5.6.2 Growth

When the residual of the data was analysed, there was no statistically significant differences between the treatment groups and the control (GLM: χ^2 (2) = 1.17, p = 0.24). There was a consistent increase in the mass of *A. aquaticus* in the control and a marginal increase in the treatment groups. The mean changes in mass were SCTR 2.28 mg ± 1.0 SD, IBU-LT 1.91 mg ± 0.39 SD and IBU-HT 1.94 mg ± 0.31 SD, (Figure 5.6.2).

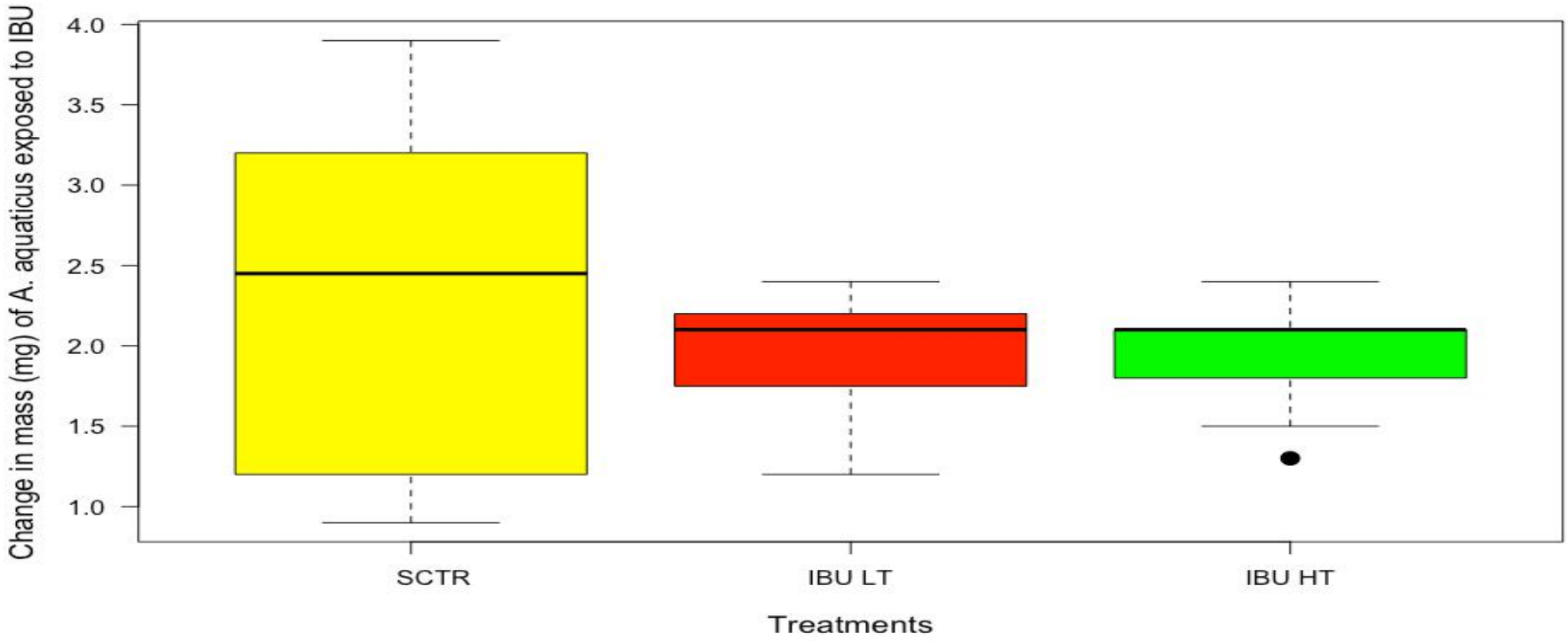

Figure 5.6.2: Boxplots displaying change in mass of *A. aquaticus* exposed to environmental relevant concentrations of ibuprofen after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (IBU-LT) and high treatment (IBU-HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There is an outlier.

5.6.3 Feeding

The effects of 4 weeks exposure of *A. aquaticus* to environmentally relevant concentrations of ibuprofen is presented in Figure 5.6.3. No statistically significant difference in the mass of feed materials of *A. aquaticus* was determined after 4 weeks exposure to ibuprofen, however (GLM: χ^2 (2) = 1.31, p = 0.35).

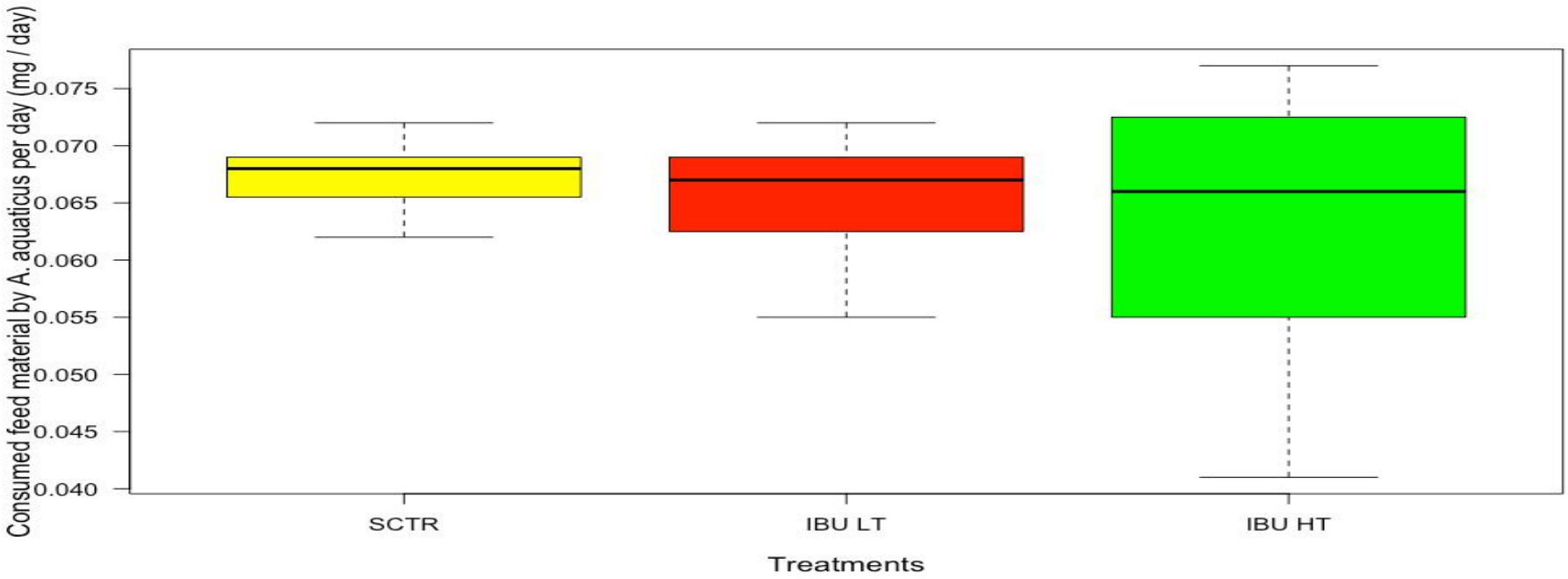

Figure 5.6.3: Boxplots displaying consumed feed materials by *A. aquaticus* exposed to environmental relevant concentrations of ibuprofen after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (IBU-LT) and high treatment (IBU-HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There were no outliers.

5.6.4 Mortality

There were no statistically significant differences between the treatments and control (GLM: χ^2 (2) = 7.42, p = 0.61) although, there was one mortality each in the control and high treatment, but this was not significant enough to cause an effect. (Figure 5.6.4).

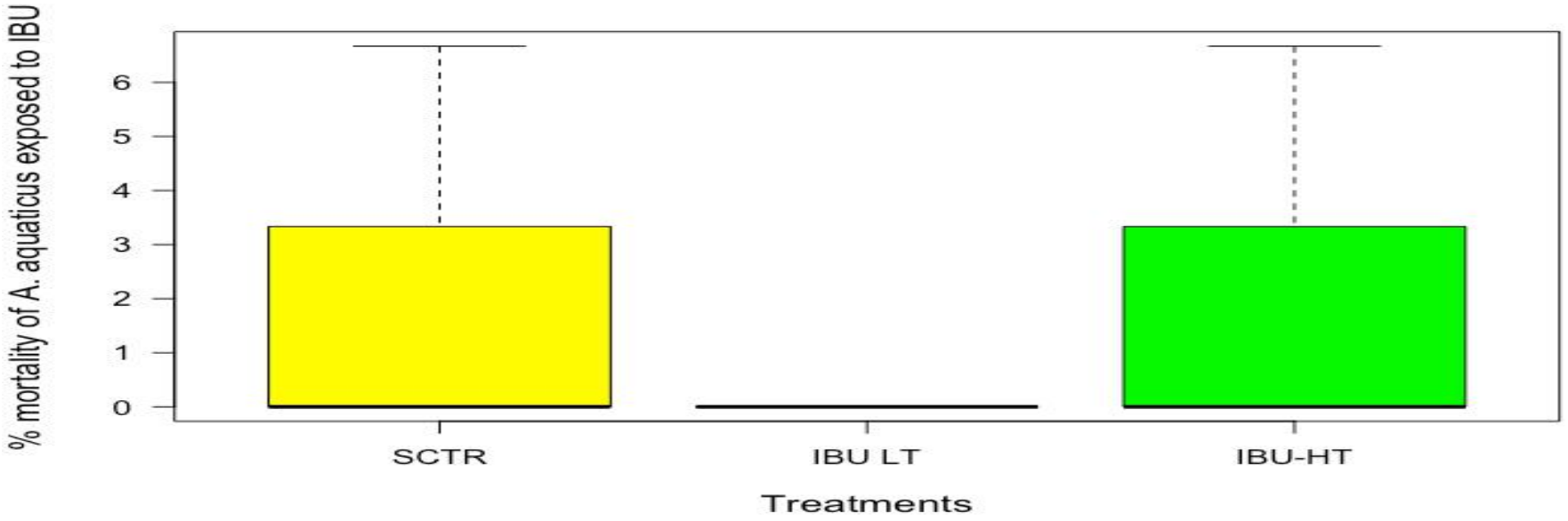

Figure 5.6.4: Boxplots displaying % mortality of *A. aquaticus* exposed to environmental relevant concentrations of ibuprofen after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (IBU-LT) and high treatment (IBU-HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There were no outliers.

5.7 Multiple mixture (ERY+DIC+IBU) experiments

5.7.1 Initial test conditions

When the experiment was initiated (day 0) the average mass of *A. aquaticus* was 22.32 mg ± 1.45 SD for control (SCTR), 22.19 mg ± 1.31 SD for low treatment (MIX-LT) and 22.37 mg ± 1.24 SD for high treatment (MIX-HT). There was no statistically significant difference in test organism mass between the treatments and the control (ANOVA: $F_{2, 42} = 0.073$, $p = 0.929$).

5.7.2 Growth

When the residuals of the data were analysed for change in mass over the course of the experiment, there were statistically significant differences between the treatments and the control (GLM: χ^2 (2) = 10.07, $p < 0.01$), (Figure 5.7.2).

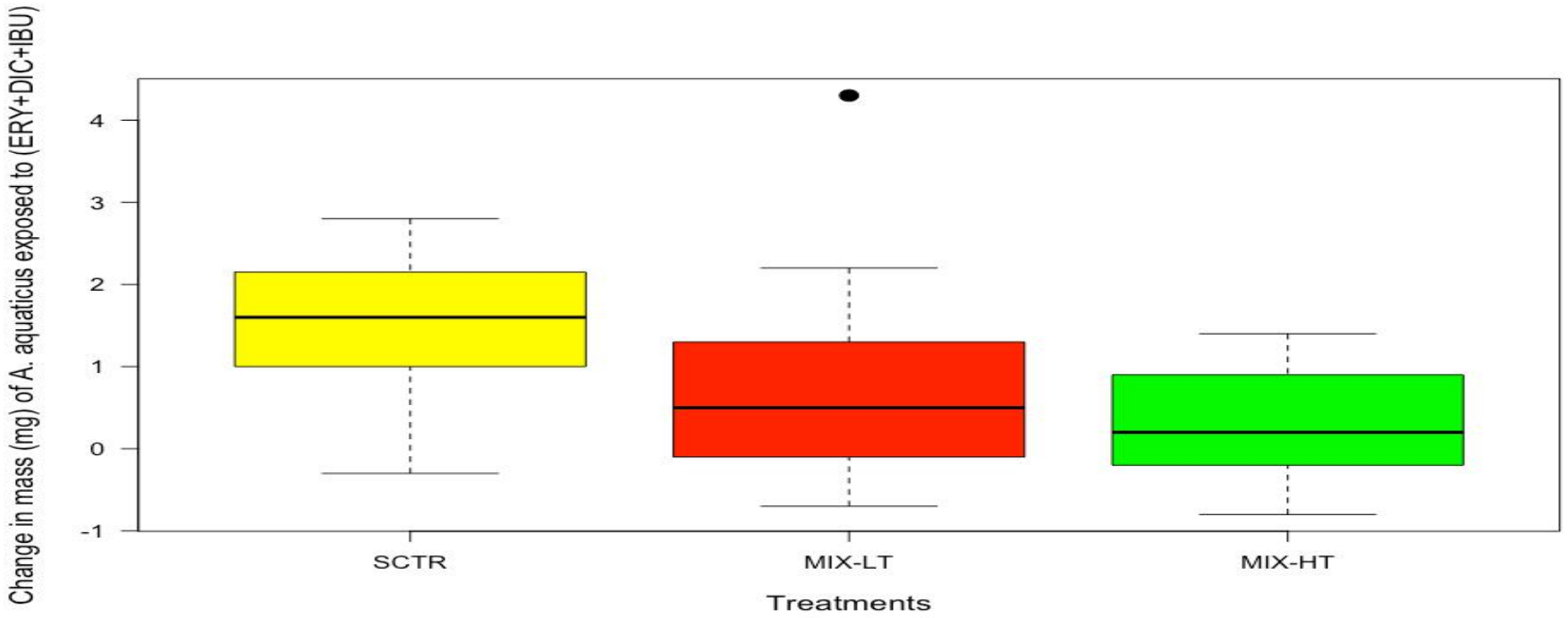

Figure 5.7.2: Boxplots displaying change in mass of *A. aquaticus* exposed to mixtures of erythromycin, diclofenac and ibuprofen after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (MIX-LT) and high treatment (MIX-HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There was outlier.

5.7.3 Feeding

There were statistically significant differences in the mass of feed materials between control and treatments (ANOVA: $F_{(2,42)} = 6.72$, $p < 0.01$). The mass loss of *Alnus glutinosa* litter by the control was higher than those in the treatment groups i. e. feeding rate in the control was higher than the treatments, (Figure 5.7.3).

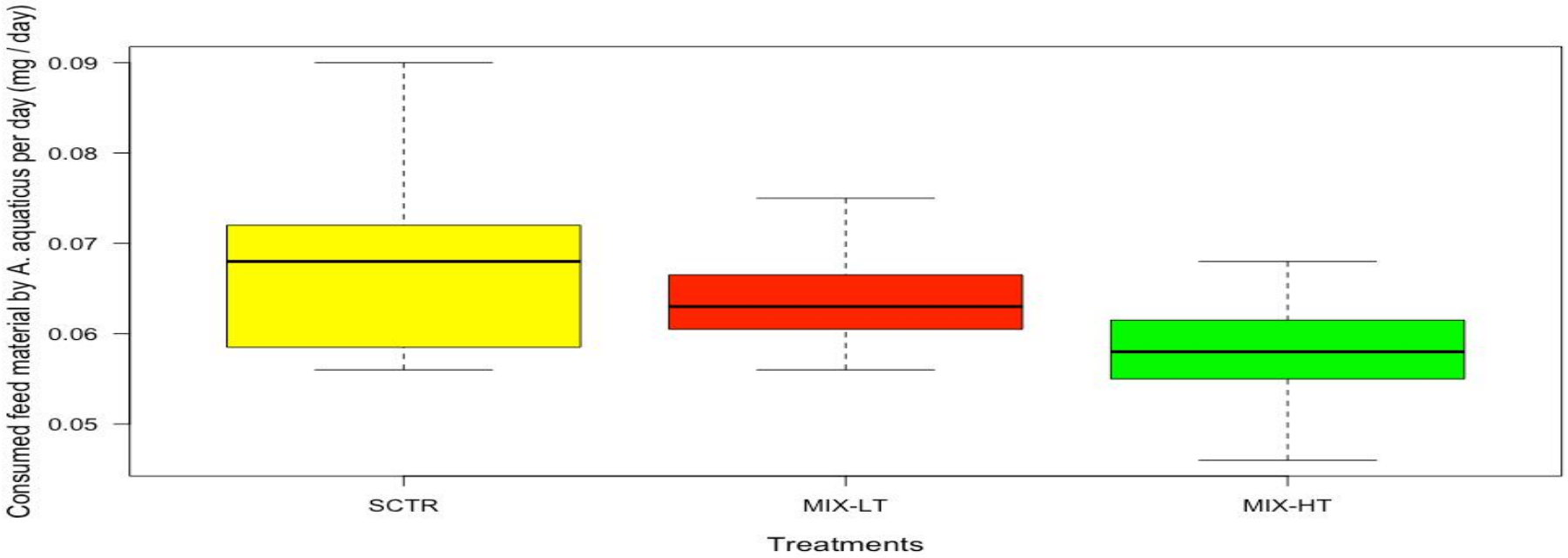

Figure 5.7.3: Boxplots displaying consumed feed materials by *A. aquaticus* exposed to environmental relevant concentrations of mixtures of erythromycin, diclofenac and ibuprofen after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (MIX-LT) and high treatment (MIX-HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} percentile). There were no outliers.

5.7.4 Mortality

Mortality did not occur in the control throughout the duration of the experiments. One and two mortalities were recorded in the fourth week of the experiments in the MIX-LT and MIX-HT respectively (Figure 5.7.4) but there was no statistically significant difference between the treatments and control (GLM: χ^2 (2) = 22.11, p = 0.56).

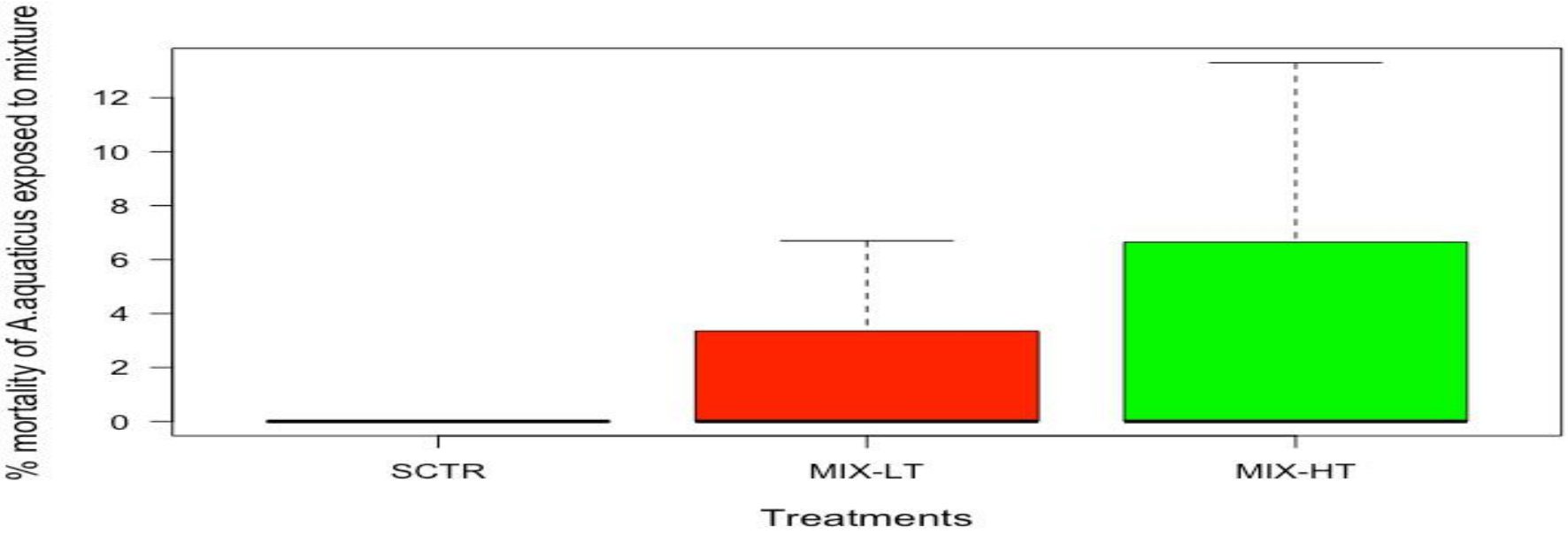

Figure 5.7.4: Boxplots displaying % mortality of *A. aquaticus* exposed to environmental relevant concentrations of mixtures of erythromycin, diclofenac and ibuprofen after 4 weeks static renewal experiments. Solvent control (SCTR), low treatment (MIX-LT) and high treatment (MIX-HT). The dark horizontal line inside the box represents the median (50^{th} percentile), top of the coloured box represents 3^{rd} quartiles (75^{th} percentile), top whisker represents 4^{th} quartiles (90^{th} percentile), bottom of the coloured box represents the 2^{nd} quartiles (25^{th} percentile) and the vertical lines represents the 1^{st} quartiles (10^{th} perc entile). There were no outliers.

5.8 General Discussion

The aim of this study was to seek to improve the understanding of the effects of prolonged low-level exposure of *Asellus aquaticus* to erythromycin, diclofenac, ibuprofen and their mixtures. Overall, there were substantial impact on growth and feeding when *Asellus aquaticus* was exposed to the erythromycin and diclofenac. Though, there was no concurrent effects due to ibuprofen.

There are few data on the use of *A. aquaticus* as a test species in pharmaceutical effect studies but there is substantial information on its use in metal toxicity. This study is one of the few in which *A. aquaticus* is used in pharmaceutical effect studies.

5.8.1 Effects of erythromycin on growth, feeding and mortality of A. aquaticus

Results obtained from this study clearly showed that growth and feeding behaviour of *Asellus aquaticus* were affected when the isopod was exposed to environmentally relevant concentrations of erythromycin, hence hypothesis $\boldsymbol{H_2}$ was upheld/accepted. Reduced feeding activity, reduced growth and increased mortality was noticed in the treatments compared to the control. These visible effects on feeding, body mass and mortality were related to concentration levels.

This present experiment was supported in a similar experiment by Plahuta et al. (2015) in which *A. aquaticus* was exposed to 2.5/5.0 mgL^{-1} of Bisphenol-A (BPA) for 21 days; even though a different drug and at higher concentrations when compared to this study, there was 75 % reduction in growth rate. In many crustacean significant growths occurs only through periodic moulting, in this instance most likely, moulting of the exoskeleton has been inhibited/disrupted by the activities of erythromycin, as well as inhibited feeding, therefore, caused a reduction in growth (D'ascenzo et al., 2003).

Another reason that may have caused reduction in body mass when exposed to erythromycin was decreased available energy for body growth (Slooff, 1983). Any organism exposed to contaminants need to apportion energy to withstand the contaminant either by evasion, expulsion, elimination, or biochemical complexation (Donker,1992), however, if the concentration levels of the toxicants is more than what the organism can cope with, effects are expected/noticed.

Other research further supported these findings, for instance, a similar result was obtained but with a different species in an investigation by Quinn et al. (2008) in which *Hydra attenuata* was exposed to 50 mgL^{-1} of erythromycin for 96 h, significant reduction in feeding activity was observed. The change in feeding rate/activity was also observed in another organism after 72 h exposure to erythromycin at the same concentration with the one used in this investigation. Investigations by Plahuta et al. (2015, 2017), has shown that the source of diet also plays a significant role in contaminants absorbing through feeding/ingestion. *Asellus aquaticus* which are bottom/sediment dwelling animals are directly exposed to pollutants from interstitial and overlying water by consuming Alder leaves. It has been reported that absorption of contaminants through dietary source could be up to 100 % of total residue by aquatic organisms, hence it is right to assume that the source of pharmaceuticals to which an organism is exposed could be significant in the effects occurrence in the animals (Landrum & Robbins., 1990; Peters et al., 2000; Orn et al., 2016).

It is a known fact (Bloor., 2010; Bloor and Bank., 2012) that fungi and bacteria are valuable parts of diet of all detritivores and a recent report revealed that *Asellus aquaticus* favoured a diet of conditioned leaf materials over unconditioned leaf materials, with natural conditioning being the preferred conditioning option (growth of fungi and bacteria on the leaf). Investigations by Bloor (2010) has equally revealed that

natural conditioning leaf materials produces noticeably softer and heavier leaf materials, which could be ascribe to the settling of micro-organisms on the leaves. *A. aquaticus* feed, by scraping the leaf surface, thereby, this feeding habit of *A. aquaticus* could have added to the negative effect found when exposed to erythromycin because the residues of the drug might have settled also on the leaf surface. Erythromycin, a narrow spectrum antibiotic works by inhibiting protein synthesis (by preventing them from building protein) in mammals, since protein do all the cell's work, a bacterium that cannot build protein cannot survive, a similar process may be found in *A. aquaticus* thereby reducing feeding activities, body weight and increase mortality.

In many cases most of the data from the literature agrees with the results of this finding. When *Asellus aquaticus* was exposed to cadmium at concentration of 600 μgL^{-1}, the feeding activities and growth of the test animal was reduced (Ebele et al., 2017; Liu et al., 2017). In another study in which *Palaemon serratus* was exposed to tetracycline a similar result inhibiting the feeding activities was observed even though different pharmaceuticals, but they are still environmental stressors (Oliveira et al., 2015). Any element impacting an organism's feeding activity may equally have strong ecological implications within the organism's biome.

Another study by Plahuta et al. (2017), in which *Asellus aquaticus* was exposed to two different waste water treatment plants influent A & B, it was observed that 100 % and 95 % mortality occurred in the undiluted samples respectively although, the pharmaceuticals in the waste water were not revealed by the researchers. This result disagreed with this present study in which mortality recorded was not significant. Mixture effects of different compounds with different modes of action and with different receptors being targeted may be a factor in this case. Plahuta et al. (2017) also observed significant changes in mortality between influent and treated effluent samples.

The mortality from sewage treatment effluents was less than 20 %. Compared to the mortality in influent samples, it indicates efficient removal of a great quantity of toxic pollutants in sewage treatment systems. This present study suggests that the decreased feeding activity directly influenced the growth rate of *A. aquaticus* but not enough to cause lethality in the test species hence, hypothesis $\boldsymbol{H_1}$ was rejected. However, the increase in food intake by the control positively correlates with the growth and final size of the test animals (Graca, 1990; Arsuffi and Suberkropp, 1986).

5.8.2 Effects of diclofenac on growth, feeding and mortality of A. aquaticus

The results of this experiment showed that environmentally relevant concentrations of diclofenac have significant effects on growth and feeding behaviour of *Asellus aquaticus,* hence hypothesis $\boldsymbol{H_2}$ was upheld/accepted. This suggest that growth and feeding behaviour are more sensitive indicators of stress in extended exposure to xenobiotics.

Diclofenac in humans inhibit prostaglandin biosynthesis. Prostaglandins mode of action are contractions of muscles in different organs (Cleuvers, 2004). Some previous works in the literature revealed the presence of prostaglandins in some invertebrates and vertebrates' animals, for example crustaceans (Bundy, 1985). A likely reason for the observed reduction in activities of *A. aquaticus* could be that the natural pattern of muscle contractions in *A. aquaticus* was interfered with by diclofenac. These may affect physiological processes such as feeding and growth.

Previous research has also shown that behavioural indicators are more useful for appraising sub-lethal effects of pharmaceutical contaminants. For instance, Hernando et al. (2006) demonstrated that a variety of contaminants interfere with normal freshwater isopods behaviour after exposures much less severe than those causing significant

mortality. Inhibition of feeding behaviour may be explained by serotonin functions as a neurotransmitter and regulate a wide range of behaviours (Fent et al., 2006; Baker et al., 2013). The results of this experiments were supported in a similar study by Richards et al. (2015b) where male Japanese medaka fish (though different test species to the one used in this experiments) were exposed to diclofenac at environmentally relevant concentrations three biomarkers were expressed, indicating that diclofenac has capacity to cause cellular toxicity. More recent studies further supported this result, Nassef et al. (2010), Brodin et al. (2014), Schoenfuss et al. (2016), Relic et al., 2017 and Conolly et al., (2017) in which Japanese medaka fish was exposed at environmentally relevant concentrations to diclofenac, the feeding rate and feeding activity were reduced.

This experiment equally compared favourably with the works of Schwaiger et al. (2004) in which the macro-invertebrate animal, *Daphnia magna* was exposed to diclofenac at concentrations similar to the one used in these experiments, reduced feeding activities and growth rate was observed. In a related study by Triebskorn et al. (2004), Zhang et al. (2012) in which rainbow trout (*Oncorhynchus mykiss*) was exposed to diclofenac concentrations similar to the one used in this report 1 µg L^{-1} to 500 µg L^{-1} for 28 days period. Mutation in the gills and kidney of the exposed fish was reported. Renal lesions and alterations of the gills was observed at 5 µg L^{-1} (LOEC) (Zhang et al., 2012). These concentrations are higher than the one used in the present study. The results further supported this present study even though different test animals but the mode of action and target receptor of diclofenac in organisms may be the same.

In this study mortality was not significant when compared with the control throughout the duration of the experiments, therefore, hypothesis $\boldsymbol{H_1}$ was rejected. The works of Dietrich et al., (2010b) was in contrary to this experiment, the embryo of zebra fish was

exposed to diclofenac after 96 h, it was recorded that the lethal concentration was 480±50 µg L^{-1}(LC_{50} 96h) and effect concentration was 90 ± 20 µg L^{-1}(EC_{50} 96 h). This may be explained by the fact that fish embryo is more sensitive to toxicants and the concentrations employed in the study is higher than the one used in this experiment.

5.8.3 Effects of ibuprofen on growth, feeding and mortality of A. aquaticus

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID), an unstable chemical that can be degraded in the environment with a t_{50} < 1 day (Richardson and Bowron, 1985). The results from this investigation showed that 4 weeks exposure of *Asellus aquaticus* to ibuprofen at environmentally relevant concentrations is of no significant consequences ecologically. There was no significant concentration response relationship for any of the endpoints examined e.g. growth and feeding, hence hypothesis $\boldsymbol{H_2}$ was rejected and for mortality, hypothesis $\boldsymbol{H_1}$ rejected also.

Heckmann et al. (2007) supported this result when *Daphnia magna* was exposed to chronic concentration of ibuprofen (20-40 mgL^{-1}) for 14 days. However, in an earlier study by Heckman et al. (2005), total mortality was recorded when *D. magna* was exposed to ibuprofen concentrations of 80-100 mgL^{-1} for 12 days. Although, one may argue that *A. aquaticus* and *D. magna* are two different species of freshwater macro-invertebrate animals, *D. magna* are more sensitivity to contaminants than *A. aquaticus*, hence the reason why no response was noticed in A. aquaticus.

This study disagrees with the works of De Lange et al. (2006), in which relevant environmental concentrations of ibuprofen were investigated on *G. pulex* at low concentrations of 10-100 ngL^{-1} and resulted in a significant decrease in activities of the benthic macro invertebrate animal. One could argue that *G. pulex* is more sensitive to environmental contaminants than *A. aquaticus*. *G. pulex* lives in oxygen rich water

column and *A. aquaticus* is a bottom/sediments dweller, can withstand low oxygen, high ammonia concentrations and highly tolerant of pollution. Both are used in bio-monitoring of the health of freshwater habitat.

This study also disagrees with the investigation of Pomati et al. (2004) where an aquatic freshwater plant, *Lemna minor* and cyanobacterium *Synechocystis sp*. were exposed to relevant environmental concentrations of ibuprofen. It stimulates the growth of cyanobacterium *Synechocystis sp.* when exposed at concentration of 1, 10, 100 and 1000 μgL^{-1} after just 5 days and show no effects on duckweed *L. minor* within 5 days of exposure. However, after changing of the test media it inhibited the growth of duckweed *L. minor* after 7 days of exposure at all tested concentrations. Ibuprofen was also observed to be embryotoxic at concentrations >10 μgL^{-1} to zebrafish (Pancharatna et al., 2015). Ibuprofen has also been observed to distress the defence system and stimulate stress in adult bivalves (Parolini et al., 2016). In another study by Furlong et al., (2014) similar to this report, where *M. galloprovinciallis* was exposed to ibuprofen, larva development only occurs at much more higher concentrations than environmentally relevant concentrations. However, additional studies involving long-term exposures (in agreement with this present study) were conducted suggesting that, exposing aquatic organisms to relevant environmental concentrations of ibuprofen may not impact on the organisms (Cleuvers, 2004; Hans et al., 2006; Heckmann et al., 2005, 2007; Pounds et al., 2008).

But, before a definite conclusion can be reached on the environmental risk posed by ibuprofen on macro-invertebrates, multi-generational exposure investigations needs to be conducted. Another investigation on *Oryzias latipes*- a killfish after exposure to chronic concentrations of ibuprofen for 6 weeks showed that although reproduction was

delayed total reproduction of *Oryzias latipes* did not differ between the treatments (Flippins et al., 2007; Heckman et al., 2007).

In a study that disagree with the results of this present study, endocrine disruption at sub-molar concentration levels of ibuprofen revealed a decrease in corticol production by 40 % in interrenal cells of *Oncorhynchus mykiss* (rainbow trout) which may impair stress response (Heckman et al., 2007). A previous study on the acute immobility of *Daphnia magna* (Cleuvers, 2004) has demonstrated that ibuprofen work by narcosis principle (Van Hecken et al., 2000). In a multi-generational study conducted by Clubbs and Brooks, (2010) in which *D. magna* was exposed to ibuprofen, there was reduced fecundity of *D. magna* in the first generation, while long term (10 days) exposure did not significantly different from the controls.

5.8.4 Effects of mixtures of erythromycin, diclofenac and ibuprofen on growth, feeding and mortality on A. aquaticus.

Going forward from the analysis of single compounds, in the present study, effects of mixtures of erythromycin, diclofenac and ibuprofen at relevant environmental concentrations via direct (waterborne) exposure pathway on *Asellus aquaticus* in a 4 weeks bioassay was investigated. Sublethal responses such as growth, feeding behaviour and mortality were analysed. It was observed that the compounds influenced the growth of *A. aquaticus* both at low and high treatments and hence discontinuous increase in body mass when compared with the constant increase in mass in the control, therefore hypothesis $\boldsymbol{H_3}$ was accepted/upheld. They are consistent with previous investigations that had showed that mixtures of some drugs can cause greater effects than when acting singly (Cleuvers, 2004). In this present study, exposure of *A. aquaticus* to mixtures of erythromycin, diclofenac and ibuprofen negatively affected

the growth and feeding activities of the test organism, but the effects were not as strong as in the single compounds of erythromycin and diclofenac. However, synergism exhibited in this present study maybe as a result of different receptors targeted by the compounds, NSAIDs targeting COX 1 & 2 and ERY targeting prokaryotic cells.

In a similar investigation by Quinn et al. (2009) in which Hydra was exposed to mixtures of pharmaceuticals for 96 h, there was reduction in the ability of the freshwater Hydra to regenerate.

Investigations by Parrot and Bennie (2009) also supported the findings in this study, although, *Pimephales. promelas* (Fathead minnow) was used to study the effects of mixtures of seven drugs at concentrations of 1µg L^{-1} for 3 months. The degree of defects observed in the fathead minnow were small. Sun et al. (2009) investigated binary mixtures of a hormone (17β-estradiol) with letrozole at environmentally realistic concentration and detected significant decrease in fertility and fecundity after 21 d of exposure.

In a similar study to this experiment, even though different compounds and test species, Dietrich et al., (2010) exposed *Lemna gibba* (Fat Duckweed) to different mixture of drugs similar sensitivity was demonstrated by the test species at concentrations 1–300 µg L^{-1}. After 7 d, the test specie showed sign of necrosis.

In a multigenerational mixture experiment in which acetaminophen, diclofenac, ibuprofen and a host of other compounds were used, it was observed that the sex ratio was altered by 17 % more males. In a binary combination (diclofenac and ibuprofen) and quaternary (ibuprofen, acetylsalicylic, naproxen and diclofenac) exposed to D. magna, a very strong additive effect was observed at concentrations of 34-54 mgL^{-1} (Cleuvers, 2004). Very strong additive effects were also observed when *D. magna* was

exposed at concentrations 10-fold lower than the quaternary concentrations. The body size and reproduction were affected (Cleuvers, 2008).

In a study carried out by Nieto et al., (2016), Carcinus maenas was shown to have significant changes in haemolymph osmolality and osmoregulatory capacity after being exposed to relevant environmental concentrations of the mixtures (10 ngL^{-1} and 17.5 psu of salinity). The osmoregulatory ability of the mixture was improved, implying a reduction in benefit by organisms and a rise in haemolymph osmolality (Furuhagen et al., 2014). The *A. aquaticus* exposed to the mixtures of erythromycin, diclofenac and ibuprofen started losing weight as a result of the exposure while the control animals are gaining weight weekly. Feeding rate was equally affected, the exposed isopod was feeding at reduced rate in the low and high treatments compared to the control. Hence there was alteration in feeding rate of *A. aquaticus* exposed to the mixture. Similar investigation on *Hydra attenuata*, showed that minimum concentrations of 10 mgL^{-1} and 50 mgL^{-1} was needed to observe a significant reduction in feeding activities when exposed for 96 h to ibuprofen and carbamazepine respectively (Quinn et al., 2008). This concentration was 1000 times higher than the concentrations employed in this study though, with different study compounds and test animals and duration.

De Lange et al (2006, 2009) established the effects of pharmaceuticals on feeding activities and behaviour of other macro-invertebrate animals, using concentrations similar to those used in this study but different pharmaceutical compounds were used.

Considering the feeding rate and growth between the control and treatments, one-way ANOVA/GLM results suggested that there was a significant interaction. When you compared this with the exposure of *A. aquaticus* to single compounds of erythromycin and diclofenac there is a decrease in growth and feeding rate by 10-fold. These showed that the single compound of erythromycin and diclofenac are more toxic than the

mixture of erythromycin, diclofenac and ibuprofen. This may be due to the fact that erythromycin and diclofenac are toxic to *A. aquaticus* while ibuprofen may be harmful. The realistic environmental concentrations of isolated compounds such as diclofenac and erythromycin do cause increase mortality, reduced feeding rate and growth. However, when they are in mixtures, these compounds may present increased (synergistic) or reduced (antagonistic) inherent toxicity. Aside this, diclofenac and ibuprofen have similar mode of action (MoA) and hence they may act (additively) synergistically. Addition of erythromycin to this mixture may cause it to act antagonistically, hence the result obtained in this study.

The low significance in this study compared to environmentally relevant concentrations of isolated compounds such as diclofenac and erythromycin may be due to antagonistic toxicity. The low and high treatments did not show any sign of increase mortality as a result of the exposure to the mixture. There were only three mortalities throughout the duration of the study, one in the low and two in high treatments and none in the control. Generally, many scientists agree that concentration addition (CA) is appropriate for estimating mixture toxicity of substances acting in a similar manner, while independent action (IA) assumes that in a mixture of different chemicals, the effects exerted by individual chemical are not dependent on others. The key limitation of the concentration addition model, as Kortenkamp et al., 2009 noted, is that differences may be detected for some mixtures containing drugs for which only low effects are detected.

5.9 Conclusion

Based on this study, it can be suggested that *A. aquaticus* can be recognized as a reference model test animal and good indicator to evaluate the potential effects of

contaminants. The results of this study showed that the toxicity of drug mixtures is unpredictable, and complex compared to effects of single pharmaceuticals. In the single compound experiments, erythromycin and diclofenac effects on *A. aquaticus* growth and feeding were more pronounced and no effect on mortality while ibuprofen showed no effects on the test animals. However, the mixtures showed concentration addition (CA) effects and one of the weaknesses of this model is that differences are sometimes seen for some mixtures containing drugs for which only little effects are detected.

CHAPTER SIX

General Discussion

6.0 Research synthesis

This PhD thesis had investigated the occurrence and effects of pharmaceuticals in African rivers (Nigeria). The main goal of the research was to quantify the occurrence of selected pharmaceuticals in African Rivers (Nigeria) and to seek to improve the understanding of the effects of prolonged low-level exposure of *G. pulex* and *A. aquaticus* to environmentally relevant concentrations of erythromycin, diclofenac, ibuprofen and their mixtures. The main objectives of the study were to: (1) evaluate the presence of drugs belonging to different therapeutic classes in previously unstudied surface water in Nigeria. (2) Quantify spatial patterns of pharmaceutical contaminations in the Odo Iya Alaro river, Lagos Southwest Nigeria. (3) Determine seasonal dynamics of contamination. (4) Assess the effects of prolonged low-level exposure to environmentally relevant concentrations of erythromycin, diclofenac and ibuprofen on growth, feeding, and mortality of *G. pulex* and *A. aquaticus*. (5) Examine the effects of mixtures of the above pharmaceuticals on *G. pulex* and *A. aquaticus* relative to individual compounds. To achieve the 1st, 2nd and 3rd objectives (Chapter 3), data were collected from Odo Iya Alaro river in Lagos State, Southwest Nigeria to find out if there are indeed pharmaceuticals in Nigeria aquatic systems. In order to achieve the 4th and 5th objectives, Chapters 4 & 5 investigated the effects of the three study compounds (erythromycin, diclofenac, ibuprofen and their mixtures) on the growth, feeding and mortality of *G. pulex* (Chapter 4) and *A. aquaticus* (Chapter 5). More detailed and specific findings in relation to the individual chapters are summarised as follows.

6.1 Occurrence of pharmaceuticals in river water and effluents in Nigeria compared to other parts of the world.

The quest by human to live a better life and increase agricultural productivity in other to feed himself led to development of modern pharmaceuticals in the eighteen century (1800s). Ever since, the use of drugs has been a blessing to human race for (i) prevention and treatments of diseases (ii) maintenance of good health (iii) increases productivity in work place by reducing man-hour spent on sick-leave (iv) longevity of life (v) increases crop yield and farm produce for human consumption. These brought unlimited joy to mankind because of its numerous benefits but not until the last 20 years when the presence of these drugs in the environment (aquatic and terrestrial) became an issue of global concern because they are designed to be biologically active hence, their unexpected presence in the ecosystem has generated worries over possible potential adverse effects on fauna and flora. Pharmaceuticals are continuously been released and expectedly pervasive and *pseudo*-persistency in the environment.

They have been found in all types of aquatic environment, i.e. ponds, streams, rivers, sewage, lakes, sea, groundwater and had equally been reported in drinking water. They enter the aquatic systems through many channels but of most important route is the STPs effluents. Also, the development of highly effective analytical instruments has aided the detection of this pharmaceuticals in nanogram per litre in the aquatic environment. However, the quantity and types of drugs in an environment depends on usage, method of disposal, life style, age of people living in that vicinity, type and quality/effectiveness of sewage treatment plants, climatic condition, government regulations/policy, physicochemical parameters of the receiving water body and

receptors in the organisms taken the drugs and ecological mechanism will all add up to play an important part in the occurrence and effects of drugs in river systems.

More than 1000 publications are available on the occurrence of pharmaceuticals in aquatic environment mostly from North America, Western Europe (Santos et al. 2013), very small part of China and India (Hughes et al., 2013). Most of this research particularly in China and India are clustered around very small number of Urban sites (Hughes et al., 2013). Large numbers of potentially high-risk pharmaceuticals are poorly or not investigated. Techniques employed during sampling in most of the research are poor for capturing the high degree of temporal and spatial variability associated with pharmaceutical pollution.

Also, as human population increases, the need to improve quality of life will increases and pharmaceutical usage will also increase. This may be a major factor for increase occurrence of pharmaceuticals in aquatic system and by extension pharmaceutical contamination of the freshwater ecosystems in years to come (Daughton, 2003). To mitigate the impact of pharmaceuticals in the environment, it is important we understand the occurrence and effect of these compounds in the environment. If these challenges are not dealt with soonest, the ever-increasing human population especially in developing countries of Africa, Asia and South America with rising economic development in these continents may contribute significantly to the ongoing contamination of the environment and substances that are detected presently at high nanogram to low micrograms per litres may soon reach mg per litre in these continents.

Most importantly, this research has actually addressed some vital components of this complex system and has established the presence of pharmaceuticals in Nigeria freshwater system (Chapter 3).

In this study, pharmaceuticals are consistently found throughout the duration of sampling across the urban and semi urban stretch of Odo-Iya Alaro river including the STPs and pharmaceutical manufacturing effluents. Verlicchi et al. (2012), in his global review of pharmaceuticals, reported effluent concentrations for many analytes some of which were also observed in this study. Paracetamol, sulfamethoxazole, fexofenadine, metformin, carbamazepine and cimetidine concentrations all of which fell above the ranges reported by Verlicchi et al. (2012). Similarly, study of effluents in the European Union (EU) reported average concentrations 3-4 order of magnitude lower than those determined here for paracetamol, cimetidine, fexofenadine, atenolol, sulfamethoxazole, carbamazepine and diazepam, while concentrations of venlafaxine, sitagliptin, propranolol and tramadol were an order of magnitude lower than those reported by Burns et al., (2018), Loos et al., (2013) and Kasprzyk-Hordern et al., (2009).

For seasonal variations, the highest six median concentrations in the dry season are: fexofenadine>metformin>cimetidine> paracetamol > carbamazepine>sulfamethoxazole and for wet season are: paracetamol > cimetidine > sulfamethoxazole > metformin > fexofenadine > codeine respectively indicating that there are no specific spatial trend and that concentrations of analytes were elevated throughout. This corroborated the fact that variety of sources existed for pharmaceutical contamination of the river system in Nigeria.

However, the maximum concentrations recorded for antibiotics, analgesic and psychiatric in this study were more than 50 times higher than those reported for other African countries and globally (Table 6.1). Furthermore, the concentrations of

pharmaceuticals such as fexofenadine, metformin, ranitidine and lidocaine are up to 3-4 orders of magnitude higher compared to global concentrations for these compounds, however, for African countries data are sparse. When the data from this investigation were compared to the concentrations reported in a recent review on the global environmental PhACs occurrence (Tim Aus der Beek et al., 2016), the values measured in this present study are of 3-4 order of magnitude higher (Table 6.1). Table 6.1 shows (this study) as far as l know and from all the literature studied/published, the first time in African river where 27 pharmaceuticals were detected and in such high concentrations.

Probable explanations might be: (i) Lagos State being the city with the highest number of human populations in Africa and the smallest state in Nigeria has the highest number of educated people and hence, usage of pharmaceuticals is high. (ii) Lagos state has more than 60 % of pharmaceutical manufacturing companies and serves as hub for distribution of pharmaceutical products in West Africa. (iii) Inadequate STPs and incomplete removal from the few available. (iv) Rivers are highly impacted by informal settlements: both urban and semi urban cities/town. (v) Multiple sources of pharmaceutical load into the aquatic systems.

Since the concentrations of pharmaceuticals monitored in river system in Nigeria were known (Chapter 3), it was then established if erythromycin, diclofenac, ibuprofen and their mixtures have subtle physiological effects on *G. pulex* and *A. aquaticus*. Although, two of the selected test compounds for the toxicity experiments (diclofenac and ibuprofen) were not monitored in this study but there is high likelihood that they are present and other studies in Nigeria had detected them in the environmental matrix.

Plate 3: Vacuum truck discharging untreated sewage to a channel that joins Odo Iya Alaro River.

Plate 4: Vacuum trucks queuing to dislodge untreated sewage.

Table 6.1: Comparison of pharmaceutical concentrations measured in Nigeria (this study), Africa and globally (global data are taken from Hughes et al., 2013 and Tim Aus Der Beek et al., (2016)).

Pharmaceuticals	Max Conc in THIS STUDY (ng/L)	Max Conc in Africa (ng/L)	Max Conc worldwide (ng/L)	Median Conc in THIS STUDY (ng/L)	Median Conc in Africa (ng/L)	Median Conc worldwide (ng/L)
Amitriptyline	248	NA	<19[e]	11	NA	<19[e]
Atenolol	68869	39000[p]	859[a]	48	NA	39[a]
Carbamazepine	82196	<1[g], 735[h], 1240[o]	12000[a], 8050[b],4609[q]	88	NA	174[a], 752[q]
Cimetidine	95690	NA	1000[a]	560	NA	97[a]
Codeine	39381	NA	1000[a], 826[q]	153	NA	49[a], 21[q]
Diazepam	75031	NA	34[a]	42	NA	9[a]
Diltiazem	28	NA	146[a], 64[q]	2	NA	13[a],6[q]
Erythromycin	15110	11[g], 240[j], 1000[l]	90000[a], 5[i]	1	NA	51[a]
Fexofenadine	93448	NA	1144[f], 1287[q]	522	NA	253[f], 59[q]
Gabapentin	590	NA	7780[a]	5	NA	103[a]
Hydrocodone	559	NA	92[f]	1	NA	22[f]
Lidocaine	2779	NA	40[f]	4	NA	11.8[f]
Metformin	80967	NA	47[d]	1877	NA	NA
Noreistherone	63	NA	<19[e]	1	NA	<19[e]
Oseltamivir	48	NA	9[f]	5	NA	<19[e]
Paracetamol	111374	5500[o], 16000[p]	15700[a], 23000[b]	8525	NA	148[a]

Propranolol	13	NA	590[a]	2	NA	18[a]
Raloxifene	15	NA	7[f]	10	NA	NA
Ranitidine	3265	NA	570[a], 44[q]	2	NA	27[a]
Sitagliptin	36	NA	121[e]	5	NA	37[e]
Sulfamethoxazole	129475	3[g], 4090[k], 6010[j], 13800[m], 38900[k], 23350[n]	11920[a], 29000[b]	1482	NA	83[a]
Temazepam	89	NA	39[c]	4	NA	17[e]
Tramadol	2924	NA	8000[a], 1166[q]	8	NA	802[a], 218[q]
Triamterene	345	NA	NA	5	NA	NA
Trimethoprim	47025	160[k], 2650[m], 400[l], 6950[k], 9480[n]	4000[a], 13600[b]	91	NA	53[a]
Venlafaxine	45	NA	4[f], 548[q]	4	NA	<19[f], 97[q]

a = Hughes et al., 2013, b = Tim Aus Der Beek et al., 2016, c = Jerker et al., 2017, d = Niemuth et al., 2015, e = Burns et al., 2017, f = Burns et al., 2018
g = Inam et al., 2015 (Nigeria), h = Li et al., 2014, i = Kim et al., 2007, j = Matongo et al., 2015b, k = K'Oreje et al., 2016, l = Olarinmoye et al., 2016 (Nigeria), m = Ngumba et al., 2016, n = K'Oreje et al., 2012, o = K'Oreje et al., 2018, p = Agunbiade and Moodley, 2014, q = Loos et al., 2013
NA = not available

6.2 Sensitivity of G. pulex and A. aquaticus exposed to ERY, DIC and IBU

The differences in the sensitivity of macroinvertebrate to pharmaceuticals have been recognized, the available information is limited to very few species. However, fewer studies have investigated the sensitivity of *G. pulex* (water column dweller) and *A. aquaticus* (sediment dweller) to pharmaceuticals and their mixtures. Both species are common invertebrate biological models that plays an important role in aquatic food webs by serving as an intermediate between primary producers and higher trophic levels (Flaherty & Dodson, 2005). They are bio-indicators of the well-being of the aquatic environment. They may also serve as the representatives of the aquatic macroinvertebrates' compartments in river because *G, pulex* is a water column dweller and *A. aquaticus* is a bottom/sediment dweller and most macro-invertebrates lives within these two compartments (plates 1 and 2).

The current environmental risk assessment (ERA) regulations greatly rely on the *Daphnia magna* results to perform hazard assessment on chemical compounds, studies exploring the sensitivity of other macroinvertebrates (*G. pulex* and *A. aquaticus*) to pharmaceutical exposures and the underlying toxic mechanisms is lacking. Further information on the effects of pharmaceutical exposure at realistic environmental concentrations on both water column dwelling (*G. pulex*) (Chapter 4) and sediment-dwelling (*A. aquaticus*) (Chapter 5) aquatic macro-invertebrate animals will assist in prioritizing compounds that are considered to be of highest risk for future interventions. A four weeks exposure of *G. pulex* and *A. aquaticus* to environmentally realistic concentrations of erythromycin and diclofenac in isolation (Tables 6.2) caused a substantial significant decrease in growth and feeding of both test species compared to the controls.

Plate1. G. pulex (pollution sensitive)

Plate2. A. aquaticus (pollution tolerant)

The growth and feeding response shown by *G. pulex* and *A. aquaticus* to erythromycin and diclofenac are similar except for mortality. Hence, *G. pulex* and *A. aquaticus* showed similar sensitivity to growth and feeding activities when exposed to erythromycin and diclofenac at environmentally realistic concentrations. Feeding activities had implications for energy reserves and growth, hence, it was logical to investigate these as part of the physiological endpoints.

Mortality endpoint was also studied as sensitive parameter in an extreme case of pharmaceutical contamination. An Increased mortality was noticed especially for *G. pulex* while mortality was not significant for *A. aquaticus*. The exposed *G. pulex* caused more than 50 % mortality. Although, both *G. pulex* and *A. aquaticus* were affected by being exposed to single compound of erythromycin and diclofenac at environmentally realistic concentrations, the results indicated that *G. pulex*, are likely to be more affected by erythromycin and diclofenac exposure than *A. aquaticus.* Hence, increased mortality noticed for *G. pulex.* However, previous studies have demonstrated that *A. aquaticus* is more tolerance than *G. pulex* (Cleuvers, 2003; Ferrari et al., 2003).

The exposure of Ibuprofen to environmentally relevant concentrations of *G. pulex* and *A. aquaticus* for four weeks caused no effects on growth and feeding, mortality was not

increased when compared to the respective controls as a result of the exposures. Furthermore, the dose levels at which effects occur in aquatic invertebrates varies drastically. Kim et al., 2009 had earlier reported that Ibuprofen appears to be amongst the least toxic of the non-steroidal anti-inflammatory drugs. However, Boxall et al., (2014) report that ibuprofen was one of the most detected compounds in UK effluent at high enough concentrations to potentially affect ecosystem.

Table 6.2: Summary of effects of erythromycin, diclofenac and ibuprofen on *G. pulex* and *A. aquaticus*

Test compound	Exposure scenario (Environmental concentration (ng/L))			Response of *G. pulex*			Response of *A. aquaticus*		
	Low	Medium	High	Growth	Feeding	Mortality	Growth	Feeding	Mortality
Erythromycin	159.7	768.7	1377.8	$p<0.001$	$p<0.001$	$p<0.05$	$p<0.001$	$p<0.001$	$p = 0.59$
Diclofenac	202.2	1596.5	2990.7	$p<0.001$	$p<0.001$	$p<0.05$	$p<0.001$	$p<0.001$	$p = 0.75$
Ibuprofen	420.8	2629.6	4838.4	$p = 0.09$	$p = 0.08$	$p = 0.53$	$p = 0.24$	$p = 0.35$	$p = 0.61$

6.3 Sensitivity of G. pulex and A. aquaticus exposed to mixtures of ERY, DIC, IBU

When pharmaceutical risk is being assessed, it is imperative to consider the possible combination effects of drugs. The mixtures of erythromycin, diclofenac and ibuprofen caused an effect on growth and feeding activities of *G. pulex* and *A. aquaticus* compared to the controls, however, the effect observed is less when compared to the effects of single compound of erythromycin and diclofenac. There was no increase in mortality when the *G. pulex* and *A. aquaticus* were exposed to mixtures (Table 6.3). Hence, similar pattern was observed in both species when exposed to mixtures. The drug mixture influenced the moulting behaviour of *G. pulex* at low, medium and high concentration levels, leading to a discontinuous increase of body length, compared with the constant increase of body length in the control treatment.

Permanent exposure of *G. pulex* and *A. aquaticus* to a wider range of pharmaceuticals in natural aquatic systems may influence moulting behaviour (growth) and followed by severe ecological consequences as gammarids and isopods play important role in freshwater ecosystems. A sustained reduction in *G. pulex* and *A. aquaticus* feeding rates as a results of exposure to these drugs causes' growth inhibition and may have wider implication for ecosystems functioning, for instance mortality of *G. pulex* and *A. aquaticus* may ultimately impact on decomposition of leaves in the aquatic ecosystem and sub-lethal changes to metabolism, energy storage and feeding may also alter shredder feeding rates, food selection and ultimately decomposition rates and hence the food chain might be threatened.

Most importantly, in this thesis concentrations that have been readily detected in the surface waters of the UK and similar western developed nations (Canesi et al., 2007; Hughes et al., 2013; Santos et al., 2010) were shown to elicit significant lethal and sub-

lethal effects on both *G. pulex* and *A. aquaticus*. Furthermore, these effects concentrations have been exceeded by several orders of magnitude in studies examining polluted rivers in China, India, the United States (Larsson et al., 2007, Li et al., 2008; Onesios et al., 2009) and even Nigeria (Chapter 3) demonstrating the distinct likelihood that such complex and negative effects are occurring in freshwater ecosystems worldwide.

Table 6.3: Summary of effects of mixtures of erythromycin, diclofenac and ibuprofen on *G. pulex* and *A. aquaticus.*

Test compound	Response of *G. pulex*			Response of *A. aquaticus*		
	Growth	Feeding	Mortality	Growth	Feeding	Mortality
ERY + DIC + IBU	$p < 0.01$	$p < 0.01$	$p = 0.57$	$p < 0.01$	$p < 0.01$	$p = 0.56$

6.4 Standardized toxicity testing procedures

Pharmaceuticals are subject to very rigorous pharmacological, mammalian toxicology and clinical testing prior to approval for human use but Pascoe et al. (2003) highlighted a paucity of data examining their effects on aquatic fauna, particularly important freshwater taxa such as aquatic macro-invertebrates. Sanderson et al. (2003) estimate that ecotoxicological data is publicly available for just 1 % of approved human pharmaceuticals. The ecotoxicological assessment of these drugs has been mostly based on short-term experiments performed in agreement with standard tests protocols according to existing guidelines (e.g. OECD, ASTM, 1997; ISO, 1996), under the same laboratory conditions described for rearing procedures. Test organisms from different trophic levels such as algae, zooplankton, invertebrates and fish (Kim et al., 2009; Gonzalez-Pleiter et al., 2013). Acute toxic effects are generally manifested through

non-specific mechanisms of action (MOAs) usually, disrupting cellular membranes (known as narcosis) resulting in cell death (cytotoxicity) or oxidative stress that results in cellular damage (Fent, 2008).

Other tests include the effects on soil microorganisms, honeybees, sediment dwelling Chironomus and respiration of activated sludge; the guidelines for these are also available freely on the OECD website (OECD, 2012).

Data from these experiments are fitted to a dose-response model from which either the LC_{50} (median lethal concentration), EC_{50} (median effective concentration), lowest-observed-effect-concentration (LOEC) and no-observed-effect concentration (NOEC) can be calculated (Nakada et al., 2006). The majority of these tests are concerned with acute toxicity which will have an effect on the organism in question within 96 hours (Sprague, 1969) although some such as OECD Reports 202 and 204 (OECD, 1984; OECD, 2004) also look at prolonged toxicity for up to four weeks. However, even 14-day tests of fish toxicity do not cover a sufficient proportion of their life cycle to be classed as chronic studies (Schmitt et al., 2005).

6.4.1 Summary of short-term toxicity data

The acute toxicity of more than 150 individual pharmaceuticals to aquatic organisms has been studied in a number of reviews by Brauschetal (2012), Crane et al. (2006), Enick & Moore (2007), Fent et al. (2006), Fent (2008), Halling-Sørensen et al. (1998), Hughes et al. (2013), Santos et al. (2010) and Webb (2001). The organisms from the majority of the main trophic groups have been subjected to acute toxicity testing of at least a single pharmaceutical compound. However, only limited data are available for benthic macro -invertebrates, bivalves, amphibians and aquatic plants and algae. The review articles show that short-term toxicity can vary markedly for the same compound

across trophic levels and both within the same and between different species. The variability may be due to different actual exposure concentrations, different sensitivity of species and laboratory performances are among the reasons for variability within the same species. Analgesic is one of the most studied pharmaceuticals and were also identified as posing a significant global risk to invertebrates. About seven different trophic groups have been investigated and of all the trophic levels examined, phytoplanktons are the most sensitive to ibuprofen toxicity, exhibiting 72-120h EC_{50} values between 1 and 315 mgL^{-1}, depending on species (Pomati et al., 2004; Lawrence et al., 2005). The acute toxicity of acetaminophen has been well studied in invertebrates; EC_{50} values (immobilization) have ranged from 13 to 290 mgL^{-1} for 24h exposures and 50 – 92 mgL^{-1} for 48 h exposures (Kuhn et al., 1989; Coyne et al., 1994; Kim et al., 2007). The acute toxicity of *D. magna* has been studied for all analgesics, and this allows for comparison of toxicity among compounds. However, none has been done for *G. pulex* and *A. aquaticus*. Ranking analgesic compounds by acute toxicity from the most to least toxic shows the following distribution: dextropropoxyphene (opiod) > paracetamol (non-narcotic) > ibuprofen (NSAID) > naproxen sodium (NSAID)> diclofenac (NSAID) > salicyclic acid (NSAID). The LC_{50} (48h) of acetyl salicyclic acid varies between 168 mgL^{-1} and 1468 mgL^{-1} for *D. magna*. Depending on quantity and quality of data, ranges of acute toxicity values span one to two orders of magnitude; in some cases, the species differences may be quite large, spanning three to four orders of magnitude. Generally, invertebrates and phytoplankton were most sensitive to the acute toxicity of analgesics, whereas bacteria, fish, and amphibians were relatively insensitive.

The analgesic diclofenac has been investigated in studies in which the liver, gills and digestive tract, histopathology was examined in rainbow trout, each were found to be

highly sensitive endpoints (Schwaiger et al., 2004; Triebskorn et al., 2004). Table 6.4.1 shows reviews of acute toxicity data for diclofenac, ibuprofen and erythromycin at different trophic levels. Diclofenac and other NSAIDs, are known to cause alterations in renal physiology and function in mammals (Rosal et al., 2010; Gasperi et al., 2014; Hoeger et al., 2005; Schmitt et al., 2010). Perhaps the most well-known and highly publicized effect of diclofenac was in 2004, when a high death rate was reported among three species of vultures in India and Pakistan leading to severe population declines. This was reported to be caused by diclofenac (Oaks et al., 2004) and the high mortality is associated with renal failure and visceral gout in exposed vulture as well as the accumulation of uric acid throughout the body cavity following kidney malfunction. A direct correlation between residues of diclofenac and renal failure exists, both by experimental oral exposure and through feeding vulture diclofenac-treated livestock (Oaks et al., 2004). The drug has come into widespread use in these countries as a veterinary medicine, but it has also been widely used in human medicine since the 1970s. Despite this such compounds and species remain relatively poorly studied in terms of their environmental ecotoxicology.

For example, studies have shown that *A. aquaticus* is much more tolerant of diclofenac when compared to the *G. pulex* and macrophyte *Lemna minor* (Cleuvers, 2003; Ferrari et al., 2003). Investigations also show that acute toxicity varies with exposure time and toxicity endpoint and that final LC_{50} or EC_{50} values can vary due to differing susceptibility of sourced test organisms and actual exposure concentrations (when nominal concentrations are used) (Fent, 2008). Studies also show that generally very high concentrations (>1 to 10 mgL^{-1}) are required to elicit an acute toxic effect over short time scales. These concentrations are generally well above those encountered in the aquatic environment, often exceeding them by several orders of magnitude (3 to 7

(Fent et al., 2006)). Webb (2001) compared the acute toxicity responses in various trophic levels and found that the antidepressant, antibiotic and antipsychotic compounds were generally the most toxic and that algae were generally the most sensitive organisms. A similar comparison by Jones et al. (2002) identified the same high-risk compound classes. Hughes et al. (2013) compared the measured environmental concentrations of major classes of pharmaceuticals and their potential risk of chronic toxicity to fish, invertebrates, bacteria, algae and plants in freshwater ecosystems. At particular risk were invertebrates and fish due to exposure to relatively high concentrations of antibiotics and cardiovascular drugs (particularly in Asia); antidepressants and painkillers pose a similar risk in North America (Hughes et al., 2013). There are some notable exceptions to the rule of low acute toxicity. For example, the antidepressants fluoxetine and fluvoxamine demonstrate acute toxicity to green algae (EC_{50} = 31 000 ngL^{-1}) and Sphaerium clam species (4-hour LOEC = 3000 ngL^{-1}) at much lower concentrations (Webb, 2001; Brooks et al., 2003). Also, carbamazepine demonstrates acute lethality to zebrafish at 43 000 ngL^{-1} (Santos et al., 2010). However, the maximum measured environmental concentration of fluoxetine was 596 ngL^{-1} taken from estuarine samples in New York, USA (Balmer et al., 2005) and the global median concentration was 17.8 ngL^{-1} (Hughes et al., 2013). Fluvoxamine has only been studied twice in the environment with median and maximum concentrations of 0.7 and 4.6 ngL^{-1} respectively (Vasskog et al., 2008; Schultz et al., 2010).

These data demonstrate that for most compounds the margin of safety for acute toxicity is high although there are exceptions to this rule. This may be of particular concern in areas immediately adjacent to large or poorly performing STPs or pharmaceutical manufacturing facilities (Hughes et al., 2013). The widely held opinion that the risk of

acute toxicity is low (Fent, 2008) does therefore hold for most compound classes but more research may be necessary.

The EU Directive 93/67/EEC classified pharmaceuticals displaying an acute toxicity (LC_{50}) higher than 100 mgL-1 as not being harmful for aquatic organisms.

Often, acute toxicity is related to non-specific mode of actions and not to mechanisms involving specific targets. The compounds are thought to interact with cellular membranes leading to unspecific membrane toxicity (Camacho-Munoz et al., 2010). This general mechanism may be only one possibility; additional ones (e.g. oxidative stress) come into play with particular pharmaceuticals.

Another group of pharmaceuticals whose acute toxicity to aquatic organisms were mostly studied are the antibiotics. Antibiotics could be grouped into antiamebics, antibacterial, antimalarials, antiprotozoal, antiseptics, biocides, and retroviral. The antibacterial compounds are the most thoroughly studied of pharmaceutical compound groups from an environmental point of view. There have been 40 different antibiotic compounds, representing different antibiotic classes, studied to date. In general, no antibiotics class is appreciably more acutely toxic than any other class, although algae are the most sensitive trophic group to antibiotics.

In conclusion, present knowledge about residues of pharmaceuticals indicates that acute toxicity to aquatic organisms are unlikely to pose a risk for acute toxicity at measured environmental concentrations, as acute effects concentrations are 100 to 1000 times higher than residues found in the aquatic environment. Therefore, acute toxicity of an individual compound seems only relevant in case of spills.

Table 6.4.1: shows reviews of acute toxicity data for diclofenac, ibuprofen and erythromycin at different trophic levels

Pharmaceuticals	Species assayed	Lethal/Effective concentrations	Value (mg/L)	References
Diclofenac	Fish	EC_{50} ECOSAR	532	Sanderson et al., (2003)
	Daphnia	EC_{50} ECOSAR	5057	Sanderson et al., (2003)
	Algae	EC_{50} ECOSAR	2911	Sanderson et al., (2003)
	Daphnia	EC_{50} (48 - mortality)	22.4	Ferrari et al., (2004)
	Algae	EC_{50} (96 h - growth)	16.3	Ferrari et al., (2004)
	Bacteria	EC_{50} (30 minutes-luminescence)	11.4	Ferrari et al., (2004)
	Bacteria	EC_{50} (15 min-inhibition)	9.7	Ra et al., (2008)
	Microtox	EC_{50} (30 min)	11.45	Ferrari et al., (2003)
	Daphnia	EC_{50} (48)	22.43	Ferrari et al., (2003)
	C. dubia	EC_{50} (48)	22.7	Ferrari et al., (2003)
	Algae	EC_{50} (96h- growth)	14.5	Ferrari et al., (2004)
	Invertebrate	EC_{50}	90	Boillot (2008)
	Algae	EC_{50} -inhibition	72	Cleuvers (2004)
	Daphnia	EC_{50} - immobilisation	68	Cleuvers (2004)
Ibuprofen	Fish	EC_{50} ECOSAR	5	Sanderson et al., (2003)
	Daphnia	EC_{50} ECOSAR	38	Sanderson et al., (2003)
	Algae	EC_{50} ECOSAR	26	Sanderson et al., (2003)
	Bacteria	EC_{50} (15 min-inhibition)	37.5	Ra et al., (2008)
	Bacteria	EC_{50} (15 min)	12.1	Farre et al., (2001)
	Daphnia	EC_{50} (48 h)	9.06	Halling-Sorensen et al., (1998)

	Invertebrates	EC_{50} (96 h)	1.65	Quinn et al., (2008)
	Invertebrates	EC_{50}	100	Boillot (2008)
	Algae	EC_{50}	500	Boillot (2008)
	Fish	EC_{50}	110	Boillot (2008)
	Algae	EC_{50} - inhibition	342.2	Cleuvers (2004)
	Daphnia	EC_{50} - immobilisation	101.2	Cleuvers (2004)
Erythromycin	Fish	EC_{50} ECOSAR	61	Sanderson et al., (2003)
	Daphnia	EC_{50} ECOSAR	7.8	Sanderson et al., (2003)
	Algae	EC_{50} ECOSAR	**4.3**	Sanderson et al., (2003)
	Invertebrates	EC_{50}	15	Boillot (2008)
	Algae	EC_{50}	0.02	Boillot (2008)
	Fish	EC_{50}	900	Boillot (2008)

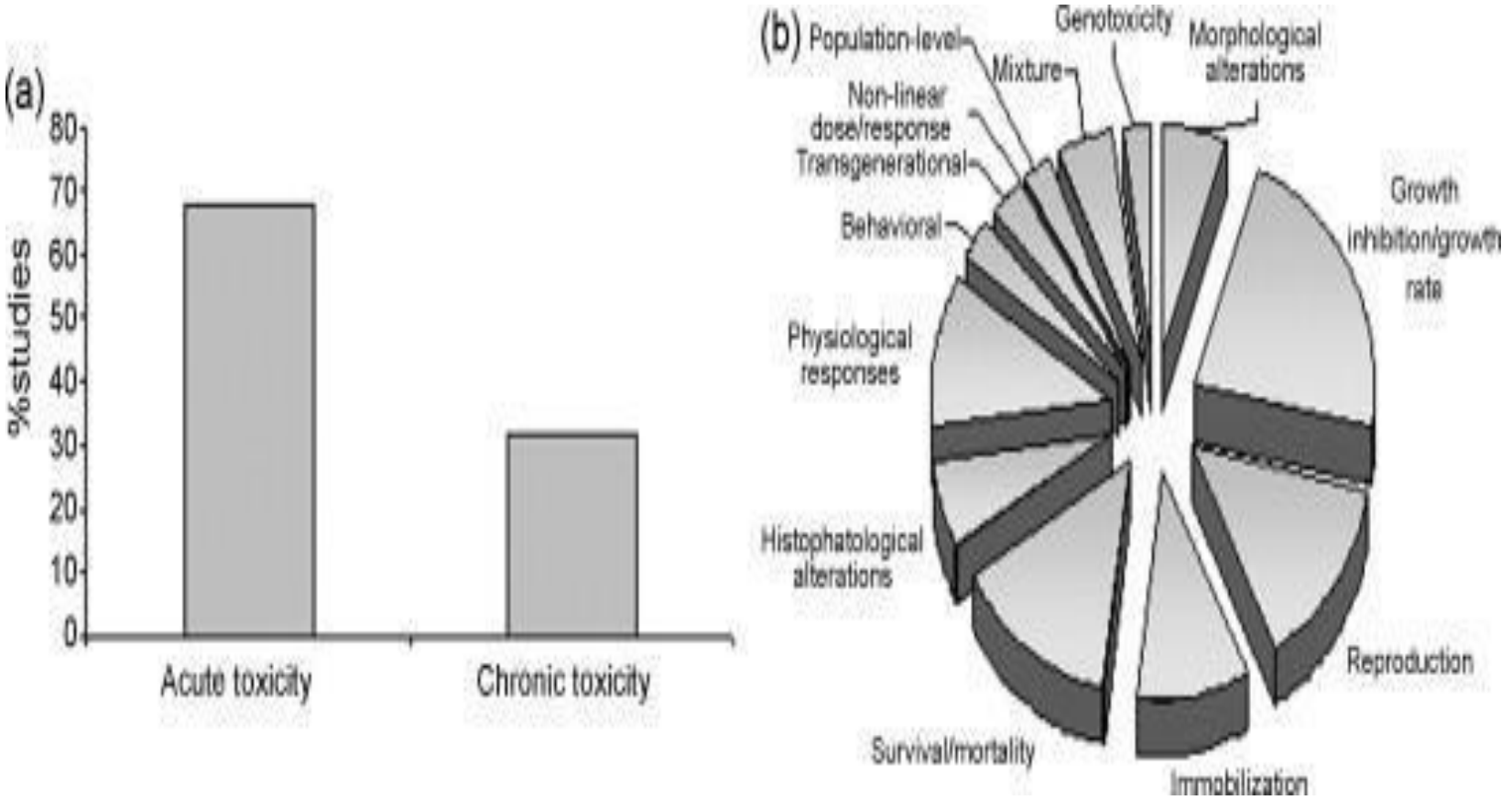

Figure 6.4.1: (a) Acute vs. chronic ecotoxicological studies (b) Principal endpoints used in ecotoxicological studies (relative %)**.**
Source: Hughes et al., 2013.

6.4.2 Summary of long-term and chronic toxicity data

Over the past few years the focus of ecotoxicological testing of chemicals on aquatic organisms has been to address the potential for long-term and chronic effects at environmentally realistic concentrations (Hughes *et al.*, 2013). Data has been collected for most trophic groups, including invertebrates, fish, amphibians, plants, and algae. However, limited data exist for benthic macroinvertebrates such as *G. pulex* and *A. aquaticus*. Furthermore, when compared with acute toxicity, there are relative shortage of chronic data (Figure 6.4.2) and this may be due to the increased costs of such investigations, logistics and analytical requirements of longer exposure times or the assessment of sub-lethal endpoints. A chronic toxicity study is defined by convention to be at least 10 % of the species' life span (Schmitt et al., 2005) but this is often not achieved, and much shorter tests are incorrectly classed as chronic (Nakada et al., 2006). More investigations into specific receptors, effects, life stages and life cycle studies are almost completely lacking for the vast majority of pharmaceuticals and some trophic levels of organisms (Fent *et al.*, 2006).

In ecotoxicology of pharmaceuticals, continuous chronic exposure is of particular concern as any negative effects may accumulate and manifest so slowly that they will be attributed to natural, ecological succession and by the time they have been identified the effects may be irreversible (Daughton & Ternes, 1999). Recent publications (Bringolf et al., 2010; Hughes et al., 2013) have shown that the majority of chronic toxicity values lie within the range of 10 μgL^{-1} to >10 mgL^{-1} which are generally at least 1 to 2 orders of magnitude greater than the concentrations measured in sewage effluent; this gap is greater when dilution in receiving waters is taken into account (Hughes *et al.*, 2013). In addition, there are some compounds which demonstrate chronic toxicity at or close to concentrations measured in the environment. For

example, ibuprofen has been shown to affect the polyp structure of *Hydra vulgaris* at 10 000 ngL^{-1} (Pascoe *et al.*, 2003) and has been detected in Turkish rivers at concentrations up to 31 323 ngL^{-1} (Loos *et al.*, 2009) with a global median of 517 ngL^{-1} (Hughes *et al.*, 2013). However, in this present study exposure of *G. pulex* and *A. Aquaticus* to environmental realistic concentrations of erythromycin., diclofenac and ibuprofen has significant effects on the test species except ibuprofen (Table 6.2). This demonstrates that the margin of safety between measured and toxic concentrations is much narrower for chronic toxicity.

Other pharmaceuticals such as acetyl salicyclic acid (NSAID) has also been shown to affect reproduction in *D. magna* and *D. longispina* at 1.8 mgL^{-1} (Marques et al., 2004). Diclofenac, commonly found in wastewater at about 0.81 μgL^{-1} (Ternes 1998b), reaches maximal levels in wastewater and surface water of up to 2 μgL^{-1} (Schwaiger et al., 2004). Chronic toxicity of diclofenac was reported in invertebrates (Ferrari et al., 2003; Ferrari et al., 2004). Histopathological effects were observed in rainbow trout; at the LOEC of 5 μgL^{-1} renal lesions (degeneration of tubular epithelia, interstitial nephritis) and alterations of the gills (Schwaiger et al., 2004) occurred. Subtle subcellular effects were observed even at 1 μgL^{-1} (Triebskorn et al., 2004). Impairment of renal and gill functions occurred after long-term exposure. In zebra fish embryos, delayed hatching occurred at 1 and 2 mgL^{-1} (Deschepper et al., 2002). Additional side effects of diclofenac have been observed in humans in the liver with degenerative and inflammatory alterations (Boyd et al., 2003). In the lower gastrointestinal tract and in the oesophagus (Bjorkman 1998), but not in fish. Brown trout showed a decrease of hematocrit levels at 0.5-50 μgL^{-1}, and signs of inflammation in gills and trunk kidney

were observed after twenty-one days at 50 μgL^{-1}, showing no dose response relationships (Hoeger et al., 2005).

Similarly, exposure to 10-100 ngL^{-1} of ibuprofen resulted in a significant decrease in behavioural activity of *G. pulex,* whereas at higher levels no significant difference was observed (De Lange et al., 2006). Environmental concentrations are in the range of 10^3 – 10^7 times lower than known LC_{50} or EC_{50} values.

Finally, current data on acute and chronic toxicity of pharmaceuticals support the conclusion that more target or bio-molecule oriented, or mode of action-based investigations, will allow more relevant insights into the effects on survival, growth, feeding and reproduction than traditional standard ecotoxicology testing.

CHAPTER SEVEN

Conclusions and Recommendations

Over the past twenty years or more there has been increasing interest in the occurrence and effects of pharmaceuticals in the aquatic environment primarily in Europe and North America. While their occurrence is now relatively well understood in these parts of the world to date there remains a scarcity of data from many African countries. Only three publications (Olaitan et al., 2014; Olarinmoye et al., 2016; Inam et al., 2015) reported the presence of drugs in the Nigeria aqueous environment despite being the hub for distribution of pharmaceuticals in West Africa and the largest consumer of pharmaceuticals in Africa because of its population (198 million) (NPC., 2018). The paucity of data on monitoring studies in Nigeria could be an imminent threat to the water resources of Nigeria because surface water serves as the main source of drinking water in many parts of the country (Aina and Adedipe., 1996).

This study has identified large number of pharmaceutical residues from various sources such as pharmaceutical manufacturing facilities, vacuum trucks, STP and urban waste collection area as sources of drugs into the freshwater habitat. Compounds such as paracetamol, sulfamethoxazole, fexofenadine, cimetidine, carbamazepine and metformin detected in this thesis are the highest ever reported anywhere in the world. The annual prescription and consumption of pharmaceuticals in Africa particularly Nigeria is unknown because of unregistered/undocumented pharmacies and a lack of proper record keeping. The over-the-counter availability of many drugs in Nigeria may have resulted in the higher levels of drug residues in the environment in addition to a lack of sewage treatment facilities, inefficient treatment methods where they do exist,

as well as the lack of regulations and political will to enforce environmental laws (Ogunbanwo, 2011)

The effects of exposure of freshwater macroinvertebrate animals (*G. pulex* and *A. aquaticus*) to erythromycin, diclofenac, ibuprofen and their mixtures at environmentally relevant concentrations illustrate that these drugs can impact non-target organisms. This implies that long-term exposure of organisms to pharmaceuticals in the environment may cause adverse effects such as reduced feeding and growth and may eventually cause mortality. Additionally, this research had shown the sensitivity of the test species to the study compounds and their potential for use in further ecotoxicological studies. The findings in this thesis provide significant information for the stakeholders- government, regulatory agencies, institutions in Nigeria and Africa to establish the minimum permissible limits of pharmaceuticals in wastewater and catalyse research on cost-effective pharmaceutical removal strategies in the Nigeria and Africa rivers.

7.1 Main findings based on this research are:

1. Pharmaceuticals are present in Nigeria rivers, and 27 out of 37 targeted analytes were detected; the highest number of compounds detected in Africa to date.

2. Pharmaceuticals such as sulfamethoxazole (antibiotic), paracetamol (analgesic) cimetidine (antacid), fexofenadine (antihistamine), carbamazepine (anticonvulsant) and metformin (antidiabetic) were detected at the highest concentrations ever reported anywhere in the world.

3. Seasonal variation existed between the wet and dry seasons, more drugs were detected in the wet season than the dry season. However, concentrations of detected compounds were higher in the dry season than in the wet season.

Seasonal usage alone may not be ascribed to this complexity, however, the diverse sources of pharmaceuticals into the environment such as vacuum trucks, urban waste sites, pharmaceutical manufacturing facilities and STP may play a part, unlike in developed economy where STP is the main source of drugs into the environment.

4. *Gammarus pulex* and *Asellus aquaticus* could be useful indicators for assessing the environmental risk of pharmaceuticals.

5. Growth discontinuation and feeding inhibition were observed in both *G. pulex* and *A. aquaticus* when exposed to erythromycin and diclofenac at environmentally relevant concentrations although ibuprofen did not cause these effects.

6. Growth and feeding were inhibited when *G. pulex* and *A. aquaticus* were exposed to mixtures of the compounds, but mortality was not observed. In the aquatic environment, chemicals do not exist in isolation but as a mixture of compounds. Perhaps studying the effects of chemicals in mixtures at environmentally realistic concentrations may be a true reflection of what happens in the aquatic environment.

7. Growth discontinuation, feeding inhibition and mortality can be extrapolated directly as toxicant-induced and varies with the concentrations of the toxicants and ability of the test animals to withstand stress.

7.2 Recommendations

The investigation described in this thesis has produced novel information on the occurrence of pharmaceuticals in African rivers, specifically Nigeria, and the effects of erythromycin, diclofenac, ibuprofen and their mixtures on the macroinvertebrate animals *G. pulex* and *A. aquaticus* using environmentally relevant concentrations. Highlighted below are a number of areas where future study should focus.

7.2.1 Specific recommendation resulting from this investigation

7.2.1.1 Pharmaceutical monitoring in Africa and particularly Nigeria: Although this research contributes significantly to data gaps in pharmaceutical monitoring in African and Nigerian aquatic systems, more rivers still need to be monitored in Nigeria and other parts of Africa. The few studies available in Africa are spatially clustered towards the big cities and the techniques employed during sampling are poor for measuring the high degree of spatial and temporal variability known with pharmaceutical contamination. Africa needs much support from Europe and North America in terms of the modern analytical instruments needed for sample analysis (LC-MS/MS). Virtually all of the publications on occurrence studies in Africa are based on samples analysed in Europe and America because of the lack of equipment in Africa.

7.2.1.2 Production of toxicity data for Africa and Nigeria: There are no environmental toxicity data for Africa and Nigeria on any of the pharmaceuticals found in the aquatic environment. The need for such data is paramount for effective monitoring and decision taken by the relevant agencies of government.

7.2.1.3 Use of OMICS Technologies: Although, this thesis had focused on growth, feeding and mortality as the endpoints in effect studies. In order to actually understand the hazards posed by pharmaceuticals in the aquatic environment. The use of metabolomes/metabolomics technologies to find the subtle effects of the pharmaceuticals in the tissues of the test animals to determine the metabolites affected/stressed when the test animals were exposed to the drugs should be part of the focus of future research.

7.2.1.4 Sensitivity of the test species should be considered: The OECD guidelines on toxicity recommend use of a small number of species which may not be representative of other organisms. Also, the sensitivity of organisms should be considered before deciding on test species; this will actually reveal the likely organisms to be most impacted by the pharmaceutical contaminants in water. Information/data on many macroinvertebrates e.g. *G. pulex* and *A. aquaticus* are still scanty in literature, and both are good indicators of water quality and should be used more regularly. The results for the single toxicity test (erythromycin, diclofenac and ibuprofen) on *G. pulex* and *A. aquaticus* indicate that the risk of some pharmaceuticals in the environment might be underestimated if hazards are not assessed by sensitive test species. It is therefore suggested that the toxicity data of pharmaceuticals should focus on other macroinvertebrates like *G. pulex* and *A. aquaticus* in order to produce more data.

7.2.1.5MultigenerationalStudies: Sub-chronic/Chronic effects of some pharmaceuticals may not be established at first and second generations especially when organisms are exposed to pharmaceuticals at environmentally relevant concentrations. However, at subsequent generations effects of such exposure of the parent organisms (1st and 2nd

generations) may be detected. This will assist the policymakers and environmental agencies to be able to really access the risk posed by the drugs in the environment. So future work should focus on multigenerational studies.

7.2.1.6 Use of environmentally relevant concentrations as exposure concentration: Should be encouraged more because this is the concentrations to which the aquatic organisms are exposed to throughout their life span. Anything short of this, is unrealistic and will amount to overestimation of the concentrations in the environment.

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