Sampling, Analysis and Fate Assessment of Pharmaceutical Pollutants in the Aquatic Environment

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"In today's highly interdependent world, individuals and nations can no longer resolve many of their problems by themselves. We need one another. We must therefore develop a sense of universal responsibility. It is our collective and individual responsibility to protect and nurture the global family, to support its weaker members, and to preserve and tend to the environment in which we all live."

The Dalai Lama

I hereby declare that this thesis has not been submitted, either in the same form or different form, to this or any other University for a degree.

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Nicola Broodbank

Abstract

Pharmaceutical drugs are widely used, yet their consequences and longevity in the environment following consumption are rarely discussed. Knowledge on the fate of these compounds within different matrices in the environment is inadequate and needs to be further improved in order to determine their concentrations and associated risks.

The determination and quantification of these compounds in water samples is already widely conducted using filtration, solid-phase extraction (SPE) and nitrogen blow-down followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). However, a method with a high degree of accuracy and reproducibility is yet to be obtained for sediment samples due to matrix complexity. Using strategically planned spot-sampling and the development of a new and optimized determination and quantification method, the analysis of sediments from the River Ouse (Sussex, UK) and River Medway (Kent, UK), allowed for the simultaneous quantification of nine target pharmaceutical compounds using ultrasonication followed by SPE, nitrogen blow-down and LC-MS/MS. Variables investigated were extraction method, solvent, sample mass and clean-up procedure; these allowed for the optimization of method development. Following these, spatial and temporal reports were concluded. Monitoring the River Medway between December 2009 and June 2010 showed stable yet high levels of pharmaceuticals in comparison to studies conducted globally. Concentrations increased the further through the year with June 2009 being the overall highest.

The method was further applied to biological matrices with excellent results. The determination of pharmaceutical compounds was extended to environmental samples from China as part of a collaboration project. It is expected that the method will be successfully used for future experimentation and analysis. Diclofenac and meclofenamic acid is overall the compound with the highest concentrations, compared to sulfamethaxazole and thioridazine which are the lowest.

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Any mistakes and inconsistencies that remain in this project are my own.

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List of Abbreviations (alphabetical)

BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
BYD	Baiyangdian (Lake)
Carb	Carbemazepine
CEC	Capillary electro-chromatography
CTC	Chlortetracycline
Diclo	Diclofenac
DOC	Dissolved organic carbon
DO	Dissolved oxygen
E1	Estrone
E2	17β-estradiol
EDC	Endocrine disrupting chemical
EE2	17α-ethynylestradiol
EM	Erythromycin
EM-H_2O	Erythromycin-H_2O
ERA	Environmental risk assessment
ESI	Electro-spray ionisation
EU	European Union
GC-MS	Gas chromatography mass spectrometry
HLB	Hydrophile-lipophile balance
HPLC	High performance liquid chromatography
Indo	Indomethacine
IS	Internal standard
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLE	Liquid-liquid extraction

LOD	Limit of detection
LOQ	Limit of quantification
MAE	Microwave assisted extraction
MAME	Microwave assisted molecular extraction
Meb	Mebeverine
Meclo	Meclofenamic Acid
MRM	Multiple reaction monitoring
MS	Mass spectrometry
NFLEX	Norfloxacin
NSAID	Non-steroidal anti-inflammatory drug
OFL	Ofloxacin
OMC	Organic Matter Content
OTC	Oxytetracycline
PFE	Pressurised fluid extraction
Phen	Phenacetin
PI	Positive ionization
PLE	Pressurized liquid extraction
POCIS	Polar organic chemical integrative sampler
POP	Polar organic pollutants
ppb	Parts per billion
PPCP	Pharmaceutical and personal care product
Pro	Propranolol
PTFE	Polytetrafluoroethylene (Teflon)
QA	Quality assurance
r^2	Regression coefficient
RQ	Risk quotient
RSD	Relative standard deviation

RXM	Roxithromycin
SD	Standard deviation
SDA	Sulfadiazine
SFE	Superficial fluid extraction
SMC	Soil Moisture Content
SPE	Solid phase extraction
SPI	Spiramycin
SPME	Solid phase micro extraction
STP	Sewage treatment plant
STW	Sewage treatment work
STZ	Sulfamethazine
Sulf	Sulfamethaxazole
Tamo	Tamoxifen
TC	Tetracycline
Thio	Thioridazine
TMCS	Trimethylchlorosilane
U	Ultrasonification
UPLC-MS	Ultra performance liquid chromatography mass spectrometry
WTW	Water treatment work
WWTP	Waste water treatment plant

Chapter 1 - Pharmaceuticals in the Aquatic Environment

1.1 Introduction

When attempting to understand the effects of pollution, it is critical to have an understanding of both the pathways through which contaminants enter the system, and their subsequent lifecycle. To do this accurate measurements of the presence of contaminants in the environment are needed. This work will focus on attempting to develop a novel method for measuring a group of pollutants known as Pharmaceutical and Personal Care Products (PPCPs), which have until now proven difficult to measure. Consequently, it is unsure how much is known about current contamination levels and the long-term impacts of this pollutant. Due to the large quantity of PPCPs in daily use and their unknown lifecycle, current contamination levels may be significant, and may potentially cause unforeseen and widespread impacts to future society.

For the purpose of this thesis, the term pollution will specifically refer to a group of selected PPCPs in aquatic ecosystems. PPCPs are continuously released into the environment at an unknown rate by anthropogenic activities. Although little is known about the long-term impacts of these compounds, evidence indicates that they may have serious impacts on exposed organisms, leading to sex change, organ failure and cancer (Daughton and Ternes, 1999). Data concerning accumulation rate, and lifetime of PPCPs in the environment is available, but rarely discussed. Consequently, further investigation into this area is needed to determine the potential long-term threat to human and ecological health.

Currently, no method exists to retrieve multiple PPCP compounds from sediment matrices with a high degree of accuracy. Simultaneous recovery of multiple PPCPs is needed due to the vast number of different compounds released in to the environment (>100,000 [(Giger et al., 2002)]), which makes non-simultaneous methods both time-intensive and costly (Zhang and Zhou, 2007, Zhou et al., 2009, Besse and Garric, 2008). Selecting particular compounds out of such a high number available, will give an insight into the wider significance through dependent sampling. Compounds will be selected based on their (assumed) environmental abundance, use and consumption, subsequent release into the environment, and coverage in recent literature and related investigations. The compounds selected will represent specific groups of compounds. It

will be assumed that the similar compounds out of the same class, will behave in a similar way and therefore logical assumptions can be made about wider importance. Such issues have made accurately gauging PPCP pollution levels in the environment difficult (Sanchez-Prado et al., 2010). A useful approach to overcome such issues may be through the use of trial and error procedures during method development to determine the accurate simultaneous recovery (Angus et al., 1998).

During method development, the possibility of low concentrations of PPCPs in samples must not be mistaken for poor recoveries. Variability in the results will primarily result from experimental methodology, not from the sample, and will therefore maximise accurate measuring capabilities. It is important to not mistake low recoveries for poor analytical methods (high variability), because detecting this correctly is not easy. Calculating precision and bias of results will help to identify these factors. To ensure that a poor recovery is not mistaken by a low concentration of target PPCPs in the solid matrix, a high initial concentration is key in limiting variance in results from sample variations. Methods will test a sample matrix where there is a predicted high initial concentration.

It is important to understand the entries and pathways of target PPCPs into the environment. These are discussed in more detail with supporting diagrams in the literature review. They indicate their introduction, pathways through and lifecycle of PPCPs in an ecosystem.

The compounds selected for testing will be carefully chosen from those which have previously come under investigation in the field, in order to maximise analysis and background information on the compounds, and provide a basis for comparison of recovery results. The choice of compounds and the number to be analysed simultaneously is stated in assessment of relevant literature.

A busy, heavily polluted and actively industrialised sampling location will be considered for analysis to meet the necessary criterion. For the purpose of this study a stretch of river water meeting those criteria has been chosen and which is also easily accessible and in constant use. The importance of using a ‘busy’ river or estuary is that it has a high boat and vessel concentration and thus high sewage outflow (which is a common source of PPCPs in the aquatic environments) should subsequently maximise the chances of attaining high PPCP concentrations in the sediment on the river bed; this

point is discussed in more detail during literature analysis. Another important consideration is that there are both Water Treatment Works (WTWs) and Sewage Treatment Works (STWs) along the banks of this river, and so an increased likelihood that chosen compounds will be present in the sampling area. This minimises the chance of low concentrations being mistaken by low recovery. This is of course secondary to the method development process, where a standard (compound) only solution will be tested for recovery. A high compound concentration is essential for testing the solid sample to minimise the likelihood of aforementioned conclusions and to also better identify a matrix effect.

The River Medway in Kent is a busy estuary catering for the South and South East of England, as well as being a tributary to the River Thames Estuary, which is highly industrialised and in constant use. This is the busiest River and Estuary in the United Kingdom, and meets all of the above criteria. It is important to remember that concentrations found on analysis may not be representative of other rivers locally. It will be used as an insight into concentrations in this stretch of water with the purpose of high initial concentration. This estuary also proves to be easily accessible and sampling certainly attainable without causing difficult or incurring unnecessary expenses due to exposed public perimeters and walkways. A method will be devised and optimised using established validation techniques, before being tested under controlled conditions. These methods will be applied to samples in the field to determine the quantification and deposition of target PPCPs in the environment.

After initial testing and method development, sampling will be conducted for method validation. The sediment samples will also be compared to water samples from concentrations in the same locations to assess the relationship between matrices, as the concentration of PPCPs in the water column may not relate to the sedimentation in a direct way.

A group of PPCPs will be selected for testing and method development application in this investigation which represent different therapeutic classes over a broad range of uses. They will possess different chemical attributes and most importantly, are heavily prescribed and consumed today. It is important that simultaneous quantification covers a broad compound range to maximise its potential relevance and application to other samples.

Following the development of suitable recovery methods, the analysis of other matrices including vegetation and biological samples will be analysed. This will not only give an indication of the dynamic behaviour and relationship of such compounds in this environment, especially those which have undergone long-term exposure to such contaminated environments as in Beausse, but also highlight if the method can be applied to different matrices (Beausse, 2004). This will also highlight if the compounds are present in these different matrices and in what level of concentrations. The possibility of success through a tried and tested method to measure and quantify contamination of such pollutants, their unknown environmental impacts are to become clearer. It is not definite that a successful method will be identified at this stage; only through laboratory experimentation will this become clear.

Following this introduction and identification of aims and objectives of this research, a relevant review of the literature will be given. This literature will be assessed and discussed in terms of critically assessing the methods already in place, limitations of existing methods, data already collected from relevant studies and other such topics such as PPCPs in the media and the law. Then the process of method development will begin, eliminating and drawing in analytical methods suitable for this simultaneous quantification of pharmaceutical compounds. Method validation and application will follow, leading to conclusions of the research and intentions for future work.

1.2 Aims and Objectives

1.2.1 Aim 1 – Pharmaceutical analysis and monitoring

This work will attempt to improve existing methods in novel ways currently used for the analysis of PPCPs and successfully apply this to sediment samples with good recoveries and high reproducibility. Current methods have either a low recovery and high variability, or high recovery and either high or unstated variability for the quantification of pharmaceuticals in sediment, giving inaccurate conclusions. Therefore an improved method is to be developed. Investigations will be conducted using microwave-assisted extraction (MAE), ultrasonication (U), solid phase extraction (SPE) and liquid chromatography tandem-mass spectrometry (LC-MS/MS) to simultaneously

determine the concentrations of nine selected pharmaceuticals from a range of sediment samples for environmental analysis and quantification.

This method will have an increased recovery and decreased variability of the selected pharmaceuticals in comparison to existing methods currently used in literature.

Once established, it will be used to determine the levels of pharmaceuticals in UK river sediment; the River Ouse, East Sussex. Several hypotheses will arise regarding the targeted chemical compounds, in particular which will be found in high and low concentrations and their effects on aquatic life. It is expected that carbamazepine (Carb), diclofenac (Diclo) and meclofenamic acid (Meclo) will be found in high concentrations throughout the samples due to the large amounts discharged into the aquatic environment and previous high quantification from studies. The PPCPs investigated also enter a river system through sewage treatment works (STWs), water treatment works (WTWs) and runoff from agricultural land.

1.2.1.1 Objective 1 – Extraction and clean-up method

To identify the best extraction and clean-up method of pharmaceutical compounds from the sample matrix. This will elucidate the solvent used during extraction which achieves the greatest recovery of the target compounds, along with sample size, extraction conditions and clean-up columns.

1.2.1.2 Objective 2 – LC-MS/MS method

To develop and refine a confident LC-MS/MS method for the simultaneous extraction, separation and quantitative analysis of a group of nine pharmaceutical compounds from river sediment samples.

1.2.1.3 Objective 3 – Environmental samples

To use the new method developed in Objective 1 and 2 to quantify the concentration of the target pharmaceuticals in the Rivers Ouse (Sussex, UK) and Medway (Kent, UK), and to determine their concentrations spatially across the sample sites identified. The new method will also be used to conduct a temporal and spatial variability report of pharmaceuticals from the River Medway, Kent, UK, and finally be applied to samples from China for analysis.

1.2.2 Aim 2 – Assessing the quality of analysis

The second aim of this thesis is to maximize the precision and reliability of the developed method in Aim 1, with a reduced variability and increased recovery. As with any analytical technique, data and sample analysis at any stage is open to a degree of variability. It is essential that this is minimized and so a set of experiments were designed to do so.

Considering the high number of variables used for the developed method, there is potentially a high degree of variability in results obtained from analysis. Quantitative results are not statistically significant unless they are accompanied by calculated errors associated with the data.

During this research a number of variables illustrating these changes were demonstrated and significant conclusions can be drawn from observations identified. With this aim complete, it can confidently be concluded that and data obtained as a result is to the highest degree of accuracy possible.

1.2.2.1 Objective 4 – Precision and bias of sampling

To identify the precision of sample recovery through triplicate sampling, including inter- and intra-day variability of the LC-MS/MS, and to show variability of bias on increasing number of replicates.

1.2.2.2 Objective 5 – Analysis of blank samples

To analyse the concentration of target compounds identified in blank samples and if this has any significant effect on concentration of target compounds in samples, and to identify background concentration of target compounds.

1.2.2.3 Objective 6 – Sample storage

Identify the best possible way to store samples to identify any significant difference.

1.2.2.4 Objective 7 – Matrix effect

To determine the matrix effect on sample analysis. Conclude the most suitable extraction method as a result.

1.2.3 Aim 3 – Applications of the developed method

Using the analytical method developed in Aim 1 to consider applications to a wider variety of sample matrices. Aside from simultaneously determining the quantification of PPCPs from sediment samples, it will be beneficial to investigate the application of this method to water samples and a variety of different sample matrices, both biological and vegetation. This will provide an insight into the suitability of the developed method to a wider range of environmental samples, without jeopardizing the recovery and reliability of the method.

1.2.3.1 Objective 8 – Biological samples

Obtain a variety of biological samples from the River Medway, UK, and determine recovery of pharmaceuticals following spiking experiments.

1.2.3.2 Objective 9 – Water samples

Analyse water samples from the River Medway, UK, for target pharmaceutical compounds and determine the successful application of the developed method used for sediment sample analysis.

1.2.3.3 Objective 10 – Geochemistry of contaminants

Determine pH, salinity (‰) and dissolved Oxygen (DO) content from water samples used in Objective 22 to identify a correlation between any of the nine selected pharmaceutical compound concentrations.

1.3 Summary

To summarise, this work will aim to;

- Develop an analytical method for the determination of a select group of PPCP compounds in river sediment. This method aims to have good high recoveries and low variability.
- Apply this tried and tested method to real environmental sediment samples.
- Use the same method to analyse water samples from the same location.
- Test this method on other environmental sample matrices; vegetation and biological.
- Test method variables such as sample storage, analysis of a blank and the matrix effect.

Chapter 2 - Literature Review

2.1 Introduction

Over the last twenty years interest in the environmental impact of pharmaceutical and personal care products (PPCPs) has greatly increased. This is apparent in an increased number of studies relating to such compounds. Publications have attempted to better understand their origin, fate and impact, but the need remains for sound and reliable method for the detection and quantification of PPCPs, with improved sensitivity, reproducibility and ruggedness. This literature review summarises the current understanding into the environmental impacts of PPCPs, but more importantly existing methods for quantification following detection, assessing their success and reliability. This will give insight into further development of a simultaneous and reliable method for this investigation of PPCP compounds in river sediments.

The compounds under investigation in this work are propanolol (Pro), sulfamethaxazole (Sulf), mebeverine (Meb), thioridazine (Thio), carbamezapine (Carb), tamoxifen (Tamo), indomethacine (Indo), diclofenac (Diclo), meclofenamic acid (Meclo). They were chosen because they are representative of PPCP classes (discussed later), and their documented abundance in the environment, rendering it relatively easy to collect accurate data with a new method.

2.2 Scientific Reviews in Literature

There have been countless attempts to consolidate and conclude research into PPCPs. However, popular topics discussed, scientific laboratory procedures, results and conclusions are so broad, that authors can only make those; reviews. Selected reviews in ascending date order follow, and their key conclusions are discussed.

There have been many reviews concerning the identification, source, fate and toxicity of PPCPs in the environment across recent decades. With each report there is an improved understanding of PPCPs in general, and in most cases, an increased environmental assessment which in turn increases a global understanding of, and builds on collected

data and information. There is also on-going improvement into methods relating to the detection and quantification of PPCPs. Later reviews still cover the same topics as earlier examples, highlighting widely unaddressed issues, not through lack of trials. This poses a big problem; that there is still much speculation as well as the aforementioned unknowns regarding PPCPs.

An older review by Halling-Sorensen *et al.* concentrate on pharmaceutical occurrence, fate and effects in the environment (Halling-Sorensen *et al.*, 1998). Although this publication is now a little dated, it deems no less important and still provides a good and interesting insight into the field of research in question as the issues remain unaddressed. At the time, pharmaceutical research was a newly popular and interesting focus for investigation. They highlight exposure routes into the ecosystem, which covers both aquatic and terrestrial environments (Figure 1). It gives insight into what was then (and still current) legislation of the topic as well as knowledge of the occurrence, fate and environmental effects. They also highlight the still important issue of determining the exact sources of such contaminants, as if the output from the source can be minimised, eventual concentration will be lowered. The report gives awareness into toxic effects of contaminants to micro-organisms, phytoplankton, plants, crustaceans and insects, but the information is not relevant as no compound data were reported.

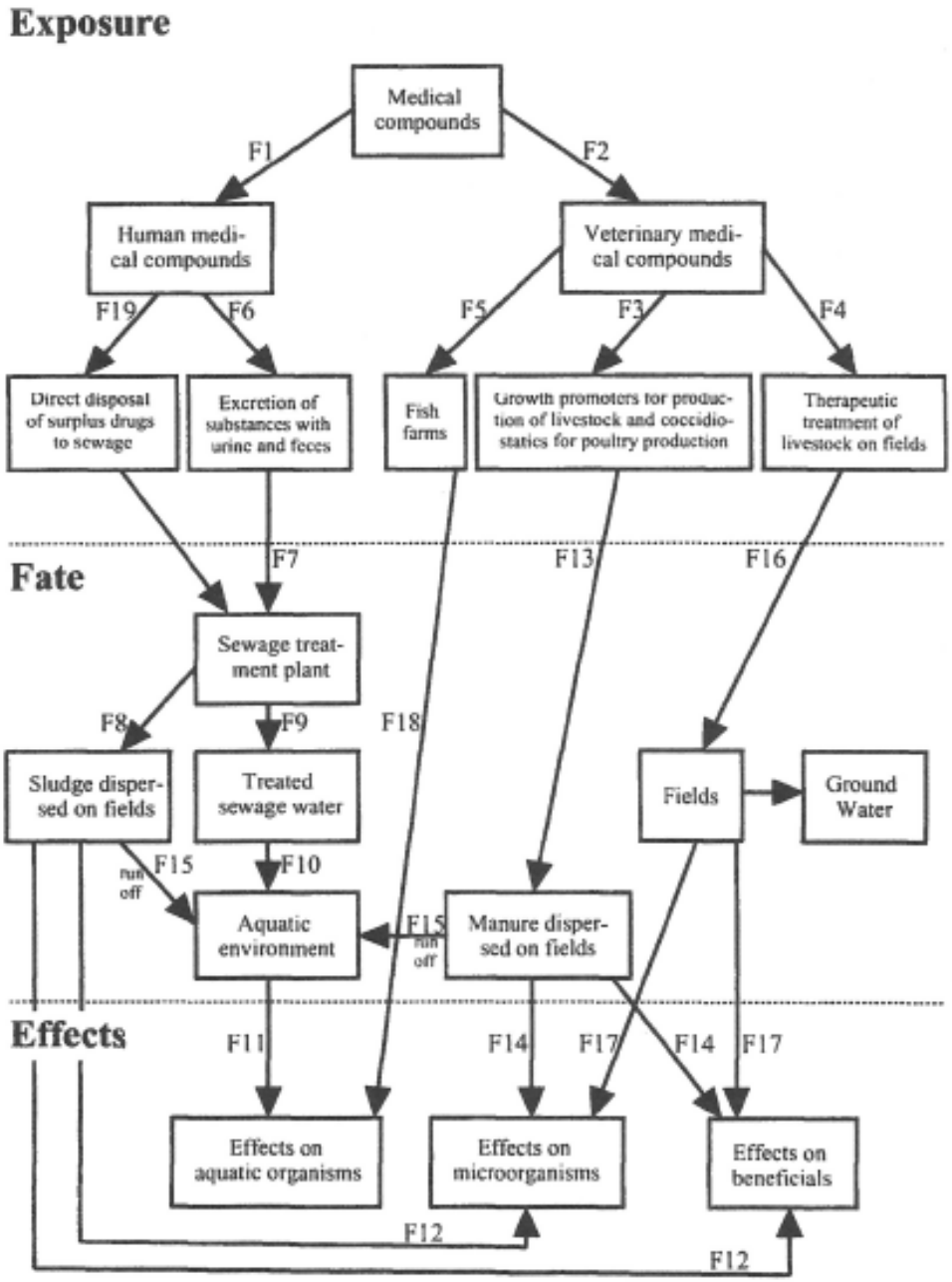

Figure 1 Anticipated exposure routes of both veterinary and human medicinal substances in the environment (Halling-Sorensen *et al.*, 1998).

Giger *et al.*highlight that over 100,000 different chemical compounds are recorded in the Europeon Union (EU) alone, with over a third released in quantities over one tonne, which is significantly high (Giger *et al.*, 2002). Pharmaceutical compounds are a major concern as they are presenting themselves as emerging, persistent contaminants. This is becoming an issue not only in scientific research, but also a public concern as reported by Donn *et al.* in The Associated Press news article;

"A recent poll indicated millions of consumers were 'very' concerned that their water supplies may be contaminated with trace amounts of pharmaceuticals" (Donn *et al.*, 2008).

This comes under scrutiny due to a lack of statistics to support the claims, as with most media attention in this research area attracts, discussed in more detail later.

Once again highlighting concerns of pharmaceutical residues in the aquatic environment, is a review by Heberer (Heberer, 2002). He calls upon papers which have recognised the importance of issues raised with concern to this environment, including Stan and Herber and Halling-Sorensen *et al.*(Stan and Heberer, 1997, Halling-Sorensen *et al.*, 1998) They discuss how unused medication being disposed of through the water and sewage system being of little importance in comparison to the wider and complex issue of excretion through the human body (through the consumption of medication) due to the compounds not being fully broken down and eliminated. Following excretion, Zweiner *et al.* also highlight how pharmaceutical substances are not completely eliminated during WWT due to inefficient techniques, and remain in the environment as they are not biodegradable (Zweiner. *et al.*, 2000). This leads to concerns regarding bioaccumulation. This and other negative knock on effects pose huge implications, discussed later. Heberer has his own diagram illustrating possible pathways and sources of pharmaceutical compounds into an aquatic environment (Figure 2). He also evaluates the occurrence of chosen PPCPs in different aquatic environments, concluding that compounds are not only widespread, but also abundant, which raises cause for further concern. In particular, those detected at trace level concentrations in drinking water pose a concern, as only a few are removed completely.

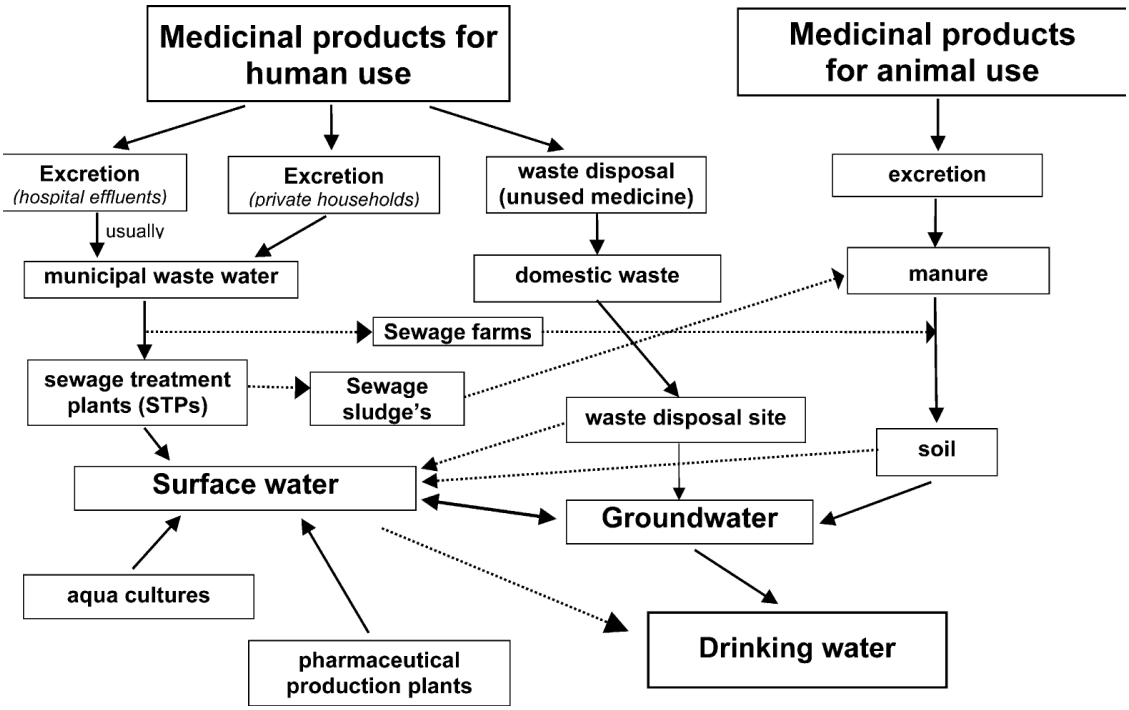

Figure 2 Scheme showing possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment (Heberer, 2002).

In 2004, Beausse published a review of target drugs in solid matrices regarding their determination, occurrence and properties (Beausse, 2004). Beausse immediately highlighted knowledge that a considerate quantity of an original substrate leaves the body untouched, and that recent developments have made it easier for compounds to be quantified to a higher degree, to parts per billion (ppb) concentration level. This gives a better insight into environmental concern due to increased accuracy. He also addressed the complex issue of how it is difficult to determine what happens in solid matrices, which is what the article focuses on. This is just one example of the documentation of the complexity of investigation a solid rather than an aqueous sample matrix, and the problems faced in doing so.

Turning focus to aqueous samples, Doerr-MacEwen and Haight gave a report on the effects of human PPCPs, as opposed to those used in agriculture or on animals for veterinary uses (Doerr-MacEwen and Haight, 2006). A figure by Doerr-MacEwen and

Haight show routes through which human pharmaceuticals reach water and groundwater (Figure 3). There still remains a high degree of uncertainty over the matter of the effects of human PPCPs, as Cleuvers discusses (Cleuvers, 2003). Focusing on human PPCPs draws on a reader's emotional connection to the subject of which importance is discussed later. They identify the potential threat to aquatic organisms as well as human health and how they are poorly understood. A different approach concerning expert stakeholders' adds another dimension to the complex issue; in total they consider 27 different opinions from the government, academia and pharmaceutical companies and give a range of assessments into PPCPs. Although those interviewed identified a concern for human health, they were even more concerned with the effect on organisms in the aquatic environment. This is possibly due to magnification through bioaccumulation through the food chain, but this is not stated. While the interviewees remain anonymous, it gives insight into a broad professional view on the issue. It was reported that the subjects were unanimous in believing a combination of strategies including improved water treatment strategies, education into the issue and a secondary wastewater treatment plant (WWTP) system, is the answer to reducing the impacts of PPCPs. However, this is not easily achievable, and interviewees may have had their opinions biased from the interviewer from question phrasing and direction, leaving them no room for their own opinions. These may have been given, but they are most certainly not portrayed in the review.

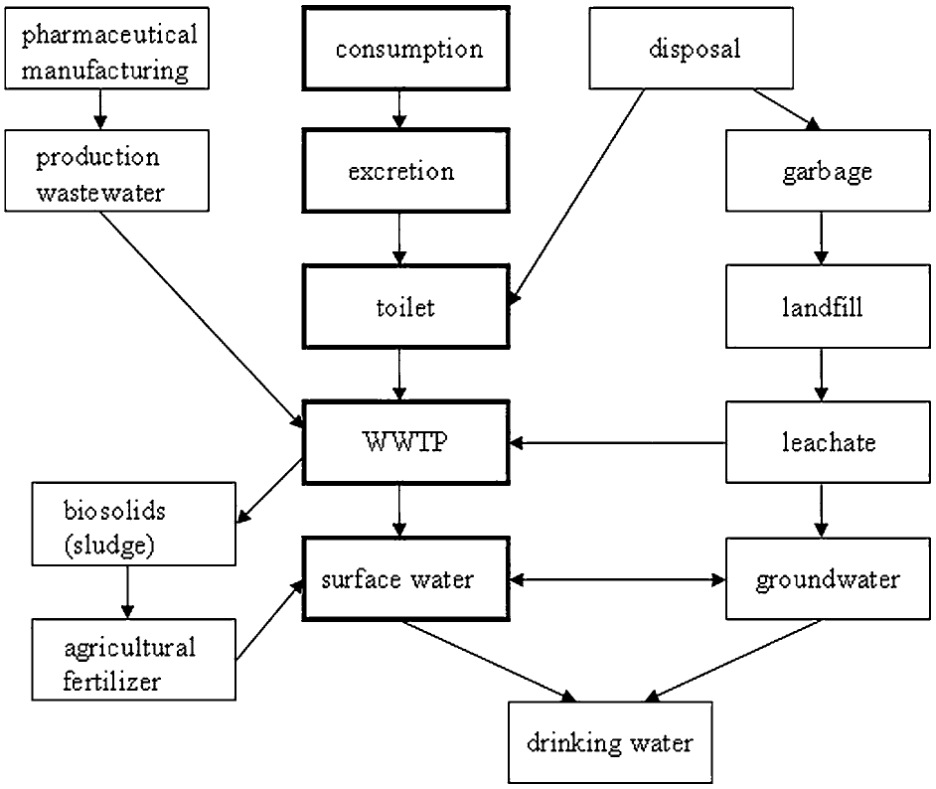

Figure 3 Some routes through which human pharmaceuticals enter surface water and groundwater. The main route of entry is through WWTPs after consumption and excretion (Doerr-MacEwen and Haight, 2006).

Although not proven to pose a threat to human health Cunningham *et al.* there have been many studies proving that pharmaceutical products contribute reports of high toxicity levels, as seen in Muñoz's findings (Cunningham *et al.*, 2009, Muñoz *et al.*, 2008). It is important to note the naivety of the general public to this growing issue, despite broad sweeping statements as mentioned previously. When asked in a study what they do with unused or expired medication, the answer was that they are disposed of in rubbish, or more concerning, washed down the sink or flushed down the toilet (Kotchen *et al.*, 2009). It seems a greater need for education and increased knowledge to the general public would be beneficial in reducing concentration of PPCPs in the environment.

A recent review by Caliman and Gavrilescu summarised PPCPs and endocrine disrupting chemicals[/agents] (EDCs) in the environment (Caliman and Gavrilescu,

2009b). Focusing on PPCPs for the sake of this research project, they interestingly report that many were unknown in terms of their impact on natural ecosystems. Many of these substances are yet to be tested for negative environmental properties, as Ingerslev *et al.* discuss (Ingerslev *et al.*, 2003). It is important to remember that these chemicals are synthetic in nature which makes them less biodegradable, and are used intensively in large volumes, further augmenting the problem. Their inability to be easily biodegraded, also raises concerns as this will lead to accumulation in any environment. This bio-accumulation is a separate issue and discussed later. Due to a lack of global regulation, it is difficult to determine widespread and long term impact of PPCPs.

2.3 Media Attention

It is already known that the fate of pharmaceuticals in the environment has attracted media attention. Ghosh wrote a short article on the negative impact of antibiotics (and PPCPs) on the environment (Ghosh, 2008). He supports Petrovic *et al.*'s argument that the fate of these antibiotic residues on entering the environment is widely unknown, and raises cause for concern (Petrovic *et al.*, 2005). In 2009, the U.S.News & World Report released a press article written by Donn concerning the release of pharmaceutical drugs into water in the United States, and claim the release of at least 271 million pounds (Donn, 2009). This of course is going to cause concern with the general public, but there is no indication of timescale of this statistic or its reliability, jeopardising the validity of the claim. Donn soon points out that consumers (the general public) are the biggest contributors to the contamination in question and so it lies in their hands to reduce this shocking figure. He talks of researchers and what they have found, but includes no direct reference links and so his claims go unsupported and untraced. There are no statistics or referenced material to support his writing and so is only assumed to be correct.

In February 2010, local newspaper Kent Messenger reported a serious environmental incident concerning the target sampling location under investigation. Alan Watkins (journalist), reported (Watkins, 2010). With this in mind, results around and after this time will pose particular interest;

"Medway faces a major environmental crisis after millions of gallons of sewage was discovered leaking for several days prior to discovery, due to a main sewage pipe rupture. The area is that of natural conservation, and as a result the Environment Agency will declare a Category One disaster. This is the second time this has happened, and evidence is currently being collected"

It can be concluded that the presence of PPCPs and related issues in the media increases public awareness, which in turn leads to an increased public concern and demand for a change in the industry, namely the decrease of PPCPs in the natural environment. The problem is, that this is a fashionable media trend, and there will soon be something else which takes its place and becomes popular with the public. At this time, PPCP attention will be abandoned, almost overnight.

2.4 Pharmaceuticals in the aquatic environment; environmental considerations

There has been a lot of investigation into substrates of this nature in aquatic environments. Reports globally include those from Spain, Italy, China, USA, UK, Canada and Germany, to name a few which implies the magnitude of the research topic in hand. Some of these reports are detailed below.

There are many ways in which PPCPs enter the natural environment, highlighted again by Caliman and Gavrilescu (2003), and in Figure 1, Figure 2 and Figure 3;

1. Direct and indirect effluents from wastewater treatment plants (WWTPs), hospitals and pharmaceutical manufacturing
2. Direct and indirect leakage from septic tanks or landfill sites
3. Direct and indirect surface water runoff
4. Direct discharge into waters.

Human pharmaceuticals are added to the environment (be it aquatic or terrestrial) through consumption followed by excretion (Farré *et al.*, 2008), often as different

forms; a mixture of, unchanged or conjured with other chemicals (Rang and Dale, 1991). These authors also state that between 30 to 90% of known prescribed doses to both humans and animals is excreted in urine, still as an active and unchanged substance. If this remains after WTW's, there is a big problem on our hands due to subsequent environmental effects. It is very common for such compounds to be used in farming, in particular fisheries, mainly to promote growth. Authors discuss how up to 80% of drugs used in such aquaculture end up in the environment, and are often also found in sediment accumulated beneath the farms. Other authors also found antibiotics in the sediment beneath fish farms, including Björklund *et al.* (H.V. Björklund *et al.*, 1991).

Highlighting the environmental risk of PPCPs in both aquatic and solid environments, Hernando *et al.* conclude that there is a high risk in particular to sewage treatment plant (STP) effluents for many identified target compounds, as well as in surface waters and sediments, whereas those not in question such as steroids, pose a low risk level (Hernando *et al.*, 2006). They report that further assessment combined with longer-term exposure is required for a more accurate assessment and better risk considerations.

Using Oasis HLB (Solid Phase Extraction) SPE cartridges and Methanol as the solvent, followed by GC-MS, Weigel *et al.* contributed a paper regarding the simultaneous determination of pharmaceuticals from water samples including Prop, Carb and Diclo (Weigel *et al.*, 2004). Seven different SPE sorbents were investigated, with a 70 to 100% recovery for some. Oasis HLB was chosen to continue the method development process further and on combination with GC-MS for analysis, gave good results of even trace amounts. In particular, Diclo was found to have a relative recovery rate of 87%, with a relative standard deviation (RSD) and regression coefficient (r^2) value of 1 during experimental stages. On the testing of Oasis HLB cartridges, Carb, Pro and Diclo had relative recovery rates of 101, 98 and 102% respectively, with very good variability (RSD) of 2, 4 and 2 (again, respectively). During the analysis of real surface water samples, 7 different locations were sampled, with concentrations between 26 and 67 ng/l. No indication of variability was noted with these results, so it is assumed that they

are as good as previously documented in laboratorial trials without real environmental samples.

The assessment of PPCPs in WWTPs is not a new approach when addressing the subject. When Lee *et al.* tested for pharmaceuticals in sewage effluent samples, they were first able to assess similar studies (Lee *et al.*, 2003). They focused on the methodology and stability of such methodologies (ie their variability and recovery rates). Employing the use of Oasis HLB SPE cartridges, 11 pharmaceuticals (including diclo and indo) were subject to recovery testing, which gave results over 80%, which supports other studies in the field aforementioned, after being analysed using GC-MS. They also tested the stability of such samples in storage conditions at 4°C for a week, and results concluded that over 75% of the known quantity in the samples was recovered. For practicality reasons, it is often important for a method to still be applicable even if samples have been stored for a period of time, as analysis cannot be carried out on site, amongst other reasons researchers commonly face such as machine downtime and batch sample processing.

A similar study by Gomez *et al.* also employed the use of Oasis HLB SPE, but followed sample preparation using LC-MS/MS for analysis (Gomez *et al.*, 2006). They were also testing water effluent samples, this time for 16 different pharmaceutical compounds including carb, indo, diclo and pro. Pharmaceutical recoveries of over 75% were recorded, and the matrix effect of these samples assessed. It is known that the LC-MS/MS signal can be supressed by the matrix of a sample (due to complexities and impurities of a matrix), and this is greater in a solid sample as opposed to liquid sample, but is still necessary to be considered, as Choi *et al.* demonstrates (Choi *et al.*, 2001).There are many reasons for signal suppression in this instance, which Choi *et al.* illustrate, along with Renew & Huang and Sorensen & Elbaek (Renew and Huang, 2004, Sorensen and Elbaek, 2005). They state;

1. Pharmaceuticals can sorb to organic matter making target compounds more difficult to detect as the concentration of free pharmaceuticals is lower. This is

why remobilization (traditionally using Microwave Assisted Extraction [MAE] or ultrasonification [U]) is important.

2. Other contaminants in the matrix may raise analyte peaks, leaving the real concentrations to be underestimated or mistaken as noise.

3. Contaminants in a solid matrix can reduce the efficiency of ionisation of analytes as they can inhibit charged sites on electrospray droplets.

Therefore, importantly, Gomez *et al.* test for signal suppression, with the majority of target compounds showing signal suppression (Gomez *et al.*, 2006). It becomes more apparent towards the end of the chromatographic separation, with suppression up to 91%. This was also noted by Hernando *et al.*, who have extensively investigated such matrix effects (Hernando *et al.*, 2004). Choi *et al.* have approached this and offer a solution to compensate for such effects through the use of internal standards in the sample solution (Choi *et al.*, 2001). They investigated the effect of LC separation on LC-MS/MS signal response for compounds in a complex (solid) matrix. It was noted that signal suppression was given. Comparisons to an internal standard-only sample, and relating this to the same level of standard spiked into samples gives a simple and effective compensatory calculation to determine loss on analysis through the matrix effect. Although timely, the process of identifying a suitable internal standard leads to benefits for final results and increases experimental accuracy and confidence in figures.

Gros *et al.* developed, optimised and validated an offline SPE filtration method combined with LC-MS/MS, for the detection of PPCPs in surface waters (Gros *et al.*, 2006). Recoveries of over 60% were achieved, with a variability of less than 15%. The method would be improved by increasing recovery and decreasing variability through further laboratory experimentation using trial and error. This is one of the best examples yet of a reliable recovery method. A justifying internal standard (IS) was used for the simultaneous determination of a very large number of PPCPs; 29. The internal standard is a known quantity of a selected compound spiked to the sample to test the percentage recovery obtained following experimental procedure. Ideally, the higher the recovery the better. The high number of compounds tested in this investigation makes the

recovery and variability even more impressive. These included Pro and Carb which were among the most frequently and highly detected compounds. Other compounds investigated which are of interest to this study are Diclo, Indo, Meclo, Sulf and Phenacetin (Phen). Their method proves to be robust and reproducible, which gives a high degree of reliability in a field where such methods have come under high criticism.

2.5 Methods for quantifying PPCPs

Many methods have already been aforementioned in the previous section when assessing PPCPs in water. After conducting research into the field, it appears there is one important factor in method development concerning the determination and quantification of such substances; simultaneousness. There are many reasons for this, as highlighted in research. A paper closely related to my investigations is that of Zhang and Zhou who investigated the simultaneous determination of pharmaceutical compounds in water (Zhang and Zhou, 2007). Method development often begins by selecting a good reagent, followed by a suitable extraction method. As previously mentioned, determination of compounds in water is common, as opposed to the complexities faced in method development of a more solid (soil) matrix. The early experimental stage of this method development began with testing 6 different reagents and 12 different solid-phase extraction methods, followed by LC-MS/MS analysis.

Zhang and Zhou concluded that the optimum method for determining simultaneous pharmaceutical compounds in water samples, was to use an Oasis HLB SPE cartridge using Methanol as the eluting solvent (Zhang and Zhou, 2007). They then used a LC-MS/MS method for the detection and quantification of target compounds. Their results broadly agreed with Liu *et al.* and Zhang *et al.* (Liu *et al.*, 2004, Zhang *et al.*, 2006). Their method proved successful with high recoveries; over 80% for most pharmaceutical compounds, of which 11 were tested. Results showed good recoveries and variability for almost all samples in both river water and sewage effluent, however none were relevant to this study. Their methods were interesting to note and bear in mind for further method development, especially variations around different methods of derivatisation (Figure 4).

With particular reference and interest to drinking water, Mompelat *et al.* consider the occurrence of PPCPs in this resource (Mompelat *et al.*, 2009). They comment on how popular documentation is for wastewater, but less common for drinking water and water resources even though drinking water has a more popular interest to the general public. Fent *et al.* are correct when stating that the quantity of PPCPs consumed and released into the environment is difficult to identify what is happening between the two; between human (or animal) intake and subsequent discharge into the environment (Fent *et al.*, 2006). To date, there is very little data available for a full assessment for pharmaceuticals in drinking water globally and so this review concluded little more than that there is simply 'some threat' of pharmaceuticals in drinking water and water resources. It still remains that there is no rapid or on-site identification and quantification of target pharmaceutical compounds. Young offers a solution regarding rapidity, although it is still a laboratory-based procedure (Young, 2009). He used Oasis hydrophile-lipophile balance (HLB) discs, as consistent with other authors, followed by ultra performance liquid chromatography mass spectrometry (UPLC-MS). The HLB system is particularly useful to identify surfactants for oil and water emulsification, and has been widely used in this type of investigation. The UPLC-MS is a highly sophisticated, state of the art analytical instrument which allows the selective and sensitive analysis of chemical compounds in a wide range of sample matrices. He reported a good success in his method, but did not go into detail of quantities found, recovery data or variability. This leads the readers to assume that the method has a high recovery and low variability, as one would assume that the method is successful and so both of these elements satisfactory. In fact, he proceeds further in the reader's assumptions, and notes significant advantages of the HLB disc format, again supporting his method, which includes that the disc rather than the cartridge can be used for the extraction of water samples containing a significant amount of particulate matter. This is particularly useful when analysing 'dirty' samples without the need for prior filtration.

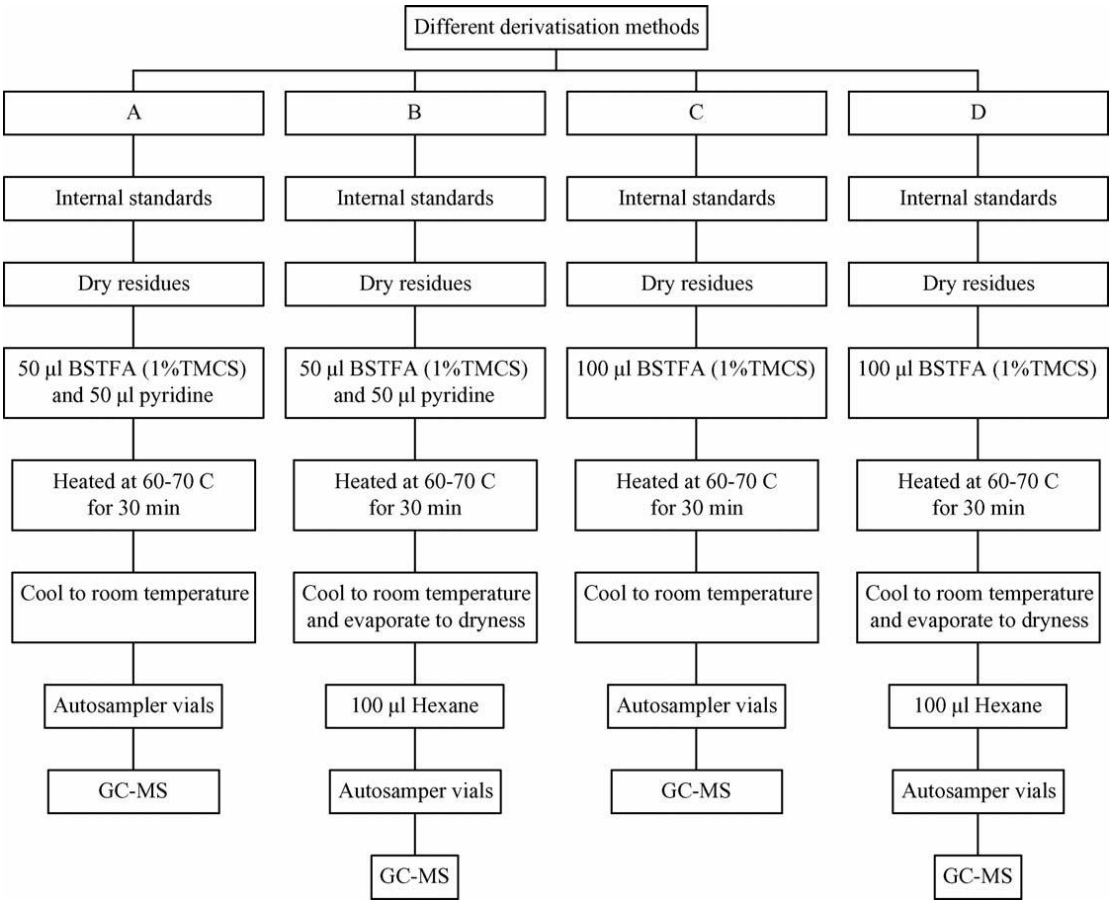

Figure 4 Schematic diagram of different derivatisation protocols (A – D) for Endocrine Disrupting Chemicals (EDC) analysis (Zhang et al., 2006).

Following the relatively simple, low-cost sample collection process, comes the more complex part of compound determination and quantification. A mass spectrometry piece of equipment is usually employed to determine compounds and then assess their quantity, namely GC-MS, or LC-MS/MS. These techniques however pose their own disadvantages;

1. Difficult to use- often require specialist training (incurring further costs)
2. Time consuming
3. Very expensive
4. Need specific procedures for complex samples
5. Require a large volume of the sample

Ternes *et al.* and Sacher *et al.* also adopt the use of LC-MS/MS and GC-MS (respectively) to detect pharmaceuticals in water samples (Ternes *et al.*, 1998a, Sacher *et al.*, 2001). Detection limits were down to 10ng/l in Ternes' investigations, even for organically highly contaminated waters like sewage treatment plant effluents. Recovery rates almost exceeded 70% for most of the compounds tested, however the determination of some compounds (including Carb) was disturbed by organic particulates in the real environmental samples; a seemingly common problem. Table 1 is a copy of the two compounds of the most interest from Ternes' 1998a study, where recoveries are both high. However the limit of detection (lowest possible detectable level) (LOD) is also high, possibly biasing results as lower concentrations could not be detected accurately. Results for Carb using LC-MS/MS rather than GC-MS prove to have a poor recovery, but a better LOD.

	Recovery (%)	RSD (%)	LOD in drinking water and rivers (ng/l)	LOD in STP effluents (ng/l)
Propranolol	91	11	5	25
Carbemazepine	99 (92)	8 (1)	20 (10)	100 (10)

Table 1 Mean recoveries (n=5) of compounds at 1µg/l and detection limits achieved by using GC-MS after using SPE. Results in brackets indicate results from LC-MS/MS detection (n=3) (Ternes et al., 1998a)

Sacher *et al.* analysed pharmaceuticals from groundwater in Germany, a seemingly popular sample location across many papers; it is unclear why this is, however it does not seem important, simply logistical. They analyse 60 different pharmaceutical compounds in aqueous samples. Their methods used SPE followed by GC-MS or HPLC mass spectrometry for analysis and separation. Pharmaceuticals under investigation which are relevant to this thesis are Diclo, Indo, Carb, Pro and Sulf. Table 2 highlights data for these compounds in question; all have a good *r*-value, as well as recovery in both tap and surface water, excluding Sulf for recovery values.

	r-value	Recovery from tap water (%)	Recovery from surface water (%)	LOD (ng/l)
Diclofenac	0.979	70	70	8.7
Indomethacine	0.990	86	114	5.4
Carbemazepine	0.796	80	74	9.6
Propranolol	0.993	84	48	4.6
Sulfamethaxozole	0.999	23	21	1.8

Table 2 Correlation coefficient, recovery in tap water and surface water (%) and limit of detection for relevant pharmaceuticals (Sacher et al., 2001).

The maximum concentration detected for Diclo, Carb and Sulf were 590, 900 and 410 ng/l respectively; all relatively high values. Interestingly, these three compounds were the only ones to be detected at least 3 times in all 105 groundwater samples with high values.

Ternes also identified concentrations above the µg/l level for Diclo and Carb, which are of particular interest in this investigation (Ternes, 1998b) (Table 3). In a paper investigating 32 compounds in German Sewage Treatment Plants (STPs) and rivers, he documented a high 6.2 µg/l for Carb in the sewage outflow, therefore leading to river contamination. Taken from his 1998b paper, Table 3 indicated a good low LOD, but low concentrations at the µg/l level. These data once again give no indication of variability, or at least not documented in the results published. Ternes also gives a diagram of the main fate of drugs in the environment, after their application. It is interesting to compare this to others in literature (Figure 5).

All conc.'s in µg/l	STP Effluents			Rivers and Streams		
	LOD	Median conc	Max conc	LOD	Median conc	Max conc
Diclofenac	0.05	0.81	2.10	0.01	0.15	1.20
Indomethacine	0.05	0.27	0.60	0.01	0.04	0.20
Meclofenamic Acid	0.05	0.00	0.00	0.01	0.00	0.00
Propralolol	0.25	0.17	0.23	0.01	0.02	0.59
Carbemazepine	0.05	2.10	3.70	0.03	0.25	1.10

Table 3 Concentrations of relevant compounds tested at both STP effluents, and rivers and streams (Ternes, 1998b).

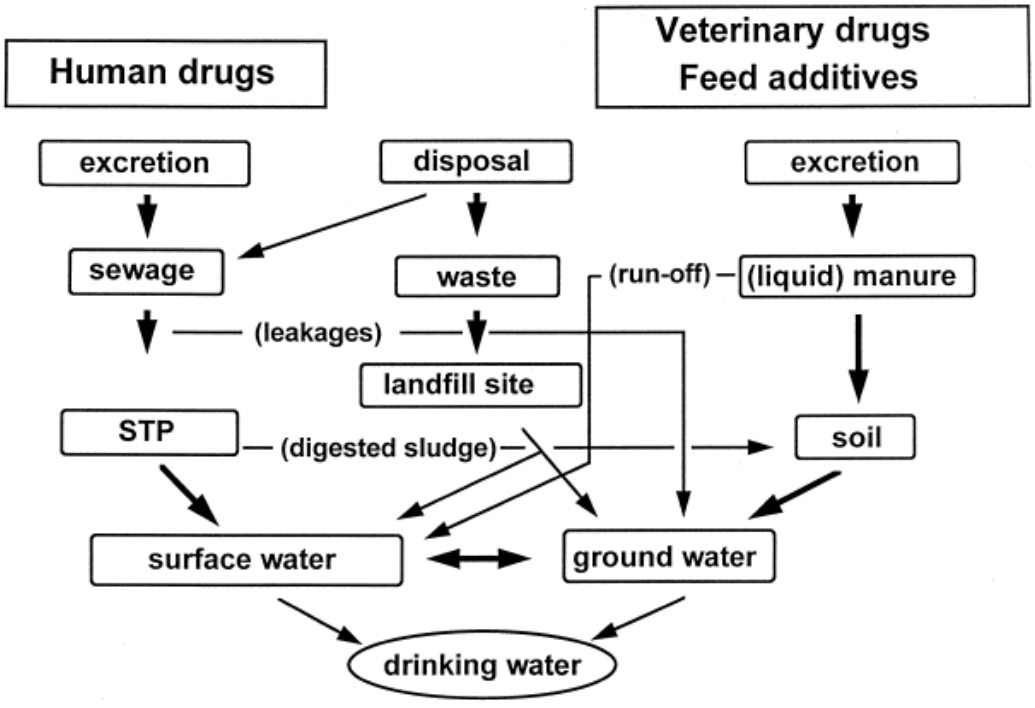

Figure 5 Scheme for the main fates of drugs in the environment after application (STP) (Ternes, 1998b).

Using a different approach entirely, Angus *et al.* developed a method for pharmaceutical analysis using capillary electro-chromatography (CEC), combining both capillary electrophoresis with HPLC technology (Angus *et al.*, 1998). Although having promising potential, this method seems short-lived in pharmaceutical testing due to lack of continued use of this method, assumed from no follow-up papers or investigations; there are very little papers using CEC in method development and subsequent continuation. Angus *et al.* used a previously validated HPLC analytical method, and so this provided a good basis for CEC investigative parameters. Their investigations at the time of publication were still on-going, although offering promising results. However, no recovery data were presented;.

Löffler and Ternes adopted the use of ultrasonication using acetone followed by ethyl acetate for the extraction of acidic pharmaceuticals from solid soil matrices, with a good limit of quantification (LOQ) of 0.4 ng/g (Löffler and Ternes, 2003). The mobile phase during LC-MS/MS analysis for this investigation comprises water/acetonitrile mixed with acetone. Three compounds of interest are highlighted from this paper; Diclo, Indo and Sulf. All showed very good recoveries between 65 and 116%, however recoveries were often high (Table 4).

	LOQ	SPE (1ug/l)		Sediment (20ng/g)		Sediment (3ng/g)	
	ng/g	Absolute Recovery (%)	Relative Recovery (%)	Absolute Recovery (%)	Relative Recovery (%)	Absolute Recovery (%)	Relative Recovery (%)
Diclofenac	8	116±25	115±25	81±18	57±12	125±85	92±48
Indomethacin	0.4	94±5	94±5	80±17	57±5	123±45	91±18
Sulfamethoxazole	20	94±7	108±6	65±8	99±22	75±41	113±93

Table 4 LOQ's, and recovery rates of three selected pharmaceuticals for SPE and sediment analysis at 2 spiking levels (Löffler and Ternes, 2003).

Prior to sample quantification, PPCPs are subjected to filtration and pre-concentration procedures due to the small level of which they are found in such samples. Methods often used are SPE or solid phase micro-extraction (SPME). In documenting LC-MS/MS methods for quantifying pharmaceutical compounds in solid matrices, Kim and Carlson identified SPE filtration as the most reliable process (Kim and Carlson, 2005).

These authors have reported on advantages of such procedures as;

1. Simple to use
2. Cost effective
3. Solvent free (less impact on the natural environment and lowers cost further)
4. Can be combined with GC-MS for a variety of compounds
5. Rapid and sensitive process.

However, this method is labour intensive when a large quantity of compounds need to be analysed, especially when trying to improve accuracy. Peck discusses further extraction methods including liquid-liquid extraction (LLE) and pressurized fluid extraction (PFE), however these are not discussed further. He simply gives the general recovery methods for the methods used in relation to the composition of the matrix ie river water or sludge (Peck, 2006).

Following the use of SPE followed by GC-MS, a third matrix has been considered by Scheytt *et al.*, in the determination of pharmaceutical compounds in sandy sediments. The porous nature of such a matrix offers its own complexities; does it initially retain compounds, and can the same methods as tested for aqueous and solid soil matrices be applied (Scheytt *et al.*, 2005). Of the three compounds tested, of particular interest in this investigation are Carb and Diclo. Recovers between 80 and 120% were presented for each respectively, and it was determined that sorption to a sandy sediment of these compounds is relatively low. Recoveries of over 100% were identified due to the samples being from real locations which may have contained the compounds in question prior to spiking with a known amount. If a compound is successfully sorbed into the matrix being tested, it makes it less available to surrounding environments ie in surrounding water. However, it then leaves the sediment environment (ie earthworms in

river sediment) more readily exposed to bioaccumulation. And to assess the concentration of compounds readily sorbed sediments proves difficult.

Sampling procedures in relation to sample collection are questionable and possess many faults. This is due to the high instability and remobilization of the compounds, which through traditional sampling procedures would disrupt the natural concentration of target compounds in question. The traditional method is through spot-sampling; using pre-cleaned bottles to take a one-off, on the spot sample of water which are then treated in the laboratory prior to investigation. However this has come under criticism due to its ineffectiveness to show concentration over time with daily fluctuations. A more recent approach is to use a passive sampling technique; Polar Organic Chemical Integrative Samplers (POCIS), developed by Alvarez, Petty and Huckins in 2002, when they also filed a U.S. patent (Alvarez et al., 2002). It is designed to sample water-soluble organic chemicals from aqueous environments, which can provide time-weighted average concentrations of chemicals over deployment periods ranging from weeks to months. As it is a passive sampler, it requires no moving parts or power supervision during use. The sorbent material are removed and set intervals, each giving an accumulative representation of the sampling period at the designated location. A photograph clearly shows the components in Figure 6. They offer a more representative alternative to traditional sample collection. When compared to traditional grab samples taken concurrently, the values identified using the POCIS were in good agreement (Alvarez, Petty, Huckins et al, 2004, Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments, Environmental Chemistry 23, 7, p 1640 – 1648).

Figure 6 Four POCIS are shown mounted in a stainless steel deployment canister. There is a white plastic outer protective canister which was removed for this photograph. (Alvarez et al., 2002)

Zhang and Zhou provide an analysis of the same 9 PPCP compounds in this work using water samples (sewage effluent and river water). They assess the difference between spot and passive sampling both in the laboratory and in the field. There was a good agreement between the pharmaceutical concentrations obtained using spot sampling and those from passive sampling, with the POCIS obtaining good results (Zhang et al., 2008).

Due to the complexity of chemical bonding and structure of pharmaceutical compounds present in solid (soil) matrices, it is essential to remobilize these as part of the experimental laboratory process, prior to sample filtration. Chen *et al.* (2008) identify the complexity of solid matrices, and agree that sample pre-treatment are required to extract and isolate target compounds. A method commonly used to remobilize and transfer said compounds from the sample matrix into the chosen solvent, is Microwave Assisted Extraction (MAE). The use of MAE for sample preparation was seen as early as the 70's however there was great progress in the late 80's when Gedye *et al.* describes the use of MAE for the rapid assessment of organic compounds (Gedye *et al.*,

1986). Even then they document how important time minimising is in such experiments, and also highlight the low running costs of the method. Although this technique has been around for decades, in 1994, Renoe highlighted the advantages of such technology in analytical chemistry applications, and its success in compound extraction from a solid matrix sample into the chosen liquid elute, ready for filtration (Renoe, 1994). Supported by Letellier and Budzinsky, the main advantages include (Budzinski *et al.*, 1999);

1. Reduction of required solvent
2. Low waste generation
3. Short extraction and preparation time
4. Small sample size required
5. Low cost and time implications

Recently, Sanchez-Prado *et al.* assessed the use of well established MAE to determine pollutants in a solid matrix, which gives a useful insight into methods already in place (Sanchez-Prado *et al.*, 2010). In comparison to other extraction techniques, Sanchez-Prado commented on the fact that due to a relatively low number of parameters, it is relatively straightforward to optimize MAE procedures. Other advantages of MAE extraction are that multiple samples can be extracted at one time, and so offers a good alternative to other methods available, and encompasses the importance of simultaneousness. Sanchez-Pedro *et al.* document the methods of different authors' with relation to simultaneous pharmaceutical extraction from a solid matrix. Of particular interest is Rice and Mitra who developed a method for 8 very diverse PPCPs in such matrices, who used MAE followed by GC-MS as their successful extraction process (Rice and Mitra, 2007). Although no specific compounds related to these investigations are relevant, the authors note that the "*concentration of PPCPs in natural solids remains largely unknown, due to a lack of methods permitting the simultaneous detection of diverse, low-level contaminants present in these complex matrices"*. Recoveries gave results as high as 89% for some compounds, but as low as 25% for others, highlighting the difficulties in simultaneously determining complex pharmaceuticals. Variability was up to almost 3% for some compounds, which is still a good result. The laboratory based recovery tests without a matrix present presented much higher and stable results compared to when target compounds were attempted to be detected in natural sediment samples. They report that matrix effects are the most

likely cause for low recoveries, which is an important factor to consider in further investigation.

Cueva-Mestanza *et al.* also targeted 8 diverse compounds (including propranolol and carbemazepine) using MAE (dried sediment mass of 2g), followed by SPE for clean-up and pre-concentration, before finally using HPLC for analysis in several types of solid sample matrices (Cueva-mestanza *et al.*, 2008a) (Table 5). Recoveries were above 70% and RSD under 11%, with detection limits between 4 and 167ng/g^{-1} obtained, however no 'real' contaminated samples were evaluated. Of the 8 compounds investigated, only two were relevant to this work; Carb and Pro. Their results from analytical testing are located in Table 5. These publications are important, as pharmaceuticals presents themselves as very difficult to extract and analyse from a solid matrix due to their complexity and chemical bonding. At present, there are few publications, some with low recovery rates, and so a further optimized method is needed.

	Recovery (%)	RSD	LOD (ng/g)	LOQ (ng/g)
Carbemazepine	78	10	15	48
Propranolol	78	11	19	62

Table 5 Analytical parameters of the proposed method result (n=6) (Cueva-mestanza *et al.*, 2008a)

Cueva-Mestanza *et al.* continued their experiments to different solid matrices, including molluscs (dried and homogenised 1g sample mass) (Cueva-mestanza *et al.*, 2008b). They tested for 6 compounds using the same method as before, and achieved recovery rates over 85%. The method showed satisfactory linear results and reproducibility of between 3 and 15%, as well as good detection limits between 30 and 220 ng/g, and applied successfully to the determination of PPCPs in mollusc samples. This gives a promising insight into method application to other solid matrices. The diagram below demonstrates the method used for the procedure to analyse mollusc samples (Figure 7); a useful insight for work later in this thesis. However out of the 6 compounds tested,

only one (Carb) is of particular interest here. At a spiking level of 0.8 and 3 µg/g (n=6), Carb produced recoveries of 98 and 90% respectively, and a RSD of 15 and 5% respectively. A LOD and LOQ were determined as 0.22 and 0.73% respectively. All indicating good stable results from the tried method.

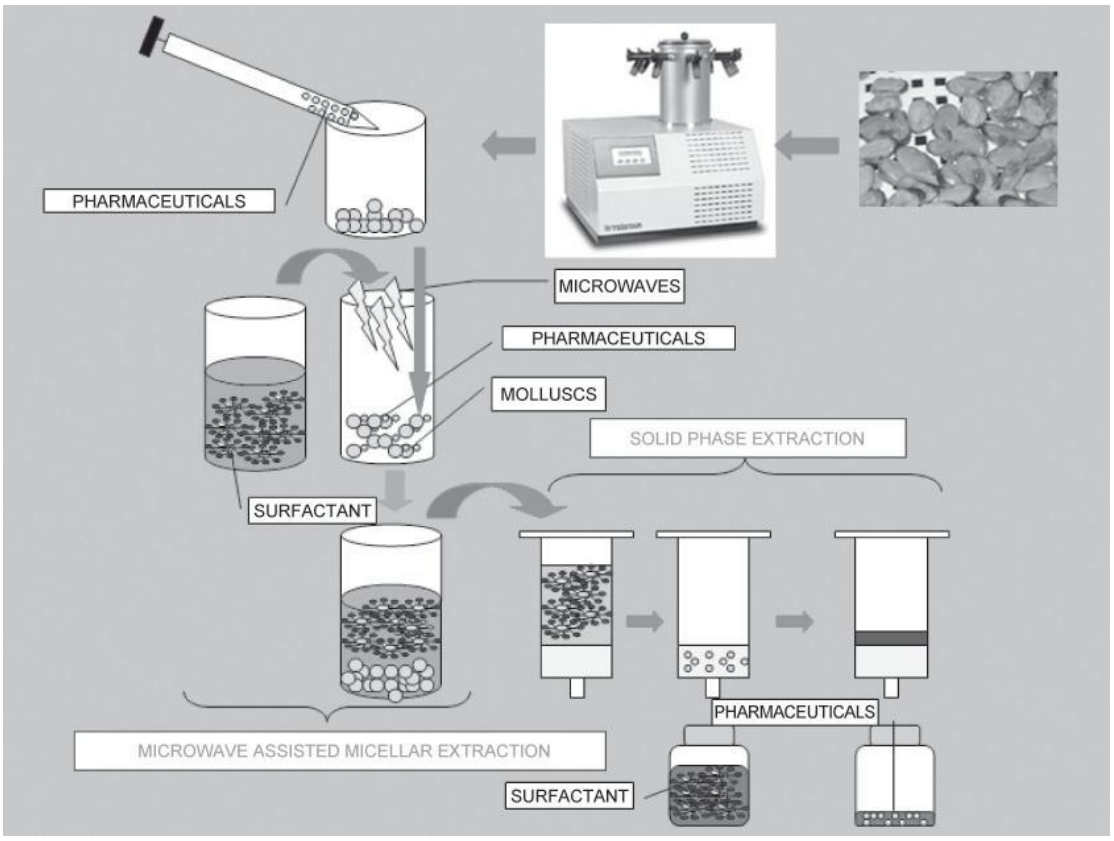

Figure 7 Scheme of Microwave Assisted Micellar Extraction (MAME) and SPE procedure in mollusc samples (Cueva-mestanza et al., 2008b)

Antonic & Heath tested a group of 4 pharmaceuticals (including Diclo), and after using MAE for the extraction process went on to use GC-MS for sample analysis (Antonic and Heath, 2007). The paper reported recoveries over 80%. Anatonic & Heath were using dried (crushed and sieved) 5g river sediment (a relatively large sample mass). High variability and room for error is normally expected from a sample of this size.

As previously mentioned, Liu *et al.* analysed river sediment samples for EDCs using GC-MS (Liu *et al.*, 2004). They used MAE as the extraction technique, with spiking

experiment recoveries over the 60% level, with good reproducibility. Although this method is for use on EDCs, it offers an interesting insight to method application and could potentially prove successful on pharmaceutical determination trials. Their preliminary results proved promising, however when applied to real environmental samples, proved less so, with recoveries regularly below the LOQ. However, some concentrations of up to 12 ng/g were detected at sewage outfall sampling locations on 2 instances. All environmental samples gave good standard deviations using a sample number of 3. Popular methods for PPCP analysis now encompass the use of MAE, SPE and MS technology, whether it be GC-MS, or LC-MS/MS.

2.6 Effects on the environment and human health

There are growing concerns over the eventual fate and consequences of named pollutants in the natural environment, and more recently, their effects on human health. Studies quantifying such compounds are vast in comparison to those determining the subsequent consequences to the natural environment or human health.

There is wide dispute over the effects of PPCPs to human health, however one thing is known; there is some cause for concern due to existing speculation and studies on fauna, but extent to this concern is unknown. PPCPs cover a wide variety of everyday household products, again highlighting the importance of their uncontrolled release into the environment; antibiotics, antioxidants, lipid regulators, psychiatric drugs, hormones, x-ray contrast media, antimicrobial compounds, insect repellents, and the list goes on (Caliman & Gavrilescu, 2003). Potential EDC and PPCPs are displayed by the authors in Figure 8. Of particular interest to this work are Tamo and Diclo which you probably at some point would have had in your home as they are common household prescription drugs.

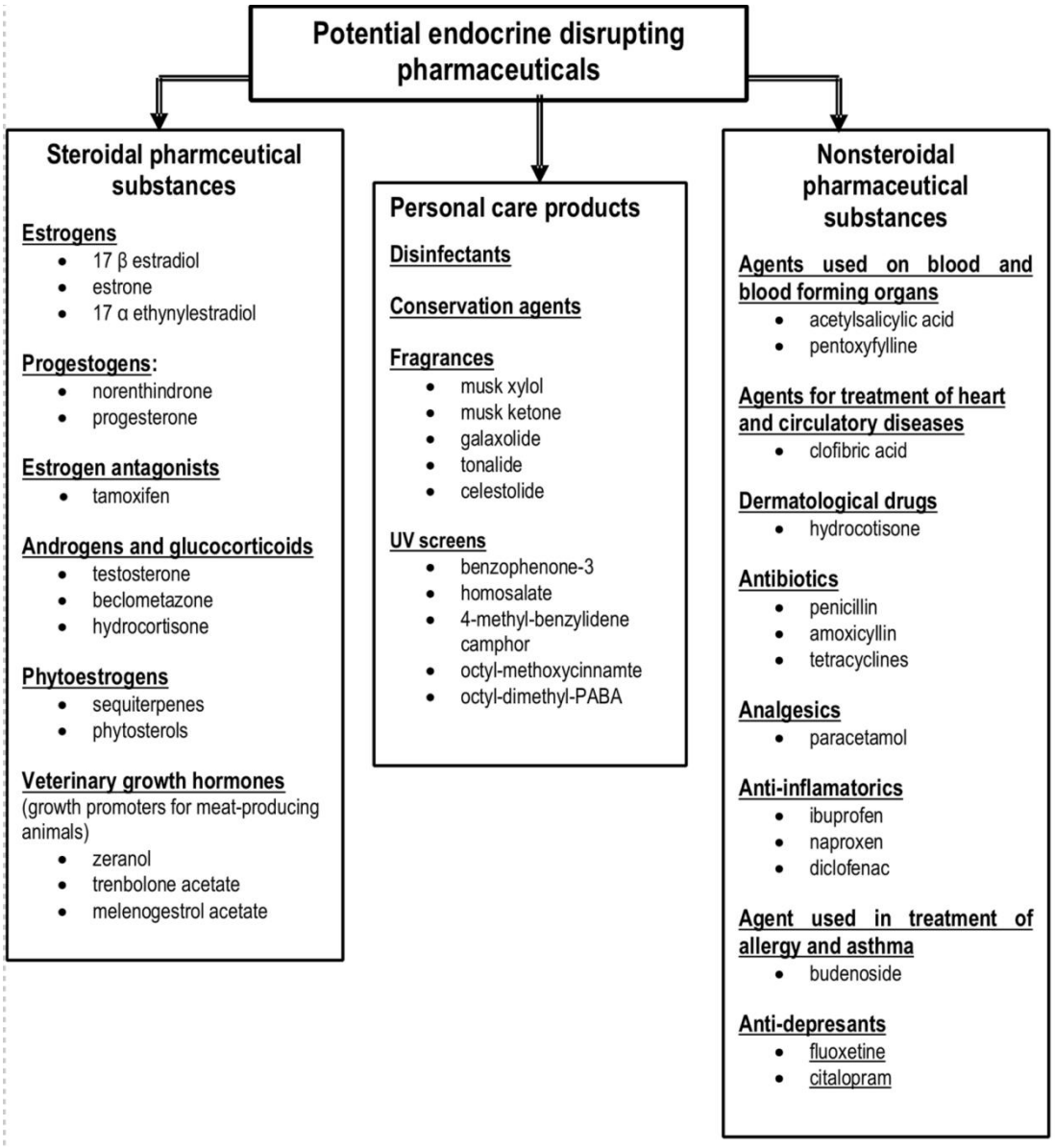

Figure 8 Scheme of the potential endocrine disruption PPCPs (Caliman and Gavrilescu, 2009a)

Although no specific pharmaceuticals to this work were discussed, Henschel *et al.* gave a strong report of common pharmaceutical compounds and their hazard on the natural environment (Henschel *et al.*, 1997). Their biological degradability and toxic effects to common algae were investigated, which are both important considerations relating to this topic. They concluded that there was no significant bioaccumulation of the four tested compounds in the algae, however there were significant effects following ecotoxicology tests, to cell structures. The extent of these concerns is however unclear.

A recent review by Crane *et al.* highlights aquatic risks of human pharmaceutical exposure (Crane *et al.*, 2006). They review current information in the field and assess the aquatic toxicology of human pharmaceuticals with relation to how it should be measured. For a long time such compounds have been detected in the environment, and more recently their associated potential risks have been of great concern, supported by Jorgensen and Halling-Sorensen when they discussed a wide variety of drugs in the environment (Jorgensen and Halling-Sorensen, 2000). There have been many water assessments globally where pharmaceutical compounds have been successfully quantified, as previously discussed. Crane *et al.* assess ecotoxicology from different sources in micro-organisms, algae, invertebrates and fish, and conclude that human pharmaceuticals pose an enormous potential threat to aquatic organisms. They highlight the need for a universal matrix method for identification and quantification, rather than different methods being used for different samples (ie algae, fish etc), as the variables between methods is too broad. This relates to the investigation preceding this review as after method development, I will go on to test application to different matrices.

There have been many other investigations into the harmful effects of pharmaceutical drugs to life forms in both aquatic and terrestrial ecosystems, and Stranchan *et al.* highlight the importance that during WWTPs, a significant proportion of the waste which includes pharmaceutical compounds, ends up in solid form, and eventually be transferred to biosoils (Stranchan *et al.*, 1983). Small terrestrial organisms within these environments can therefore act as good indicators to the wider significance of the issue and offer an insight into what could happen when infiltrated to the human body. A study focusing on earthworms found in pre-treated agricultural soil assessed the bioaccumulation of pharmaceuticals (Kinney *et al.*, 2008). Both the earthworms and the soil they were found in were assessed, and unexpectedly, over 20 compounds were identified in earthworms which were also in the soil samples. Interestingly, 28 of the detected compounds identified in the earthworms were below detectable levels in the corresponding soil samples, which highlights the bigger issue of bioaccumulation. There remains an unanswered question, of how this affects human and ecological health. Although earthworms are at the bottom of the food chain, they soon become prey and eventually work their way through the food chain until they reach us (humans), and so the problem is rapidly magnified through bioaccumulation. Further studies are

needed to assess the effects of bioaccumulation before human impact can be assessed. A globally important issue has been addressed by the aforementioned authors and their publications.

It is clear that at present, there is not enough strong statistical evaluation of the toxic effects of compounds in question to human health due to the lack of information from solid matrices, highlighting the importance of method development (Brooks *et al.*, 2003). This is critical because the effect on human health are of the upmost importance with relation to scientific investigation of this kind, and may result in a dramatic change in manufacture, disposal, prescription and use of PPCPs. However once again, evaluations in the aquatic environment are present, and studies indicate that effects at trace levels as found are rare.

2.7 The Law

Halling-Sorensen report on the legislation relating to drugs of this nature (Halling-Sorensen *et al.*, 1998). It states that;

"In Europe legislation was first initiated in the early 90's and it distinguishes medical substances into two groups; those which contain Genetically Modified Organisms (GMOs) and those which do not. These are then further divided into veterinary, and human medicinal products."

There is now an environmental dimension to the equation, which now means that the environmental impact is now also considered. It is still unclear why there is a separation between veterinary and human impact of such compounds, as in most cases they are considered equally as important (Irwin, 1994).

At present, pharmacies, doctors and others in the medical profession are largely unaware of the eventual environmental fate of the drugs (PPCPs), or at least they have no choice to act upon this knowledge if to the contrary, otherwise the situation would be

different. No limiting dispensary limitations are in place to minimise this problem. It will require an alteration in the law to enforce such a change.

2.8 Conclusions

In light of discussing the relevant literature, it can be concluded that PPCPs are currently concerning emerging pollutants in the natural environment. It is known that they pose a threat to the environment, be it aquatic or terrestrial, however the extent of this threat is largely unknown. Concerns to these environments in question raises greater concerns to the effects on human health; something which is rapidly changing.

Investigations regarding method development leading to detection and quantification of the target pharmaceuticals are unanimous in efforts to determine an effective (regarding both cost and time), sensitive method which yields good recoveries of multiple compounds. A further consideration is to whether this method is applicable to aqueous, solid, and biological samples with good, reliable recoveries and low variability.

The preferred techniques involve SPE for filtration, having prior chosen a suitable solvent for elution. It has been widely accepted that an effective method is now in place for water samples following literature discussed perviously, however methods for solid matrices are still ineffective and vary greatly between investigations, with the same method for water currently not applicable. A variety of MS techniques have been employed to effectively quantify compounds, including GC-MS, and LC-MS/MS, and conclude that for the nature of pharmaceuticals, LC-MS/MS is by far the most effective.

Chapter 3 - Development of an LC-MS/MS method for the extraction of Pharmaceutical compounds from sediment samples.

3.1 Introduction

Research into PPCPs as environmental pollutants has grown significantly as advances in analytical procedures have allowed their measurement, increasing our understanding of their occurrence and persistence (Maskaoui et al., 2007). Prior to 1990, the major focus of research was on so-called 'traditional' pollutants, such as POPs and heavy metals, and very little attention was given to PPCPs (Ellis, 2006). However, since PPCPs are used and discharged continuously, leading to pseudo-persistent occurrence and chronic exposure in the environment, they have become a focus of scientific research and attracted attention worldwide (Nikolaou et al., 2007). Currently there are over 4,000 pharmaceutically-active compounds consumed by humans and animals, of which approximately only 160 have already been studied to any extent in the natural environment (Mompelat 2004).

Knowledge of the environmental concentrations and behaviour of various pharmaceuticals is essential to understanding their fate and risk as they have been shown to have deleterious impacts on the environment. Currently, there is limited data available on their occurrence or toxicity. One study highlighted the harmful nature of exposure to a popular human and veterinary pharmaceutical, Diclo. It was found that vultures feeding on dead livestock that were treated with Diclo in Pakistan were suffering renal failure which resulted in significant decreases in the population. It was also observed that the reduction in the population of vultures caused an expansion in other species, including those which carry human-communicable disease such as rabies, thus posing a secondary threat to the local human population (Green et al., 2004). Another potential health risk is the levels of antibiotics in water due the increased occurrence of antibiotic resistant biofilm in hospital and municipal wastewater, as well as drinking water (Schwartz et al., 2003). To summarise, the concern about potential harm that these compounds may impose on humans and wildlife are based on their being biologically active by definition. Little is scientifically proven about the occurrence or health risks posed by each pharmaceutically-active compound and moreover, a mixture of pharmaceuticals may pose a greater potential health risk

compared to exposure to single compounds, since certain combinations of drugs can interact; however those effects have not been quantified or confirmed (Caliman and Gavrilescu, 2009, Jones et al., 2006).

Due to emerging evidence that pharmaceuticals can have negative effects on the environment, several methods have been proposed to measure their presence. The majority of studies have focused on analysis of water samples, leaving sediment analyses largely neglected (Nikolaou et al., 2007, Loffler et al., 2005). However, owing to the large range in the properties of pharmaceutically-active compounds, it is possible that sediment may act as a significant sink for some or all of these compounds. Sedimentary analyses may also prove more useful as they provide an understanding of the longer-term occurrence of the pharmaceuticals rather than the snapshot obtained with spot-samples of water (Antonic and Heath, 2007). Previously, methods such as U (Spongberg and Witter, 2008, Xu et al., 2008, Löffler and Ternes, 2003), soxhlet extraction (Buyuksonmez and Sekeroglu, 2005), PLE (Nieto et al., 2009, Jacobsen et al., 2004, Schlusener et al., 2003, Gobel et al., 2005), SFE, and MAE (Raich-Montiu et al., 2007, Rice and Mitra, 2007) have been used for the extraction of PPCPs from sediment (Antonic and Heath, 2007). In one study, all of these methods were compared using non-steroidal anti-inflammatory drugs (NSAIDs) and MAE was chosen to be the best method even though PLE and SFE produced higher efficiencies given that they are time-intensive and employ the use of large volumes of toxic solvents (Antonic and Heath, 2007). MAE has also been shown to be useful for endocrine disrupting chemicals (Liu et al., 2004), although to date, there are no published techniques for the simultaneous extraction of a range of pharmaceutical classes from sediment. Thus, a method is been developed here to measure nine pharmaceuticals covering three chemical classes using MAE, SPE, and LC-ESI-MS-MS in positive ionization (PI) modes. The compounds were selected so as to represent differences in pharmaceutical class, physicochemical properties, and occurrence in the environment (Liu et al., 2004). The technique was optimised by assessing different factors such as sample mass, extraction solvent, microwave temperature and subsequently validated with environmental sediment samples from the River Ouse, UK.

3.2 Experimental

3.2.1 Chemicals and Standards

All of the solvents used, including methanol, ethyl-acetate, acetonitrile and hexane (Rathburns, UK) were of distilled glass grade. Pro, Sulf, Meb, Thio, Carb, Tamo, Indo, Diclo and Meclo were purchased from Sigma, UK. Internal standards (diuron-d_6 and ^{13}C-phenacetin were purchased from Cambridge Isotope Laboratories, USA. Diuron-d_6 and ^{13}C-phenacetin were used following considerable existing literature reviews which used these standards for the same or similar groups of pharmaceuticals used in laboratory experimentation. Separate stock solutions (1000 mg/l) were prepared for individual compounds and internal standards by dissolving an appropriate amount of substance in methanol, then further diluted before using. From these standards, a mixture containing each compound (20 mg/l) was prepared weekly and used to spike the sediment samples. All standards and IS stock solutions were stored at -18 °C prior to use.

3.2.2 Microwave-Assisted Extraction (MAE)

Sediment samples from the River Ouse, East Sussex, UK were used for spiking experiments. First, sediment samples were extracted to determine the concentrations of target compounds in the matrix. Sediment samples were firstly frozen for 24 hours and then dried for 24 hours using a freeze-dry machine and were subsequently homogenized and sieved. Dry sediment samples (2 ± 0.2 g) were weighed into PTFE-lined extraction vessels and spiked with 200 ng each of the pharmaceutical standard. The samples were covered and allowed to stand for one hour in the MAE vessels at ~24 °C to allow sorption processes to occur before the solvent or solvent mixtures were added. Extraction solvents being studied included methanol, ethyl acetate, acetonitrile, hexane and ethyl acetate: hexane (1:1). Extractions were performed by a MARS-X laboratory microwave (CEM Corp., USA) at 30, 60, 90, 100, 110, 120 and 130 °C for 15 minutes (following 7 minute temperature ramp) with 100 % power or 600 W and 200 psi (1378 kPa) with 15 ml of solvent or solvent mixture (Liu et al., 2004). Following the extraction, samples were cooled to room temperature for 1 hour before they were opened to ensure the glass vessel would not break with temperature change and the sample lost. The supernatants were transferred to round-bottomed flasks (250 ml) and the sediments were washed with 3 x 15 ml rinses of the same solvent or solvent mixture.

The supernatants were combined, then evaporated to nearly 1ml by rotary evaporation. Each sample was then subjected to cleanup procedure, discussed in 1.2.4.

3.2.3 Ultrasonication Extraction of Sediment Samples

For comparison with MAE, dry sediment samples (2 g) from the River Ouse were spiked with 200 ng each of the pharmaceutical standard, allowed to stand for one hour to fully interact with sediment, and extracted using an ultrasonication bath (Decon Laboratories, UK). The samples were sonicated for 30 minutes three times using 15ml of methanol and were subsequently centrifuged at 2500 rpm for 5 minutes. Due to the absence of pressure as with the MAE, a run time of 30 minutes was used to ensure fairness when compared with MAR. The supernatants were collected in 250 ml round-bottomed flasks and rotary evaporated to about 1 ml. Each sample was then subjected to cleanup procedure (see 1.2.4).

3.2.4 Clean-Up Procedure

Due to the complexity of sediment matrix, it was necessary to develop a cleanup procedure to remove interfering substances from sediment extracts in order to improve compound separation in HPLC. A variety of approaches were tested including silica, alumina and solid-phase extraction columns. The different procedures were tested and are detailed below.

3.2.4.1 Preparation of silica gel and alumina oxide column and cleanup of extracts

The silica gel and alumina oxide were heated overnight in an oven at 500 °C (>18 hrs) and cooled to room temperature. Glass wool (pre-ashed at 500 °C for 3 hours) was inserted into the bottom of 5 ml graduated pipette to which the solid phase (silica, alumina, 50:50 silica alumina, 25:75 silica:alumina, 75:25 silica:alumina) was added to the 5 ml mark. Another piece of glass wool was inserted in the top of the column and it was conditioned with the extraction solvent of choice. Once the columns were ready, the sediment extracts were quantitatively transferred to the columns using a Pasteur pipette. The columns were then eluted with 20 ml of a solvent or solvent mixture, with the elutions finally blown down to 0.1 ml under a gentle N_2 flow.

3.2.4.2 Solid-phase extraction

For comparison with alumina and silica columns, SPE cartridges were also used as a cleanup procedure following previous work by Zhang and Zhou (Zhang and Zhou, 2007). Briefly, SPE cartridges from Waters (HLB Oasis) and Supelco (DSC-18) were pre-conditioned with methanol (2 x 5 ml) and subsequently ultrapure water (2 x 5 ml). The sediment extracts (1 ml) were transferred to amber glass bottles (1 l), rinsed with methanol (3 x 5 ml), and diluted with 500 ml of ultrapure water. Two 5ml methanol and ultrapure rinses were used due to previous successful trials of this method by Zhou et al. (2009). This is also the case for the dilution with 500ml of ultrapure water. The diluted sediment/water solutions were subsequently extracted by SPE. Once completed, the SPE cartridges were dried under vacuum for 30 minutes, from which the target compounds were eluted by methanol (15 ml) before LC-MS/MS. The solvent extracts were reduced to approximately 0.1 ml under a gentle N_2 flow. (Zhou et al., 2009).

3.2.4.3 Microfugation

The sediment extracts (1 ml) were pipette into 0.2 µm VectaSpin Micro centrifuge filters and spun at 8000 rpm in a Beckman Microfuge 1l until all of the solvent had passed through the filter.

3.2.5 LC-MS/MS analysis

Quantitative analysis of sediment extracts by LC-MS/MS was completed following a previously developed method using a Waters 2695 HPLC separation module (Milford, MA, USA) and a Waters Symmetry C18 column (4.6 mm x 75 mm, particle size 3.5 µm) (Zhang and Zhou, 2007). All samples were spiked with 200 ng of internal standards prior to analysis. The mobile phase was composed of eluent A (0.1 % formic acid in ultrapure water), eluent B (acetonitrile) and eluent C (methanol) and flowed at a rate of 0.2 ml/min. The sample injection volume was 10 µl.

A Micromass Quattro triple-quadruple mass spectrometer was employed for tandem MS analysis using a Z-spray electrospray interface. All samples were analysed in positive-ionisation mode. The temperatures for the electrospray source block and the desolvation were 100 and 300 ˚C, respectively. Nitrogen flowed at a rate of 25 and 550 l/h to function as a nebulising and desolvation gas, respectively. The mass spectrometer

operated in multiple reaction monitoring (MRM) mode in order to further validate the presence of the analytes.

An example of a chromatogram produced by the LC-MS/MS to then analyse results is displayed in Figure 9.

Compound	Therapeutic class	Ionisation mode	Retention time (mins)	Molecular mass	Collision energy (eV)	Precursor ion (*m/z*)	Product ion (*m/z*)
Pro	Anti-hypertensive	ES+	9.95	259	20	260	116(100), 183(56)
Sulf	Antibiotic	ES+	11.2	253	15	254	92(100), 108(65), 156(16)
Meb	Gastrointestinal	ES+	12.2	429	25	430	101(100), 135(3)
Thio	Anti-depressant	ES+	14.3	370	25	371	126(100), 98(75)
Carb	Anti-epileptic	ES+	14.8	236	20	237	194 (100)
Tamo	Anti-cancer	ES+	16.8	371	25	372	72(100), 208(1)
Indo	Analgesic/Antipyretic	ES+	22	357	25	358	139(100), 141(20)
Diclo	Anti-	ES+	22.1	295	30	296	214 (100)
Meclo	Anti-inflammatory	ES+	24.1	295	25	296	243 (100)

Table 6 LC–ESI-MS–MS conditions for the analysis of pharmaceuticals by MRM in positive ion modes

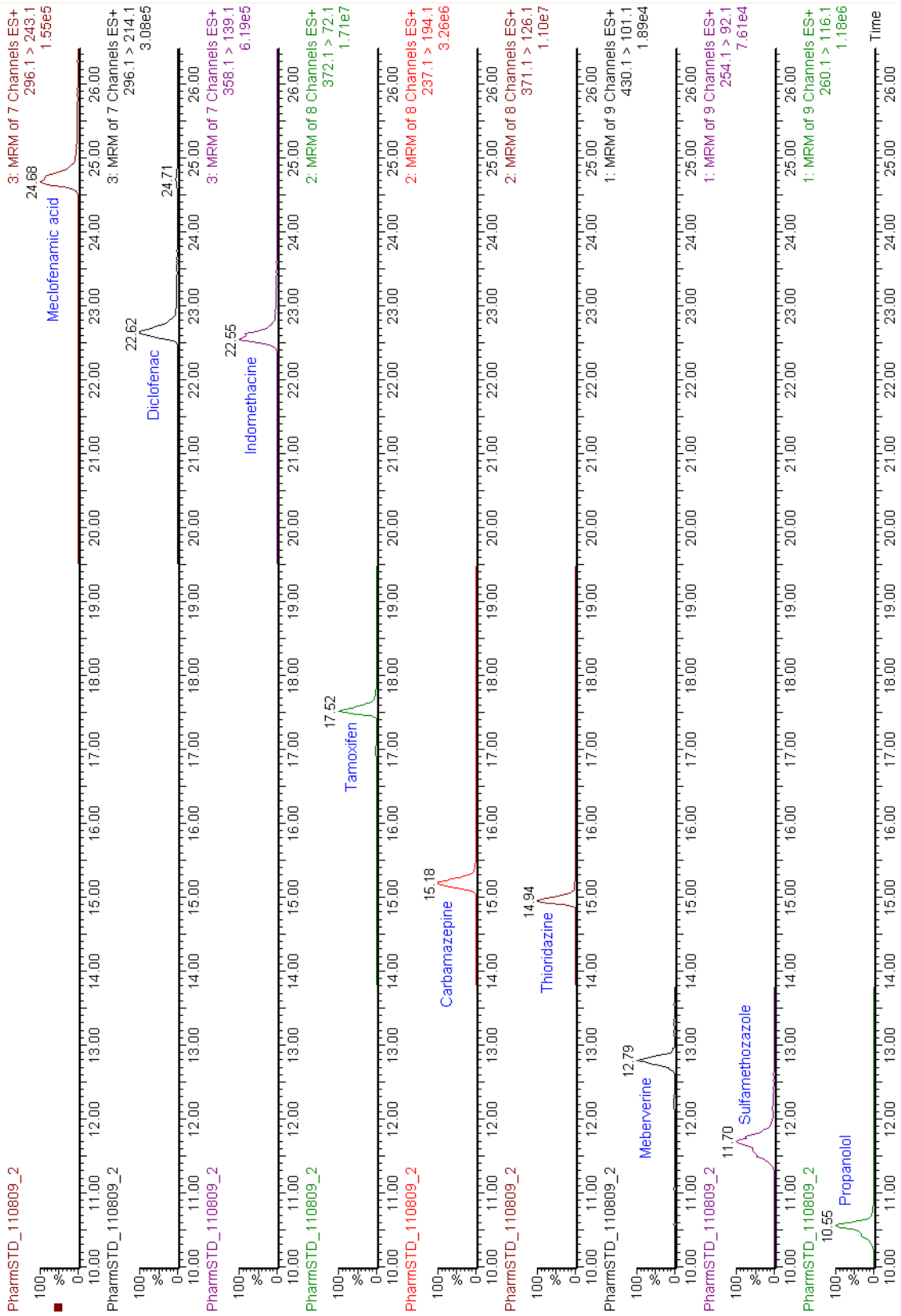

Figure 9 Chromatograms of each compound and their retention times.

3.2.6 Analysis of Environmental Samples

Once validated, the method was used for the analysis of environmental samples. Sediment samples were collected using a Van Veen sediment grab at the sewage outfall at Sheffield Park STP in the River Ouse, UK, between July 2009 and June 2010. In addition, sediment samples were collected at 500 m and 10 m upstream, as well as 100 m downstream, of the STP, to assess the impact of STP on river water quality. The top oxic fraction of all sediment samples was removed with a stainless steel spoon and stored in pre-cleaned glass jars. The sediment samples were frozen at -18 ˚C, then freeze-dried and homogenised. The dry sediment samples were then subjected to extraction, cleanup and finally analysis using LC-MS/MS.

3.3 Method Development Stage 1 – Preliminary investigations

3.3.1 The effect of changing cleanup method on extraction

To enhance the sensitivity and overall quality of analysis for pharmaceuticals, sample cleanup is often essential prior to chromatographic separation. The clean-up method can have a significant effect on the recovery of desired compounds due to their unique physicochemical properties, therefore it was the first experimental factor tested. Currently different cleanup methods have been used for this purpose, the most widely used being SPE cartridges and silica gel-packed columns (Antonic and Heath, 2007, Liu et al., 2004). The full method is given in section 3.2.4.2.

In this work, sediment extracts from MAE were purified by a range of cleanup techniques, and the recoveries for each compound over different methods were as follows: Pro 4.7 - 70 %, Sulf 0.1 - 88.2 %, Meb 3.9 - 73.7 %, Thio 1 - 51.9 %, Carb 14.4 – 98 %, Tamo 3.1 - 71.7 %, Indo 0.2 - 57.2 %, Diclo 0.1 - 82.4 %, and Meclo 1.7 - 61.7 %, indicating very different performances from different cleanup methods (Table 6). Similar results were obtained from the cleanup of sediment extracts by employing U. Overall, it was observed that SPE on Water Oasis cartridges was the method that produced the best recoveries (between 52 – 98 %) for the target compounds under the chosen conditions and variables, of which 6 compounds showed a recovery >70 % (

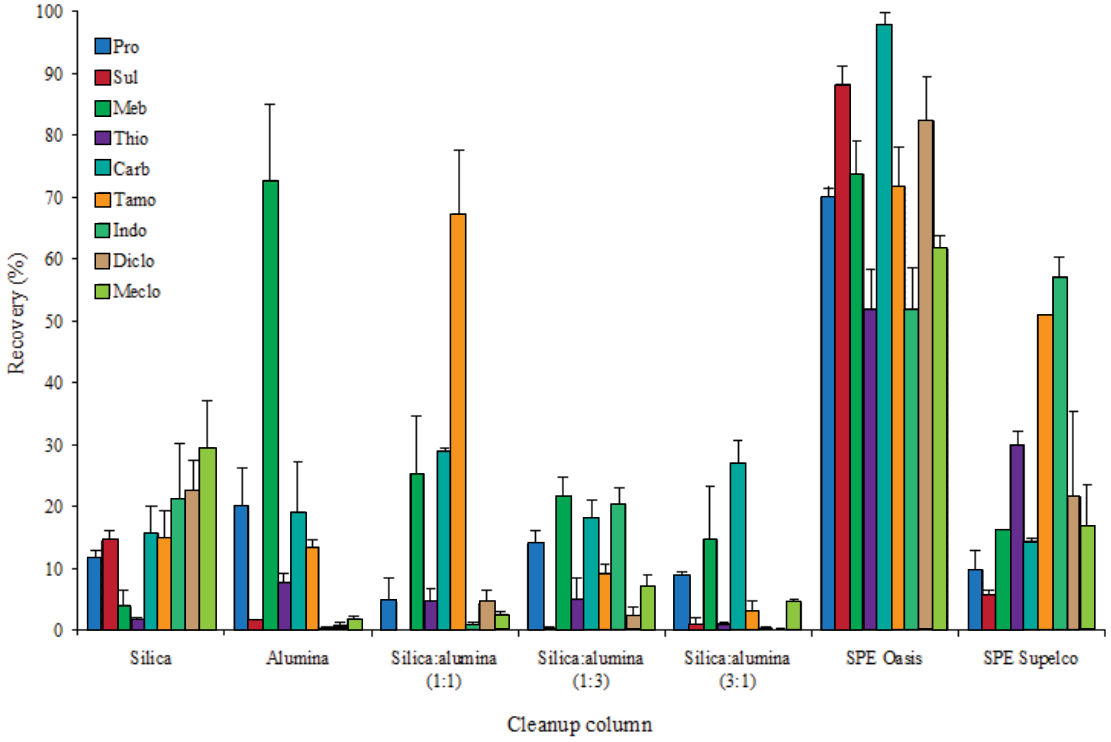

Figure 10). It is important to note that these recoveries may not be as desirable if other conditions are investigated. The results are consistent with those by Zhang and Zhou who showed Waters SPE to be the most effective sorbent during extraction of the pharmaceuticals from water samples (Zhang and Zhou, 2007). This method was favoured over a method that produced the best recovery for a single compound, since the goal of this research was to test multiple classes of pharmaceuticals. Aside from microfugation, none of the other methods showed recoveries for all compounds. Microfugation is not a desirable method because it does not completely remove interfering compounds from samples, which may reduce sensitivity therefore SPE was preferred.

	Pro	Sul	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Silica	11.01	13.17	9.89	1.48	7.10	14.27	21.24	22.60	29.43
Alumina	20.53	1.02	72.94	8.85	48.27	12.08	0.22	1.4	1.72
1:1	4.65	0.10	25.08	3.36	35.14	67.34	1.05	4.60	2.32
1:3	13.63	12.83	22.62	4.85	18.05	9.44	20.87	2.04	7.81
3:1	9.30	9.03	14.02	1.04	28.30	3.09	0.62	0.69	4.85
SPE Oasis HLB	70.00	88.20	73.72	51.92	98.35	71.67	50.99	82.38	61.65
SPE Silica C_{18}	9.73	10.69	17.99	20.01	14.37	50.12	57.21	21.56	16.75

Table 7 Recovery of pharmaceuticals (%) with different cleanup methods tested (ratios are using silica and alumina respectively ie 3:1 silica:alumina)

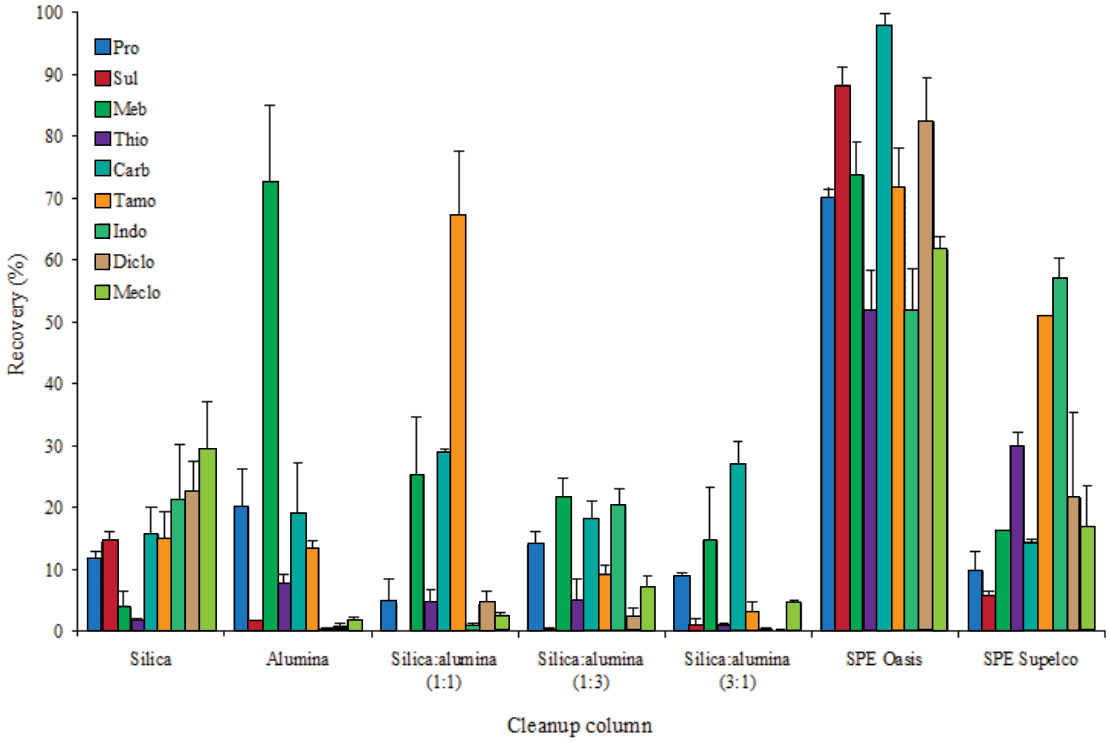

Figure 10 Comparison of different clean up methods on recovery of pharmaceutical compounds (n=3)

Another interesting finding that resulted from testing cleanup method was the relationship between silica/alumina columns and SPE products. Specifically, when SPE (Waters) and silica/alumina columns were compared, the SPE outperformed the silica/alumina columns with higher recoveries. Pharmaceuticals could have possibly interacted with the silica/alumina columns and the SPE sorbent differently, leading to a disparity between recoveries. An explination for this could be adsorption. In theory, there is no scientific reason as to why the silica columns are any less efficient at recovery of compounds than the manufactured SPE silica column, however this seems to be the case in these experiments. The poor recovery of pharmaceuticals from silica gel is in contrast to very high recovery of EDCs such as estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) from the same column obtained by Liu et al. (Liu et al., 2004). The difference is expected to be due to higher polarity of pharmaceuticals than

EDCs. There were also unexpected trends in the recoveries of various types of column material being used. It was expected that a mixture of silica and alumina would produce better recoveries than alumina or silica alone, because the mixture would interact more effectively with a greater range of chemicals with different polarity; however the alumina/silica mixture columns showed poorer recoveries for several compounds than alumina alone. The pure silica and alumina columns produced superior recoveries to the mixtures and each effectively eluted different compounds. This suggests that the pharmaceuticals interacted with the glass in the column and the plastic in the cartridge differently, leading to a disparity between recoveries. In this instance adsorption is a plausible conclusion. To summarise, the findings support the conclusion that SPE on Waters Oasis cartridges is the preferred method of cleanup for multiple classes of pharmaceuticals because it produced the best recoveries for most.

3.3.2 The effect of changing MAE temperature on extraction

A variety of extraction temperatures in MAE were investigated for suitable recovery of the target compounds (Figure 11). Overall optimum conditions for the suite of compounds was MAE at 110 °C for 15 min using methanol. Recovery was calculated at the end of the experimentation process as with all considerations in this chapter; therefore all results from samples treated fairly. High recoveries (>50%) for most compounds including Pro (84.6 %), Sulf (50.4 %), Carb (76 %), Tamo (71.3 %), Diclo (69.1 %) and Meclo (74.1 %) were obtained, although it was not as effective for other compounds, notably Thio with a recovery of only 4.5 %. All of the remaining extraction temperatures tested showed poorer recoveries for most compounds, in particular Thio and Tamo with recoveries of only 0.1 - 0.8 % and 0.5 - 10.1 % respectively. The higher recoveries at 110 °C may be explained by the favourable condition for the efficient extraction as well as relative stability of the target compounds. At temperatures higher than 110 °C, some of the compounds e.g. Meb and Tamo may have become degraded. At lower temperatures, the release of pharmaceuticals from sediment matrix may become retarded.

Also shown in Figure 11 are recovery results from the U method; 30 minutes at room temperature. In comparison to MAE, U produced overall higher recoveries of between 19.3 % and 97.6 %, with seven compounds achieving a recovery above 60 %, leaving

only two compounds with low recoveries (Meb at 43.9 % and Thio at 19.3 %). Hence, U is an effective method, in comparison to MAE at varying temperatures.

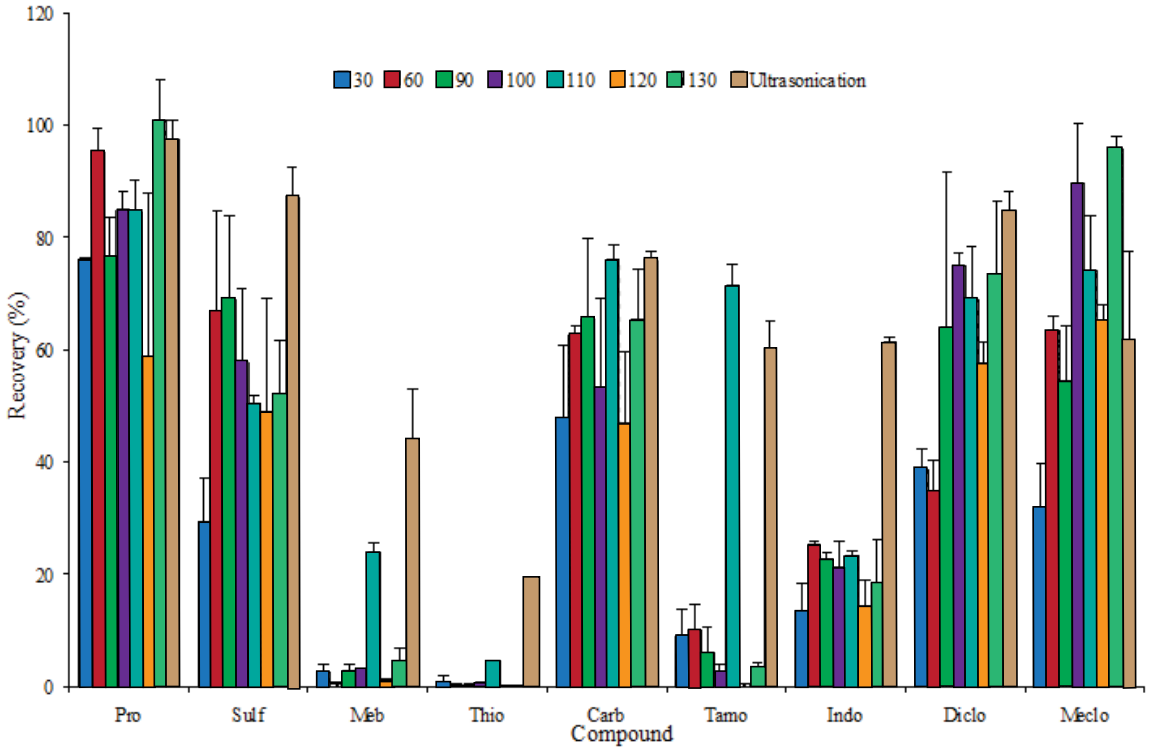

Figure 11 Comparison of different extraction techniques (MAE temperatures and Ultrasonication) on recovery of selected pharmaceutical compounds (n=3)

3.3.2.1 Considerations of a matrix effect on method development

Recovery of individual compounds may be affected by properties of the sediment matrix, inducing disparities in results. The matrix effect was investigated using the two different extraction techniques, in comparison to a solvent only blank. Matrix effect of samples on recovery of Diclo and Pro are illustrated in Figure 12 and Figure 13 as examples, and it can be concluded that there is no significant matrix effect from U, with MAE extracts showing significant interference in the response of the compounds. Similar results were found for the other compounds, suggesting that MAE is more destructive than U in causing dislodging of sediment components. It can therefore be concluded that U is more suited for the extraction of pharmaceutical compounds from

aquatic sediments. However due to other variables and considerations, U was not taken further in the investigation.

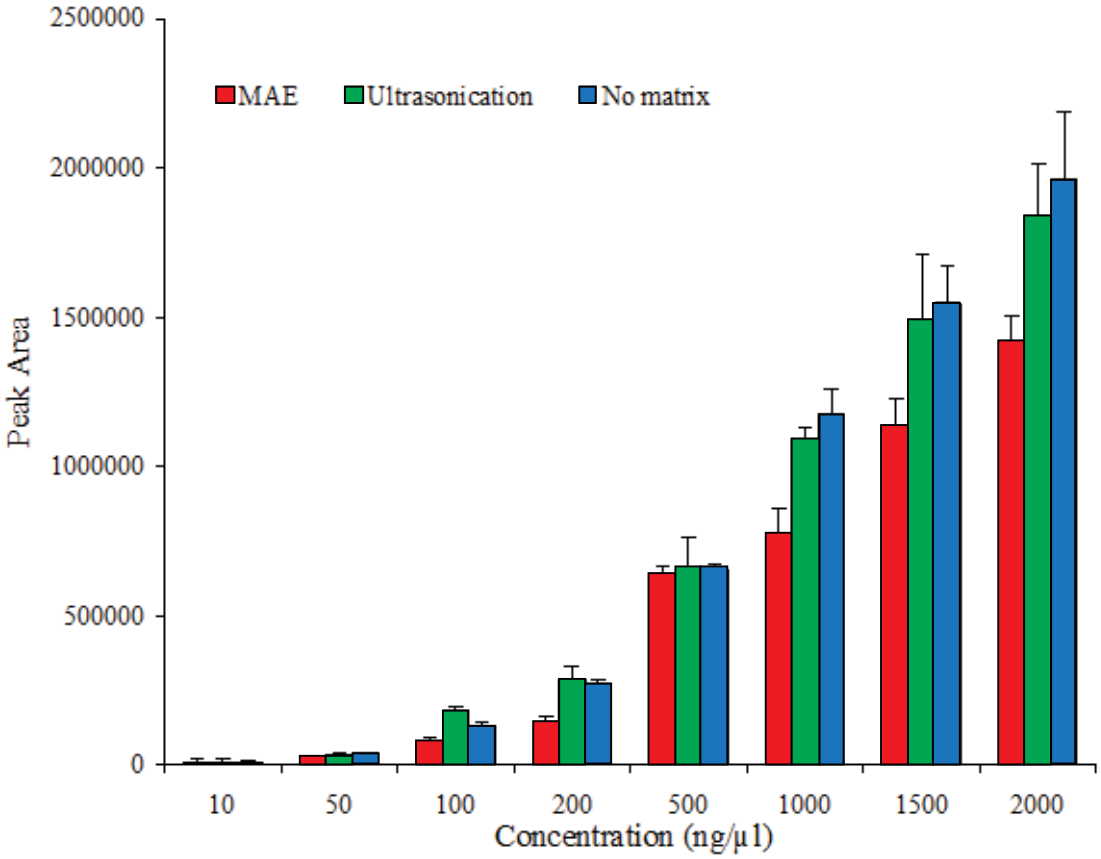

Figure 12 Effect of sediment matrix on the respoinse from LC-MS/MS analysis of Diclo (n=3)

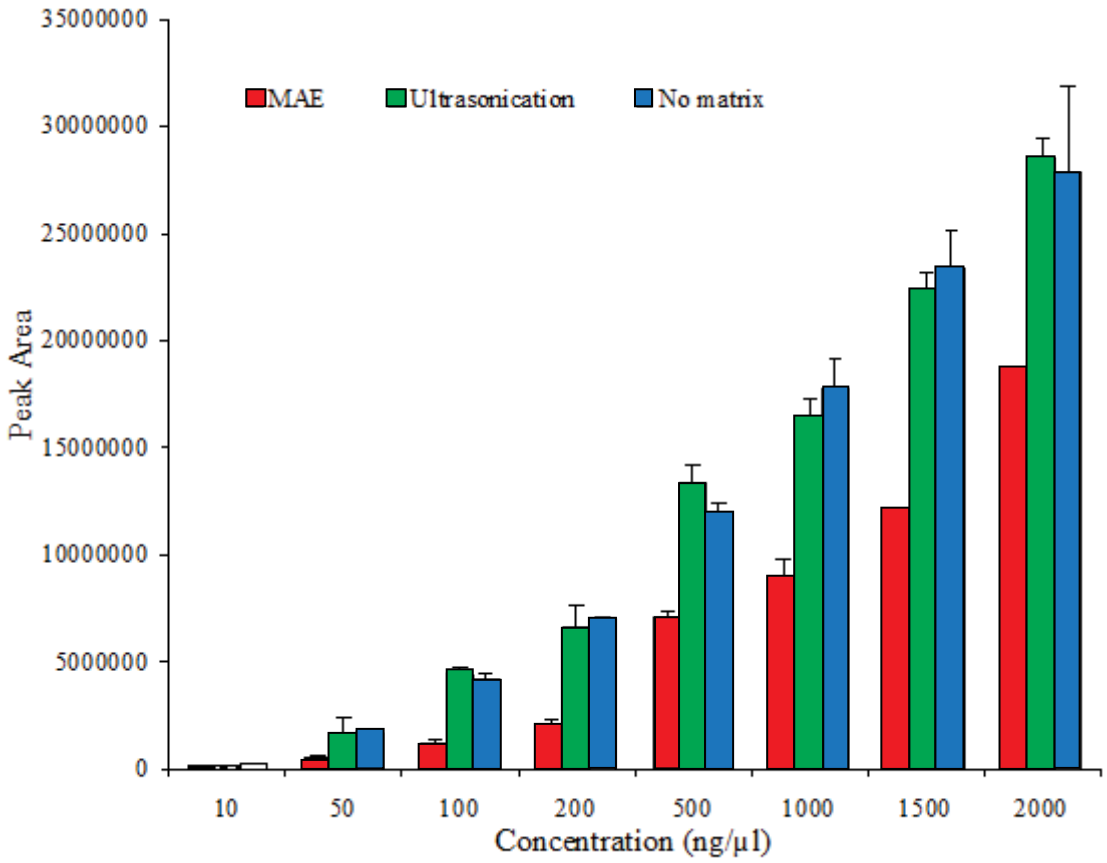

Figure 13 Effect of sediment matrix on the respoinse from LC-MS/MS analysis of Pro (n=3)

3.3.3 The effect of changing sample mass on extraction

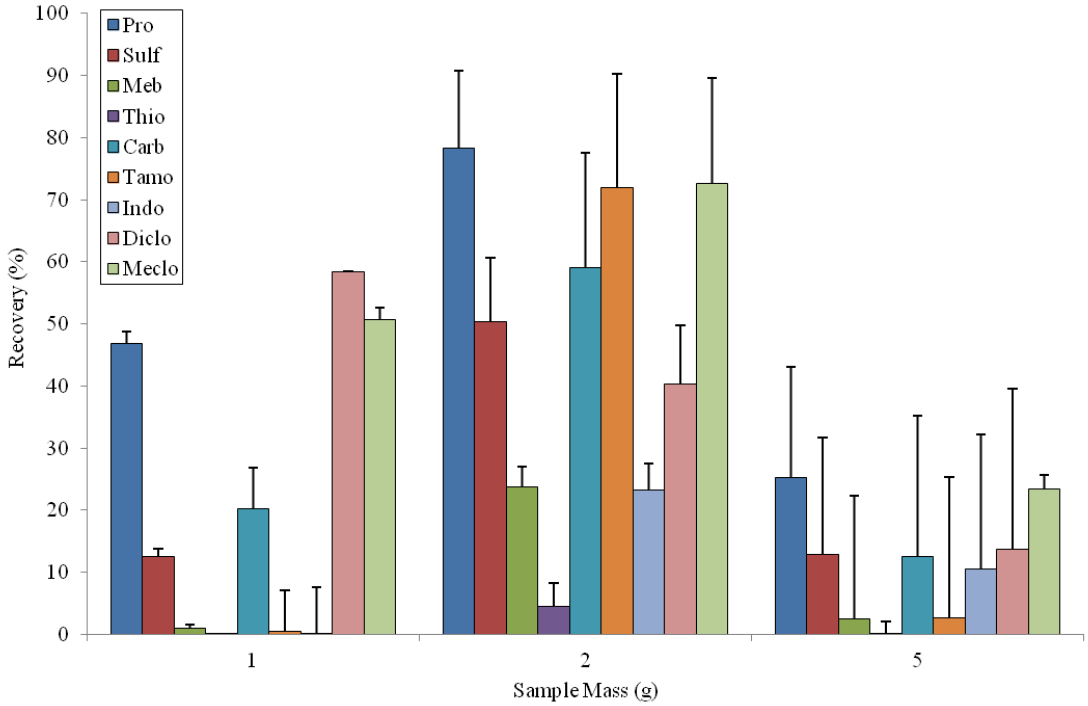

Figure 14 Comparison of three different sample masses on the recovery of 9 selected pharmaceutical compounds (n=3)

It is not evident from Figure 14 that the preferred sample mass is 2 g, however there are recoveries of almost 80 % for some target compounds. An improved analysis is displayed as a box plot, where it is clearly identified (Figure 15). Although some lower recoveries, overall this sample mass provided the highest compared to other masses tested. Recovery range was as follows; 0.01 – 58.37 % for 1 g, 4.54 – 78.28 % for 2 g and 0.02 – 25.30 % for 5 g.

Interestingly, a sample mass of 5 g produced lower recoveries of target compounds compared to 2 g, with no single compounds producing a good result. A sample mass of 1 g proved effective, but had a much lower recovery range than a sample mass of 2 g. It was expected to find the compound recovery increased with an increased sample size, which the results did not show. This is possibly due to the fact that a higher sample mass is required to extract a sufficient concentration of pharmaceutical compounds and a mass greater or equal to 5 g causes retention of compounds due to chemical bonding.

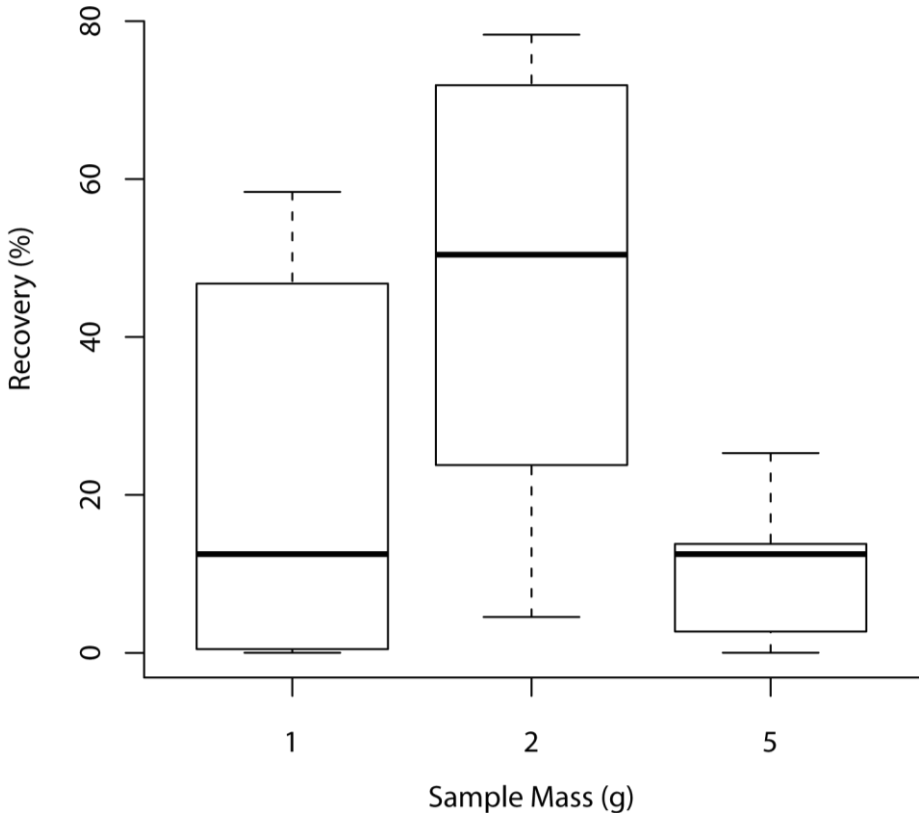

Figure 15 A box plot of three tested sample masses recovery results (n=3)

3.3.4 The effect of changing solvent on extraction

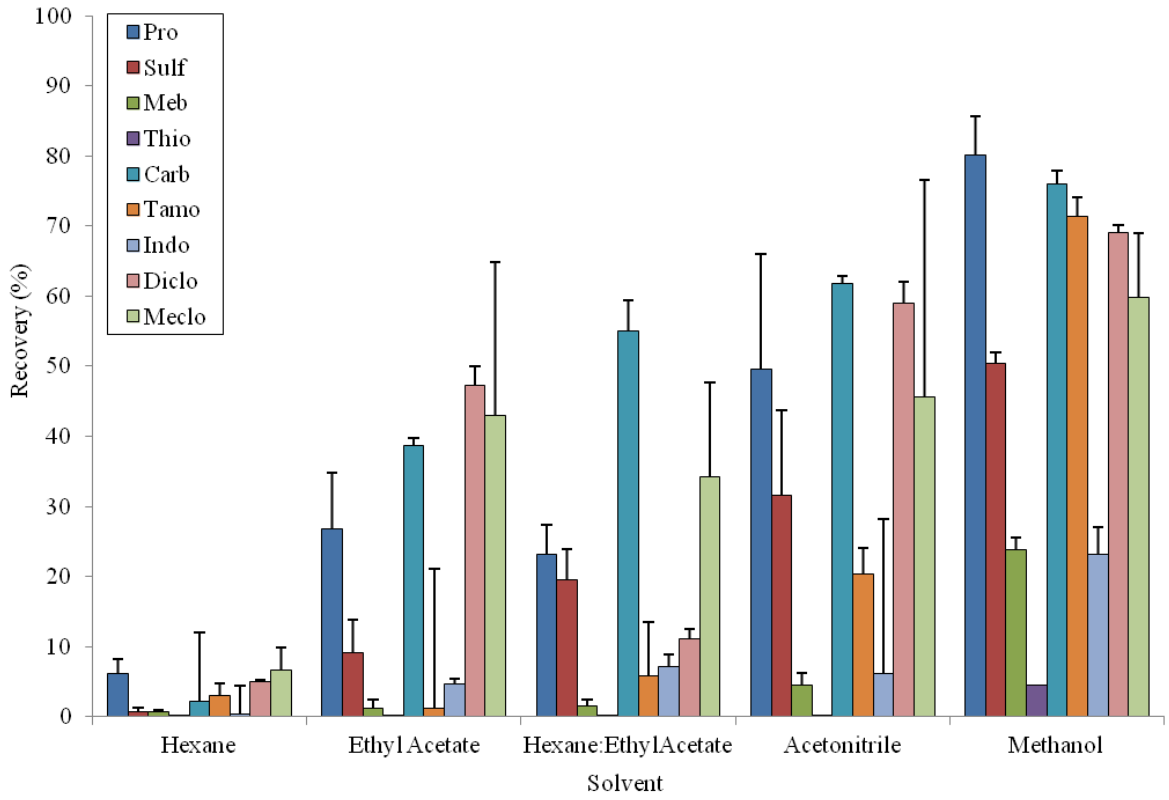

Figure 16 Comparison of 5 different solvents used for the extraction of selected pharmaceutical compounds (n=3)

A range of solvents were tested for the extraction process, to cater for compounds of different polarity. With over 70 % recovery for four compounds, methanol is clearly the best solvent to be used for the extraction of these compounds from sediment samples (Figure 16). Overall, methanol produced the highest recoveries of between 23.2 % for Indo and 84.6 % for Pro. This excludes the anomalously low recovery of 4.5 % for Thio, which despite its relatively low recovery, is surprisingly still the highest in comparison to all other solvents used. Hexane gave the lowest recoveries of between <1 % for Thio and 6.7 % for Meclo. Ethyl acetate and acetonitrile produced recoveries of between 0.1 % for Thio and 59.8 % for Diclo, and between 0.1 % for Thio and 61.8 % for Carb, respectively. Of all the compounds, Thio was the most difficult compound to be extracted. Again, as a bar chart it is not easy to identify the best solvent for extraction, however it can be hazardous in assumption that Methanol provides best overall results (Figure 16).

Further testing with a mixture of solvents (hexane:ethyl acetate, 1:1) did not significantly increase percentage recovery, with recoveries between 0.1 % and 49.5 %. For some compounds e.g. Diclo, their recovery was significantly reduced compared to ethyl acetate alone, while for others e.g. Sulf, their recovery was significantly enhanced.

A study by Liu et al. (Liu et al., 2004) also found that methanol was the best solvent for the extraction of EDCs, in particular for polar compounds such as EE2, from sediment samples. For other EDCs such as E1 and E2, the difference in recovery between different solvents such as ethyl acetate and methanol was insignificant. The findings further suggest that the polarity of extraction solvents should match as much as possible that of the compounds to be extracted, in order to ensure a more satisfactory extraction from sediments. Figure 17 not only clearly displays that Methanol is the most effective solvent to use, with the highest overall recovery compared to any other, but also that the mean recovery is the only over 50 % (also, over 60 %). The combination of hexane:ethyl acetate has very high outlier recoveries, which suggest anomalous results, probably for Carb as shown in Figure 16 as it has the highest recoveries for this solvent tested.

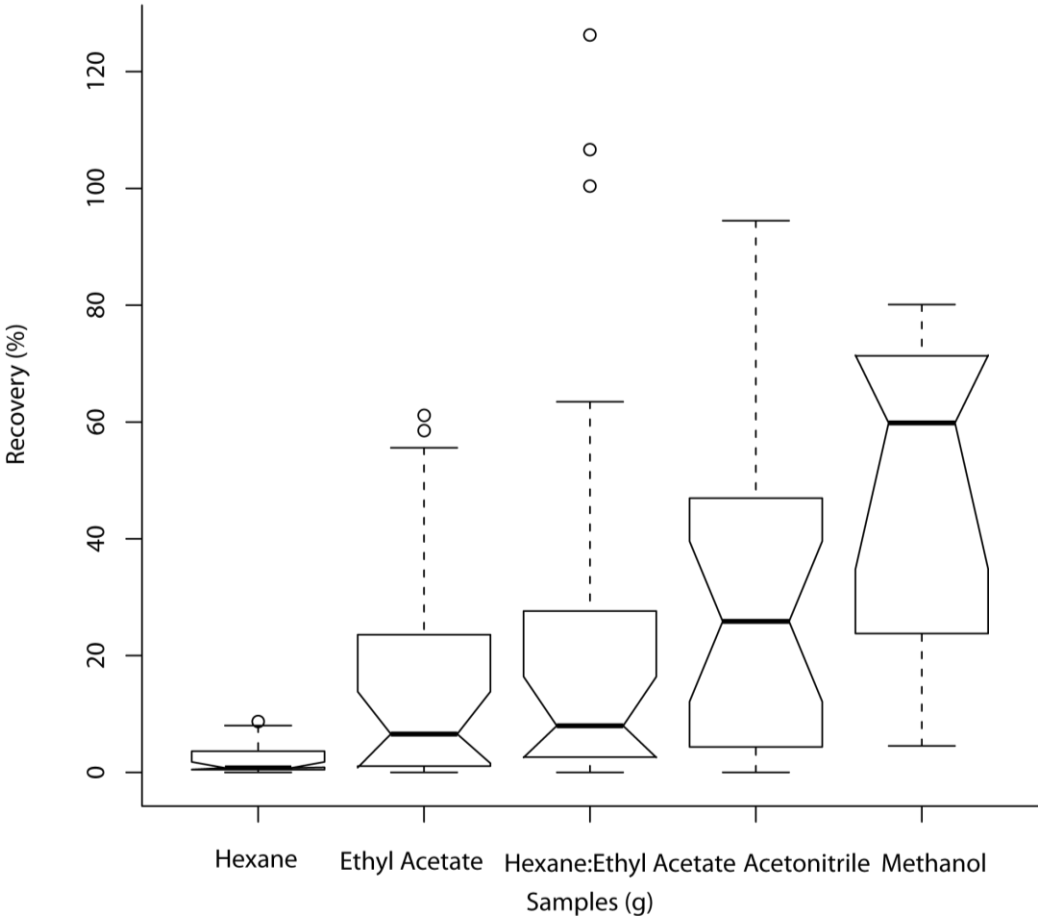

Figure 17 A box plot demonstrating the five different solvents (n=3)

3.4 Method development progress

It can be concluded from method development in sections 3.2 to 3.3.4 that the best developed method is as follows;

To firstly use a prepared sample mass of 2 g (dry weight). This, combined with using Methanol as the extraction solvent and SPE Oasis as the filtration stage, and finally using MAE at 110 °C for 15 minutes, proves to be the best parameters for a high recovery Figure 18.

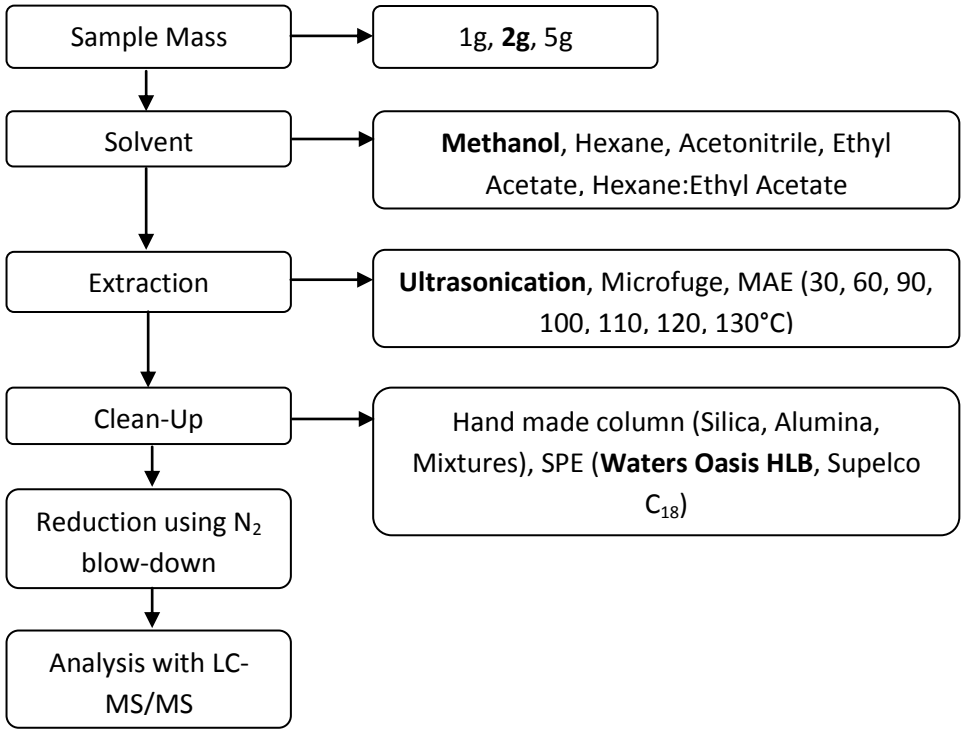

Figure 18 Flow diagram to illustrate the new method for analysis of pharmaceuticals

Other variables must now be tested in order to fully conclude the best parameters and variables for the quantification of pharmaceutical compounds in sediment.

3.5 Method Development Stage 2 – Method refinement

To ensure that the developed method is as accurate and optimal as possible, different variables were considered to further refine the method. These are detailed throughout section 3.4, and the optimal conditions applied to the developed method.

3.5.1 Triple injection

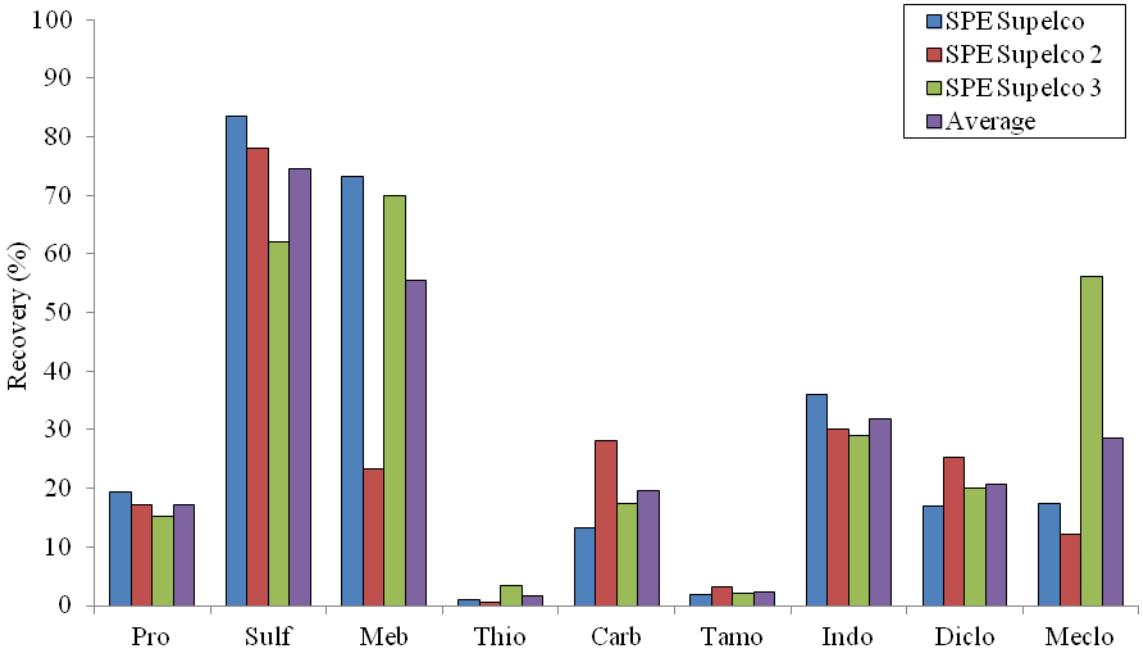

Figure 19 Comparison of variability of recoveries using triple samples (n=3)

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
SPE Supelco	19.30	83.57	73.30	1.08	13.20	1.85	36.10	17.00	17.30
SPE Supelco 2	17.10	78.00	23.40	0.64	28.10	3.19	30.12	25.30	12.10
SPE Supelco 3	15.20	62.10	69.90	3.29	17.30	2.15	29.10	20.10	56.10
Average	17.20	74.56	55.53	1.67	19.53	2.40	31.77	20.80	28.50
stdev	1.68	9.10	22.76	1.16	6.28	0.57	3.09	3.42	19.63
rsd	9.74	12.20	40.99	69.37	32.17	23.89	9.71	16.46	68.88

Table 8 Recoveries for nine selected pharmaceuticals for triplicate recovery

It is clearly illustrated in Figure 19 that there is a degree of variability from what is essentially the same sample tested. A stock solution was prepared and three sub samples

taken for the use of a triplicate experiment in the laboratory. Results shown in Table 8 clearly highlight the variability between samples with statistical analysis. It is also useful to use triplicate samples as this experiment shows, is to identify outliers which may bias results. It can be seen that the third (SPE Supelco 3) sample for Meclo is an anomalous result with a recovery of 56.10 % compared to the others of 12.10 % and 17.30 %, and therefore if not identified and removed, will subsequently increase statistical bias as seen in the standard deviation and relative standard deviation. These values would be significantly lower and accepted if this was the case. There is also another outlier identified in the Meb trials from the second sample (SPE Supelco 2) of 23.40 % compared to the others of 73.30 % and 69.90 %. Once again this affects statistical tests.

3.5.2 The effect of removing MAE on IS Phenacetin recovery

During sample data analysis for section 3.3, it can be concluded that Phenacetin was the better IS to use than Diuron. This is possibly due to a characteristic difference in the compounds such as melting or boiling point (Table 9) or other properties (**Error! Reference source not found.** and Table 10). If one has a higher or lower melting or boiling point than another it may me more susceptible to the MAE treatment and provide better results to use as an IS for the developed method. It was concluded that. An experiment was conducted using both Phenacetin and Diuron and how they performed with and without the use of MAE. The protocol used for this set of experiments followed that developed previously.

	Pro	Sul	Meb	Thio	Phenacetin	Carb	Tamo	Indo	Diclo	Meclo	Diuron
molecular mass (g/mol)	128.99	253.28	429.56	370.57	179.22	236.28	371.53	357.8	295.15	296.15	233.1
Boiling Point	189.2	414.01		476.89	341.09	410.02	468.2	514.5	423.77	2.18E-06	353.86
Melting Point	-23.56	172.43		201.8	115.17	162.35	180.85	219.37	174.6	257	126.39
Experimental Boiling Point	176	3.79E-06	488.1	230	243.5	4.74E-06	1.73E-07	1.18E-08		4.24E+02	6.90E-08
Experimental Melting Point	0.4	167	200.12	73	137.5	190.2	97	158		176.99	158

Table 9 Pharmaceutical compounds and their physiochemical properties (USEPA EPI Suite 2009)

Compound	log(K_{ow})	log(K_{oc})	K_d (soil water)	Reference
Pro	1.2-3.48	2.45-2.96	9.6/37.6	Zhang 2007, USEPA KOCWIN, Beausse
Sulf	0.5	1.54-2.41	0.22/1.8	Zhang 2007, USEPA KOCWIN, Beausse
Meb	3.82	3.02-5.31		USEPA KOWWIN, KOCWIN
Thio	5.9	4.06-5.09		USEPA KOWWIN, KOCWIN
Carb	2.45	2.23-3.12	1.4/4.4	Zhang 2007, USEPA KOCWIN, Beausse
Tamo	6.3	4.4-6.42		USEPA KOWWIN, KOCWIN
Indo	4.27	2.34-2.90		Zhang 2007, USEPA KOCWIN
Diclo	4.5	2.61-2.66	0.8/5.9	Zhang 2007, USEPA KOCWIN, Beausse
Meclo	5.12	2.62-3.44		Zhang 2007, USEPA KOCWIN

Table 10 Physicochemical properties of pharmaceuticals analyzed in this study

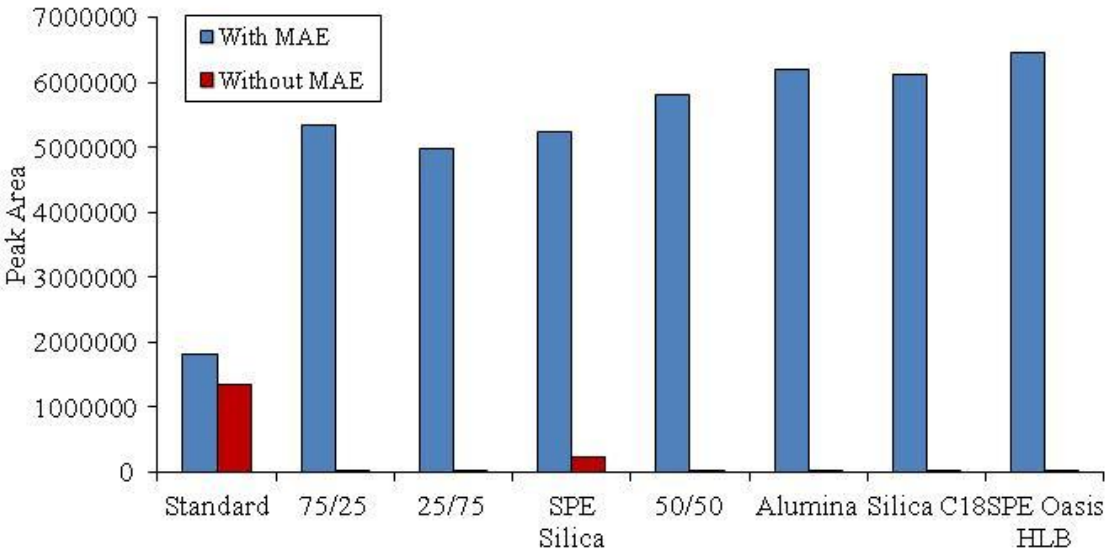

Figure 20 Comparison of using internal standard Phenacetin with and without MAE (n=3)

With the inclusion of MAE on sample compound extraction it is obvious that Phenacetin outperformed Diuron (Figure 20). Diuron produced much lower recovery of the selected compound, of which this was without the use of MAE (Figure 21). Therefore the use of Phenacetin (with MAE) was used in further uses of the developed method. This is not only for the reason of a greater recovery but also its similarity to analytes used for experimentation.

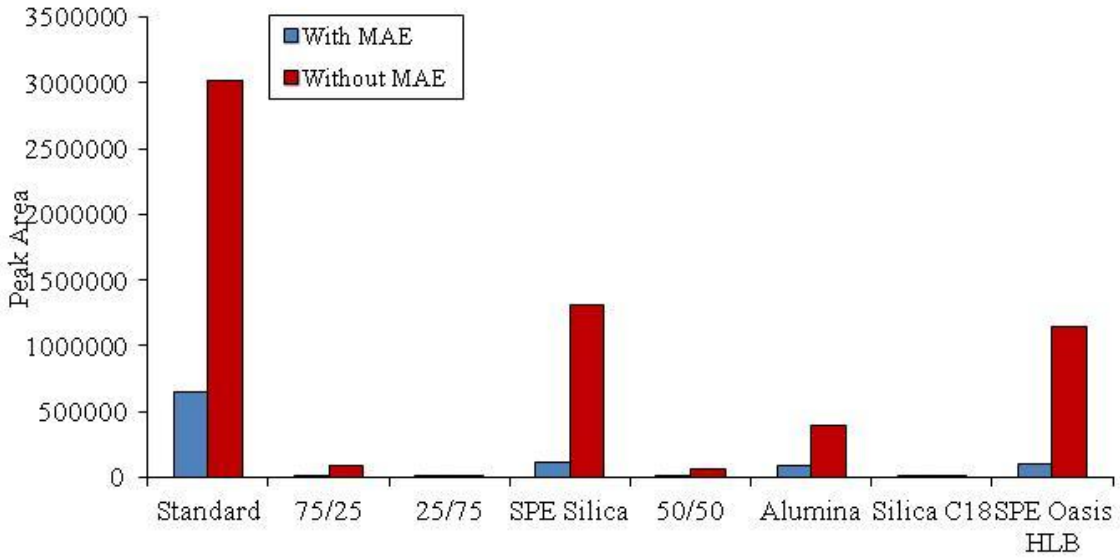

Figure 21 Comparison of using internal standard Diuron with and without MAE (n=3)

3.5.3 Triple vs. quintuplicate LC-MS replicates to test variability of samples

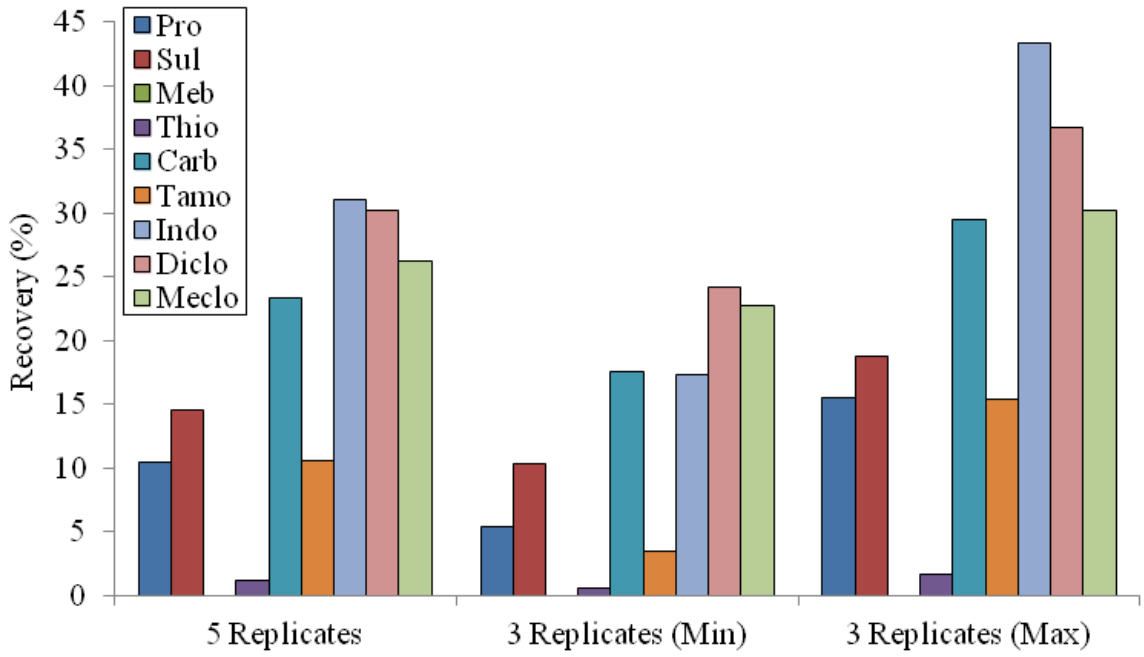

Figure 22 Comparison of using three and five replicates of the same sample on recovery (n=5)

Experimentation for further method development required increasing duplicate number to improve overall accuracy of results. Standard practice in science concludes that there is increased accuracy with a higher number of duplicate samples tested. The method validation protocols and quality assurance is in line with EU protocols.

Following the realisation that a subsample can vary upon laboratory analysis (3.4.1), it must also be considered that a subsample from each of these used during LC-MS/MS analysis may also vary significantly. To illustrate this, a sample from 3.4.1 was analysed 5 times and the recoveries displayed in Figure 22. The number of replicates will inevitably give a discrepancy in results with even the same sample, as this highlights. To illustrate this, the minimum combination of the average of these replicates were taken as if only 3 were taken as opposed to 5, and the same done for the 3 maximum results. There is a considerable difference in results. This highlights that the more

replicates are taken, the more accurate results are and anomalous results more easily identified. Table 11 highlights all possible combinations (recovery averages %) of results with minimum and maximum results.

Rep. No.	Short I.D.	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
1,2,3	1	8.3	12.0	0.0	1.6	19.3	15.3	17.4	24.2	23.6
2,3,4	2	12.2	16.4	0.0	1.0	25.8	12.8	28.7	30.3	27.3
3,4,5	3	15.5	18.8	0.0	0.6	29.5	12.6	35.6	36.7	30.2
4,5,1	4	10.7	15.5	0.0	1.3	24.8	6.1	42.0	34.3	27.8
5,1,2	5	5.5	10.4	0.0	1.6	17.6	6.0	32.0	25.5	22.7
1,3,5	6	11.5	14.4	0.0	1.2	23.0	15.2	24.2	30.6	26.5
1,3,4	7	13.5	17.1	0.0	1.2	26.4	15.4	27.3	33.0	28.7
2,4,5	8	9.4	14.8	0.0	1.0	24.1	3.4	43.4	31.6	26.4
5,2,3	9	10.3	14.7	0.0	0.9	22.3	12.5	25.7	27.9	25.0
4,1,2	10	7.4	12.2	0.0	1.7	21.1	6.2	35.1	27.9	24.9
1,2,3,4,5	11	10.4	14.6	0.0	1.2	23.4	10.5	31.1	30.2	26.3
Minimum		5.5	10.4	0.0	0.6	17.6	3.4	17.4	24.2	22.7
Maximum		15.5	18.8	0.0	1.7	29.5	15.4	43.4	36.7	30.2

Table 11 5 replicates with different combinations of 3 replicates to show variance and inaccuracy of low replicate number. Average percentage recovery

3.5.4 Percentage recovery; the use of calibration curves as a measure of increased accuracy

Following method development, the possibility of using calibration curves were considered for the analysis of results. This is due to the instability and uncertainty of using either Phenacetin or Diuron as an IS. There have been occasions where there are high discrepancies in results, and of other compounds in comparison being over 100 % (some instances of > 3000 %). This was because the IS compounds were not being recovered effectively in the first place which meant that in comparison, the rest of the compounds in samples were unreliable. Figure 23 illustrates the R squared (r^2) values of results from using different concentrations (ng/l) of compounds for the use of calibration curves. All ten compounds (including Phenacetin) gave highest values (most accurate) at 200ng/l, excluding Sulf (500 ng/l) and Diclo (1000 ng/l), however their

values for 200 ng/l were still very acceptable and so 200 ng/l was used for all calibration curves.

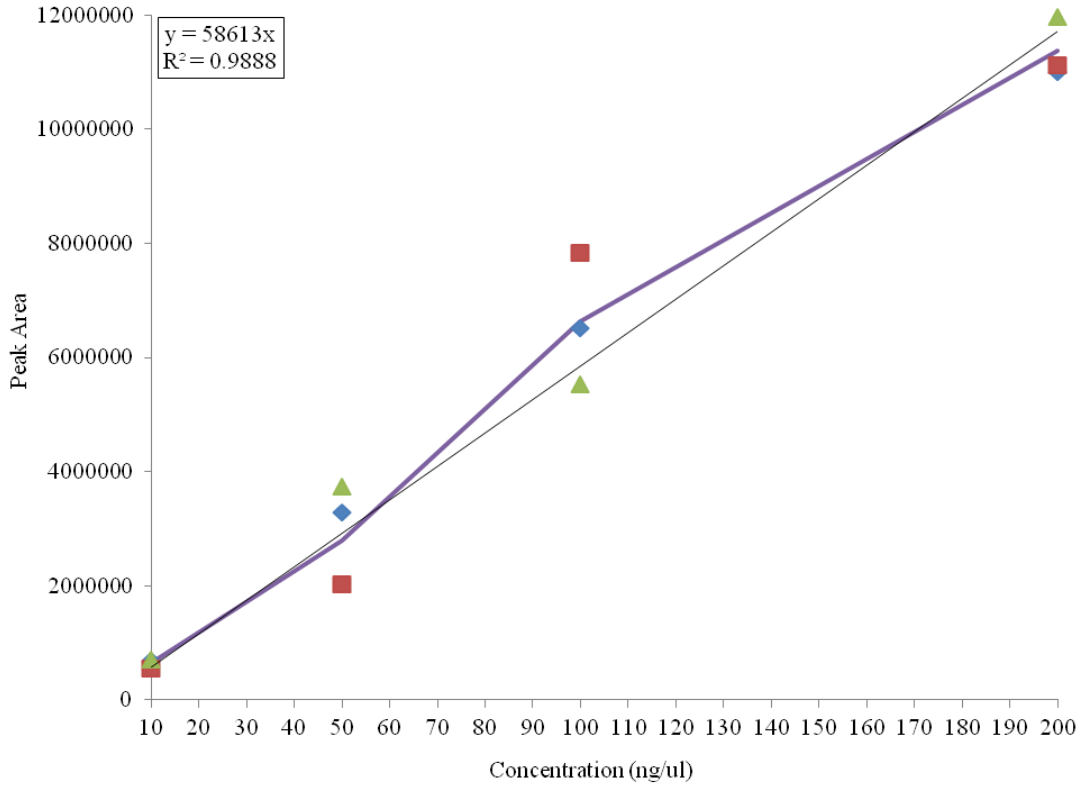

Figure 24 to

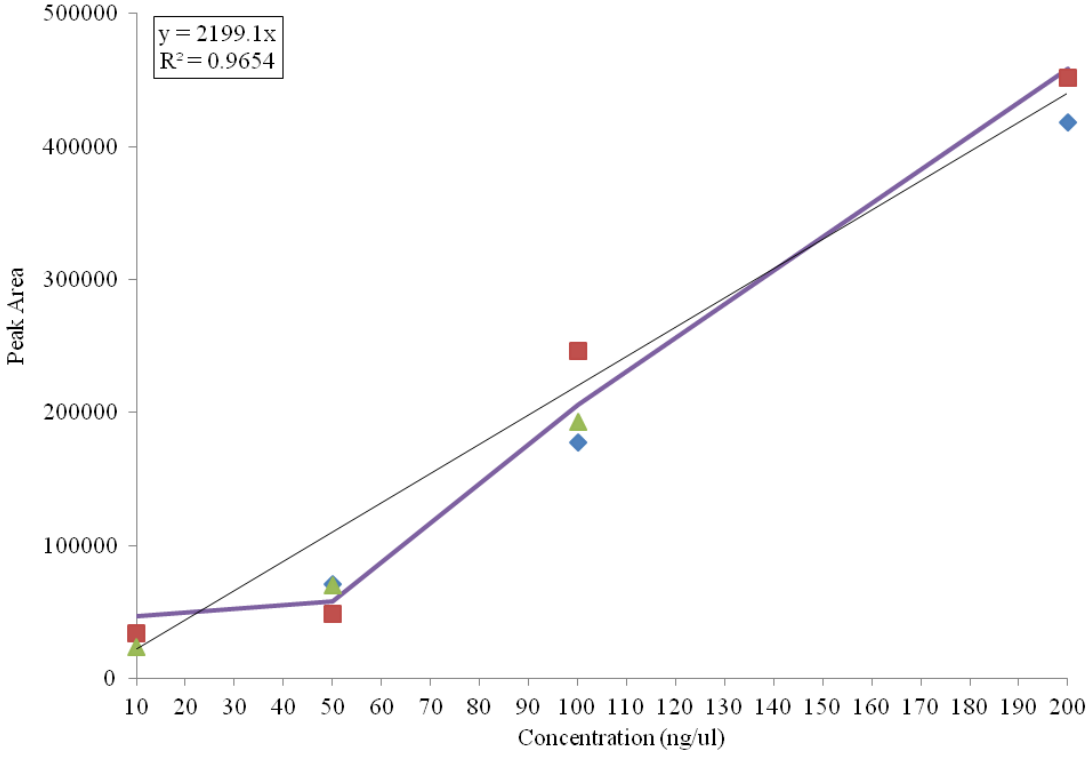

Figure 32 illustrate the calibration curves for all compounds. The dark line indicates average value of triplicate data points and crosses indicate each replicate value. The dashed line represents regression line, with r^2 value on top left of graph.

Conc (ng/l)	Pro	Sulf	Meb	Thio	Phen	Carb	Tamo	Indo	Diclo	Meclo
2000	0.81	0.9762	0.7355	0.0805	0.8566	0.8703	0.185	0.9104	0.9882	0.9388
1500	0.7711	0.9752	0.5694	-0.2518	0.8498	0.8543	-0.2379	0.7853	0.9918	0.9138
1000	0.5986	0.9684	0.2363	-1.3318	0.7207	0.7202	-1.3521	0.4226	0.9923	0.7234
500	0.7735	0.9768	0.4006	-0.0949	0.8141	0.9127	-0.2417	0.5553	0.9874	0.9483
200	0.9917	0.8988	0.8438	0.5856	0.9885	0.9386	0.5912	0.9674	0.9404	0.9654
100	0.9918	0.8186	0.7087	0.6463	0.9935	0.8277	0.3347	0.8389	0.8847	0.8505
50	0.9982	0.9981	-1.3495	-1.8065	0.9915	0.9965	-7.0842	-8.73	0.3983	-
10	1	1	-	-	-	1	-	-	-	-

Figure 23 R squared values for calibration curve data

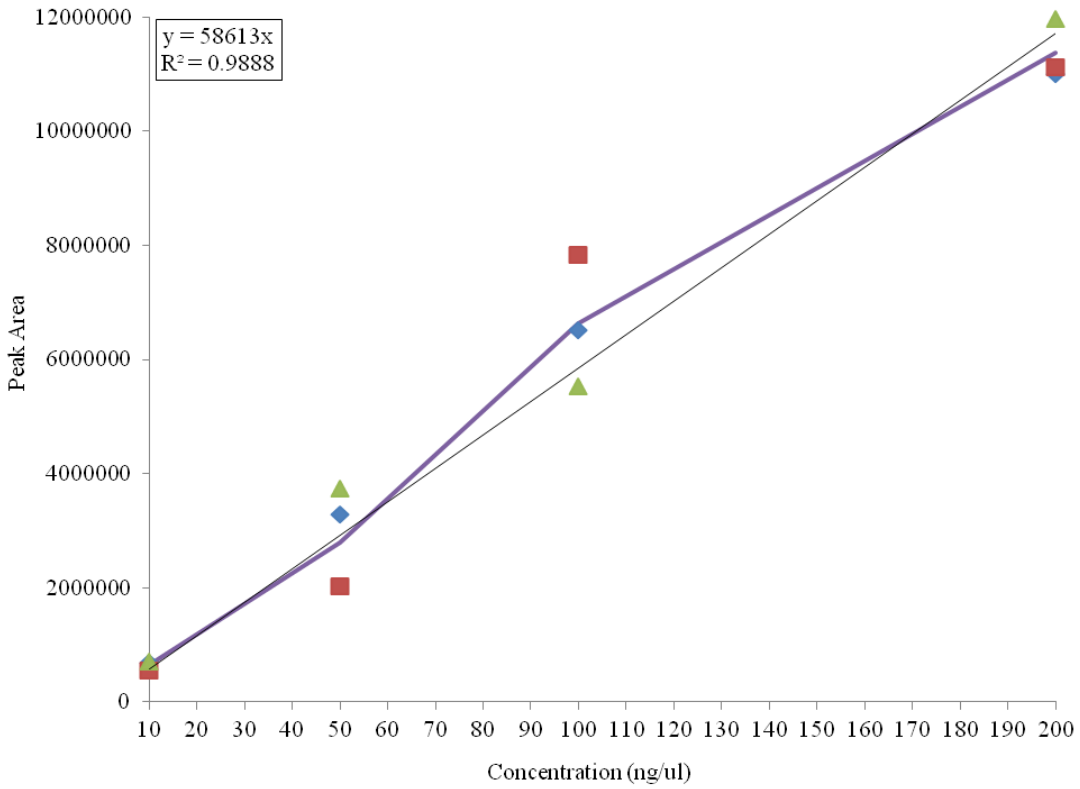

Figure 24 Calibration curve for Propanolol

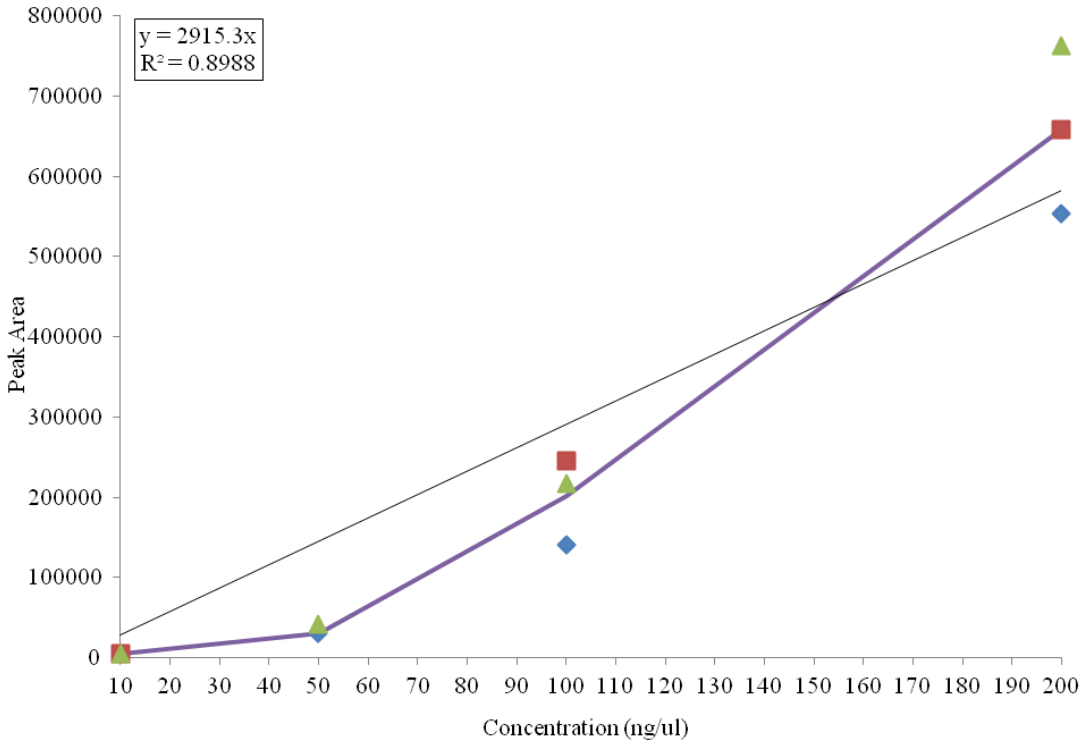

Figure 25 Calibration curve for Sulfamethaxazole

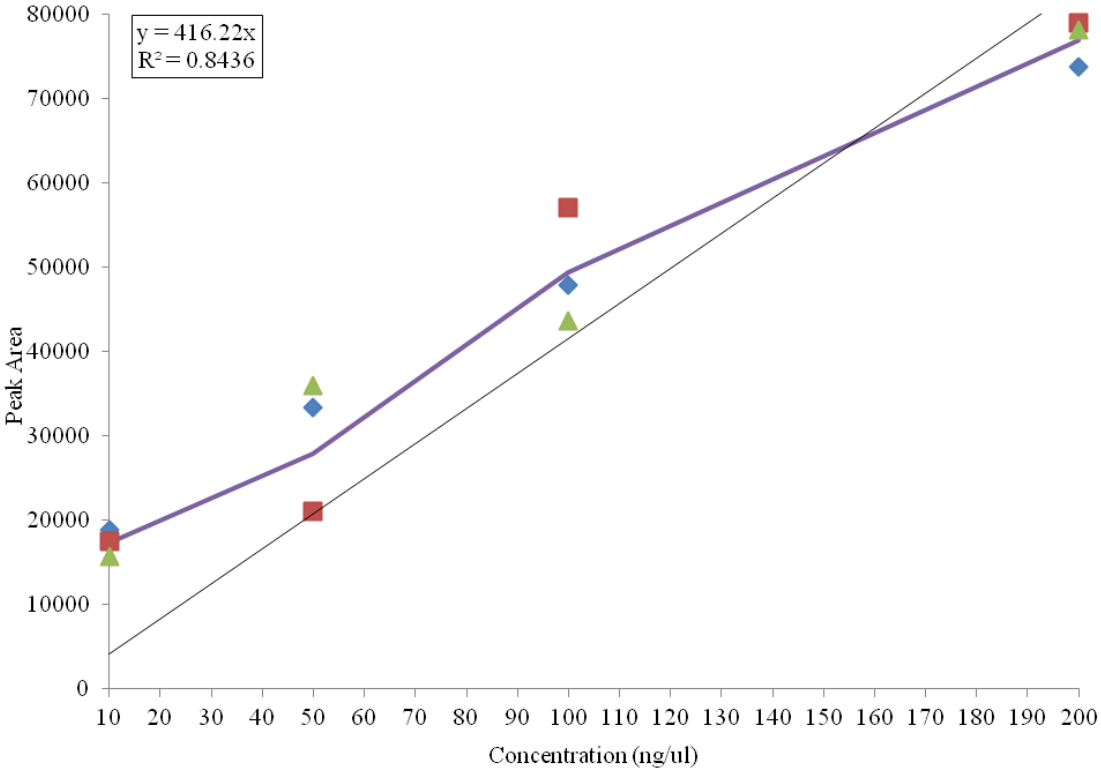

Figure 26 Calibration curve for Mebeverine

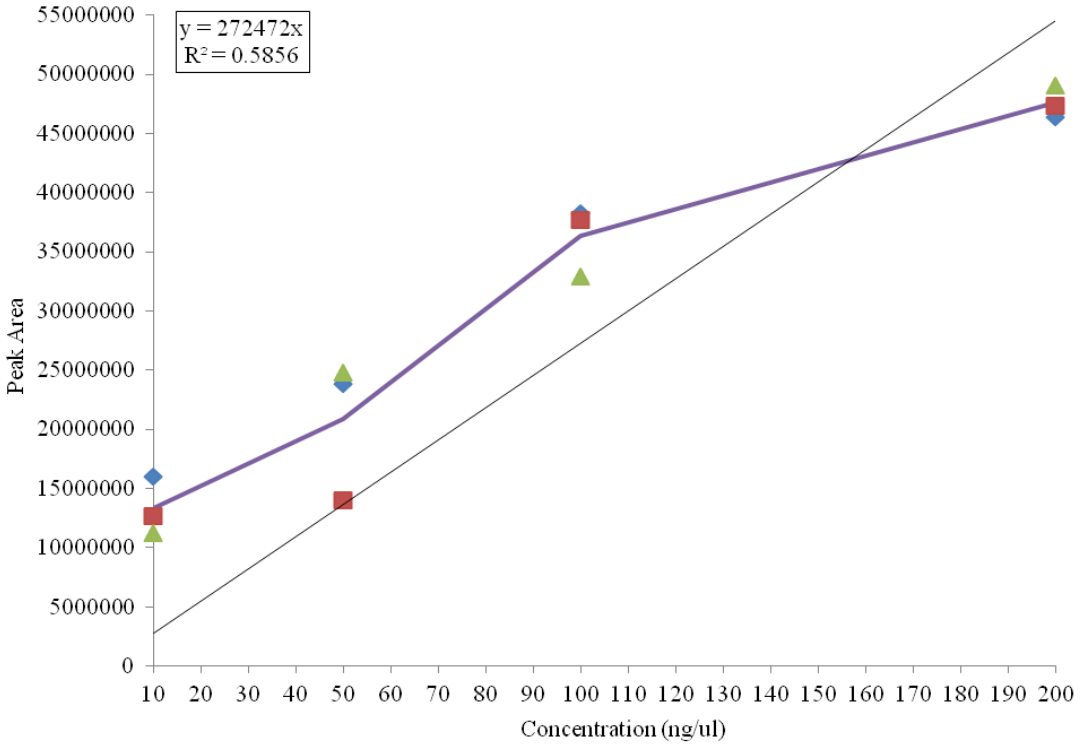

Figure 27 Calibration curve for Thioridazine

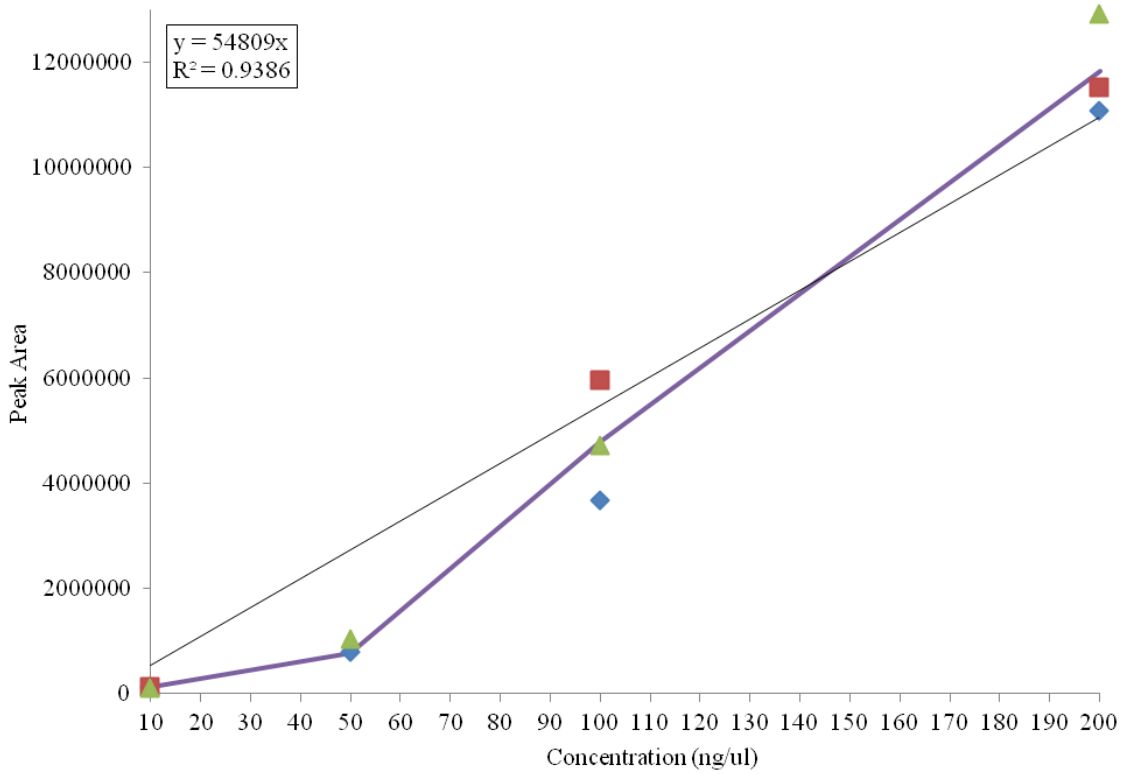

Figure 28 Calibration curve for Carbemazepine

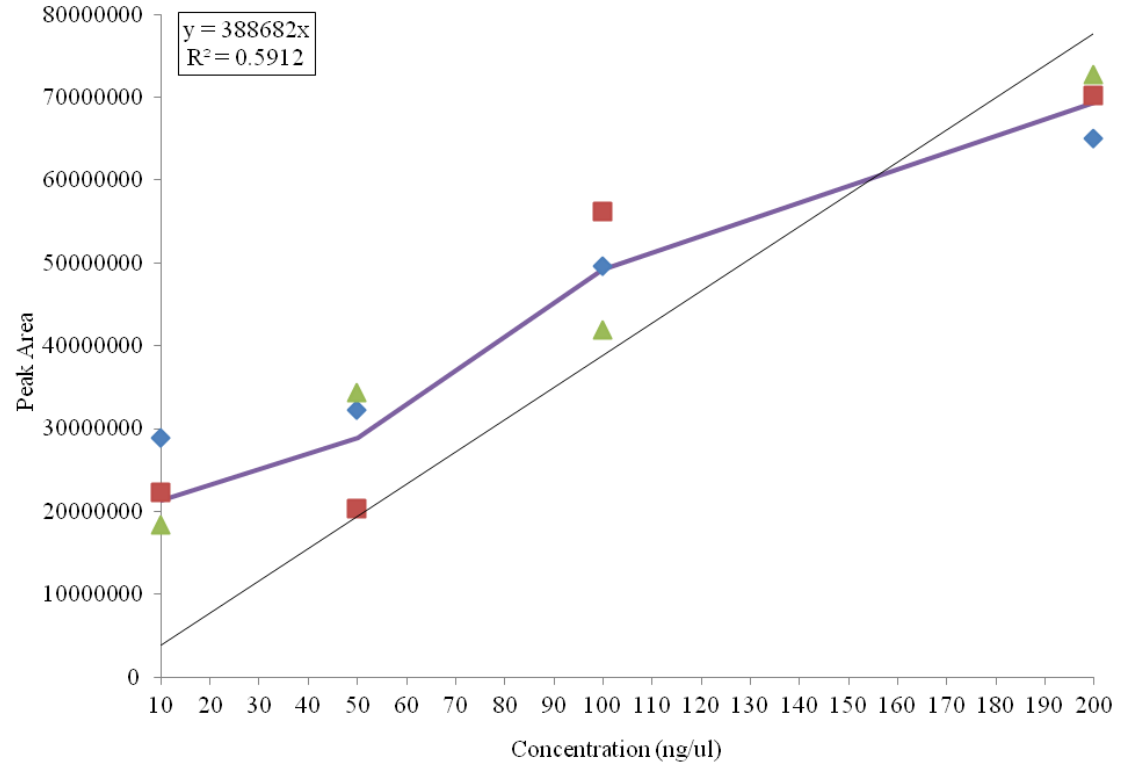

Figure 29 Calibration curve for Tamoxifen

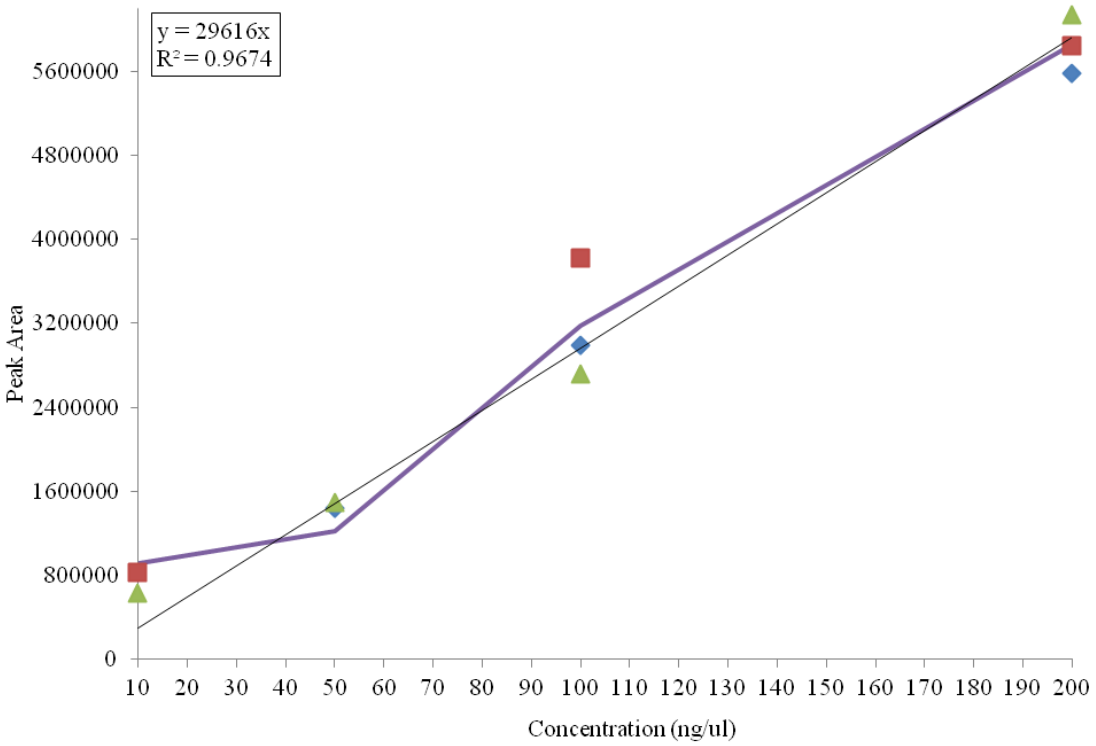

Figure 30 Calibration curve for Indomethacine

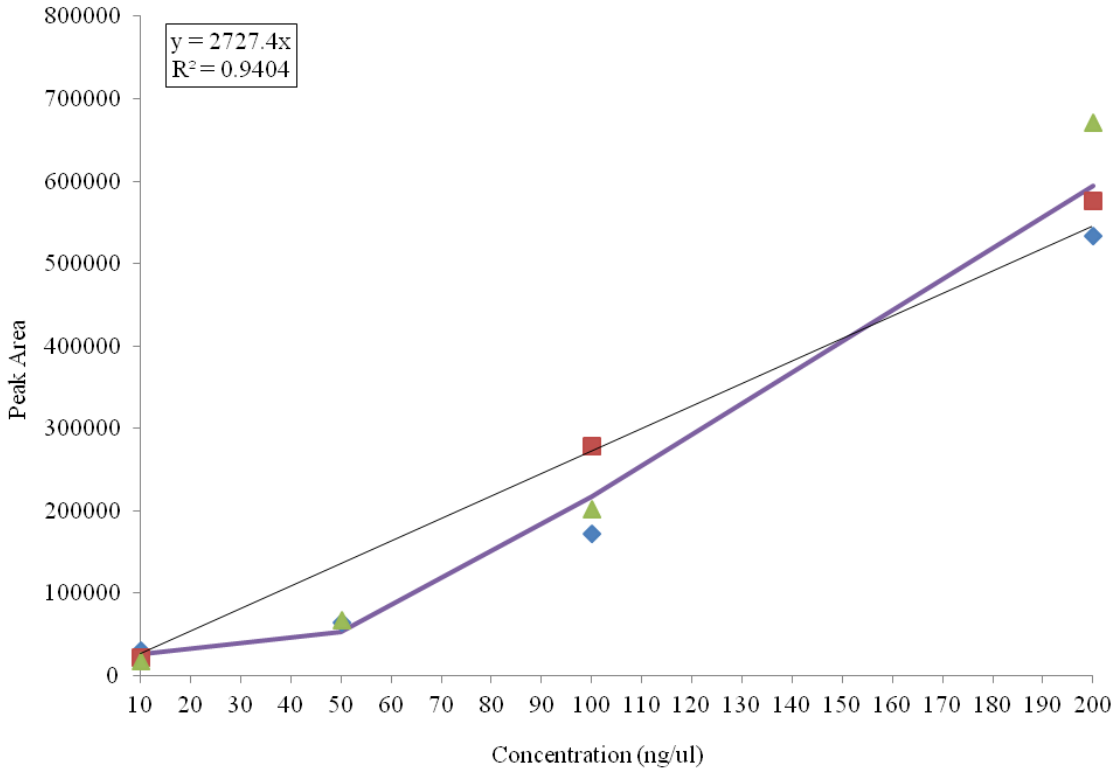

Figure 31 Calibration curve for Diclofenac

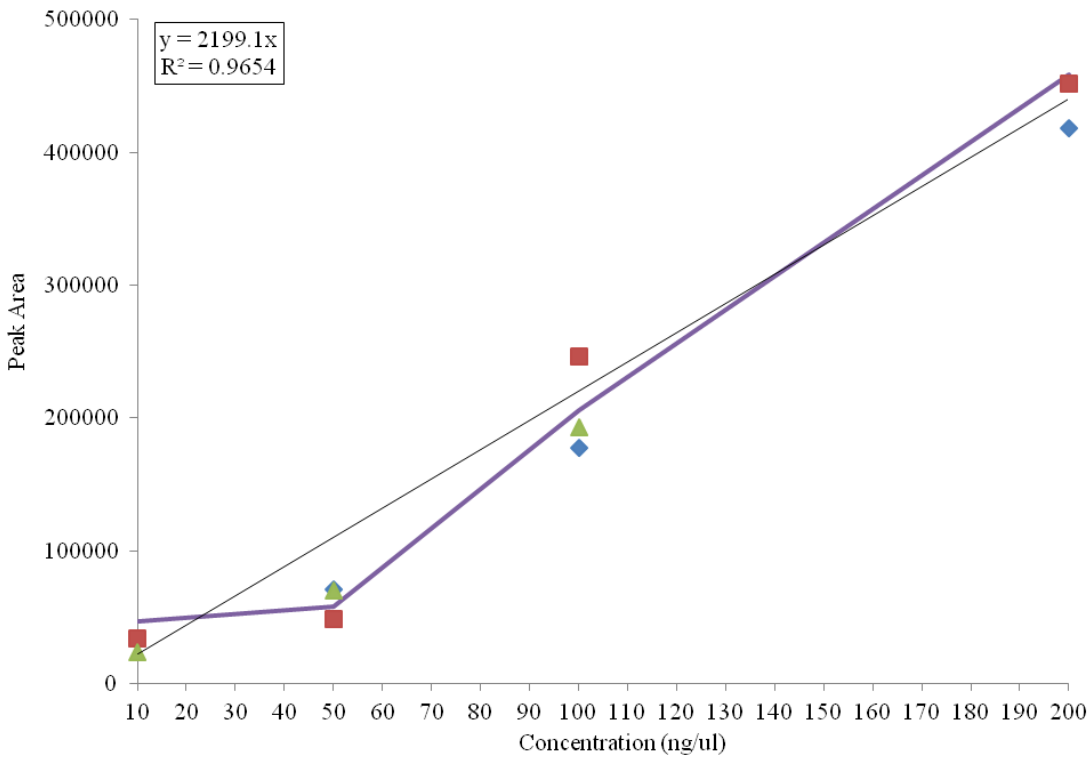

Figure 32 Calibration curve for Meclofenamic Acid

3.5.5 Analysis of a blank

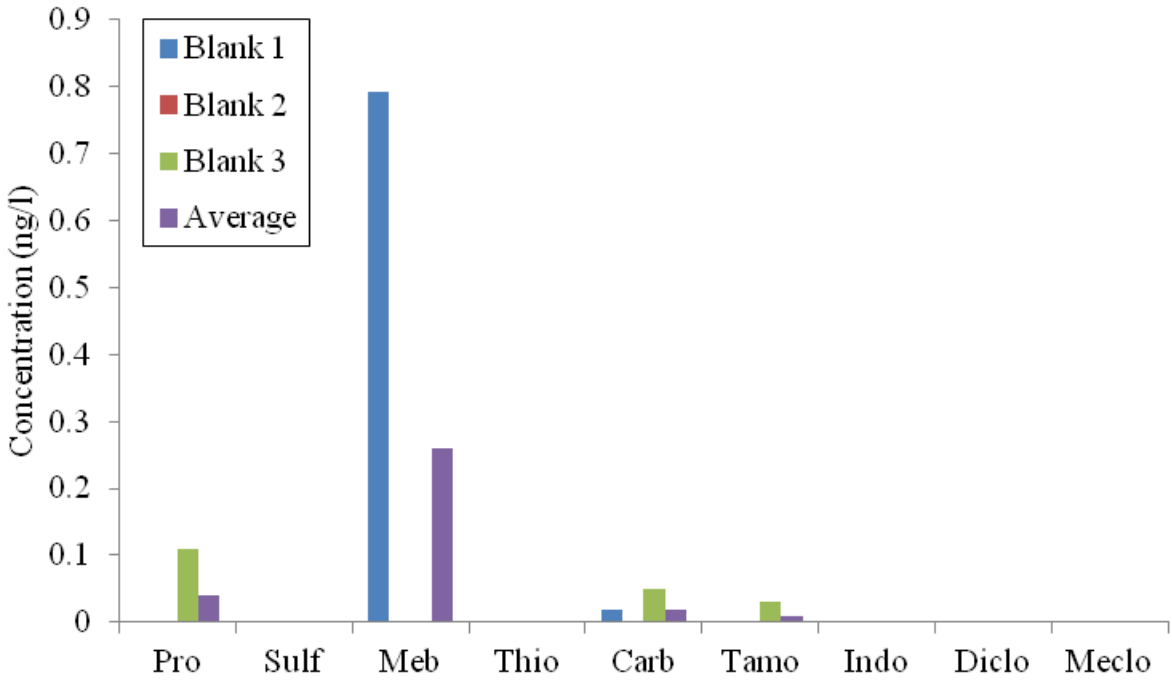

Figure 33 Analysis of a blank for target compounds

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Blank 1	0.00	0.00	0.79	0.00	0.02	0.00	0.00	0.00	0.00
Blank 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blank 3	0.11	0.00	0.00	0.00	0.05	0.03	0.00	0.00	0.00
Average	0.04	0.00	0.26	0.00	0.02	0.01	0.00	0.00	0.00
stdev	0.05	0.00	0.37	0.00	0.02	0.01	0.00	0.00	0.00
rsd	141.42	0.00	141.42	141.42	93.48	141.42	0.00	0.00	0.00
LOD	0.15	0.00	1.11	0.00	0.06	0.03	0.00	0.00	0.00

Table 12 Data for concentration of target compounds found in analysis of a three blank samples

It can be concluded that the analysis of a blank showed no significant background levels of any compound being investigated (Figure 33 and Table 12). Mebeverine has not only the highest concentration identified in a blank (0.79ng/l, table 8) (which in the scheme of this thesis is a very small scale amount), it also has the highest variability between replicates of the same sample, with a standard deviation of 0.37, which suggests this

could also be an anomalous recording of result. Mebeverine, Thioridazine, Indomethacine, Diclofenac and Meclofenamic acid all showed no background concentration in the blank sample. Remaining compounds had concentrations between 0.01 and 005ng/l, which renders the data to be irrelevant in comparison to sample analysis.

3.5.6 Direct vs. indirect injection

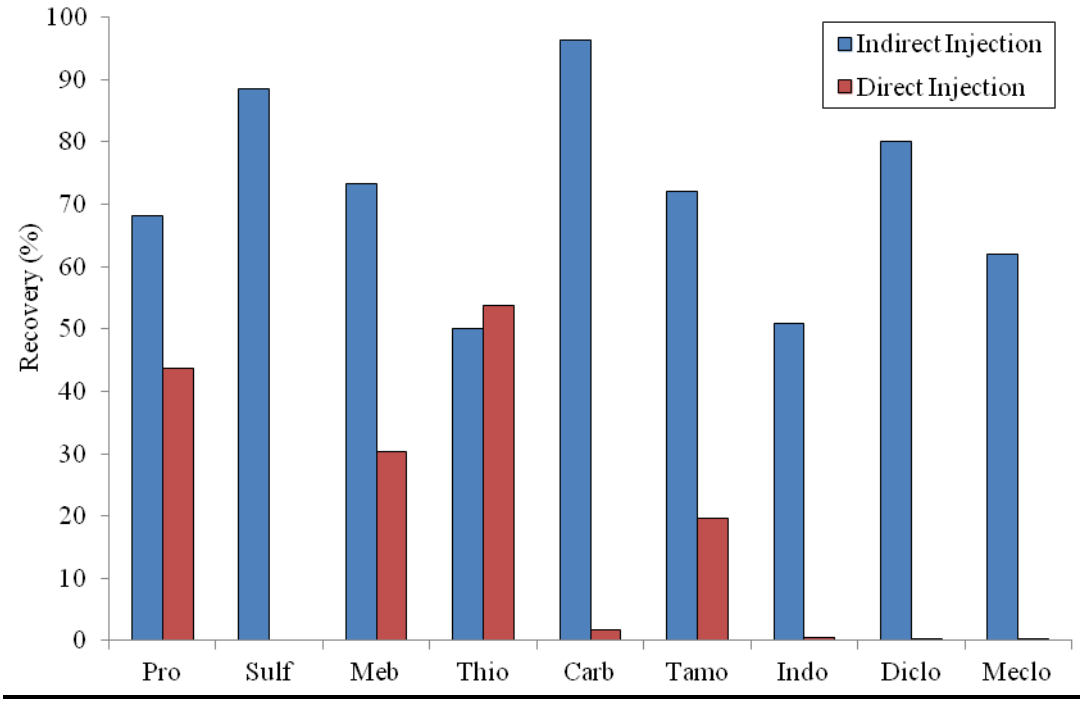

Figure 34 A comparison of all coumpounds using direct and indirect injection to SPE cartridge

When performing SPE on sample analysis it was apparent that removing the process of diluting the sample in 500ml of deionized water would clearly hasten this stage of investigation. This would only affect results, so to prove this I tested direct and indirect injection to the SPE column. Direct injection was to add the standard pharmaceutical stock solution straight onto the SPE column and indirect injection involved the process of diluting this in 500ml water, and then to draw through a vacuum so compounds would be held in the SPE column. After elution with methanol and nitrogen blow down, samples were analysed using the tested LC-MS/MS method and recovery of target compounds quantified.

Recoveries between 52 – 98 % (Thio and Carb respectively) illustrates that indirect injection outperformed direct injection, with between only 0 and 55% recovery (Figure 34). Explanation for the discrepancy between injection recoveries indicates loss of compounds via evaporation between release from syringe and absorption to SPE cartridge or immediately after the solution is injected to the SPE column. Diluting the

target compounds in 500ml deionized water ensures retention of compounds so maximum recovery is produced from the SPE filtration process.

Interestingly, Thioridazine produces the lowest recovery with indirect injection (52%) and maximum recovery for direct injection (55%). Surprisingly, direct injection outperforms indirect injection, and that the results are fairly similar. This suggests properties of Thioridazine prevents loss or retention of compounds in comparison to the two different techniques.

3.5.7 Particle size

Sample I.D.	Sample Name	Median Size (µm)	Mean Size (µm)	Average Median Size (µm)	Average Mean Size (µm)	StDev Median	StDev Mean
5up1	500m Upstream	11.12	28.50	14.96	37.48	3.87	8.30
5up2		14.92	39.07				
5up3		18.85	44.88				
up1	Upstream	20.76	75.72	18.86	78.45	4.75	39.57
up2		22.37	119.31				
up3		13.46	40.31				
effluent1	Effluent	7.78	19.37	6.95	17.47	1.17	2.68
effluent2		6.13	15.58				
effluent3		439.16	430.47				
down1	Downstream	14.10	57.71	13.56	42.20	2.56	15.92
down2		10.76	25.91				
down3		15.80	42.99				

Table 13 Particle size analysis data for River Ouse sampling locations

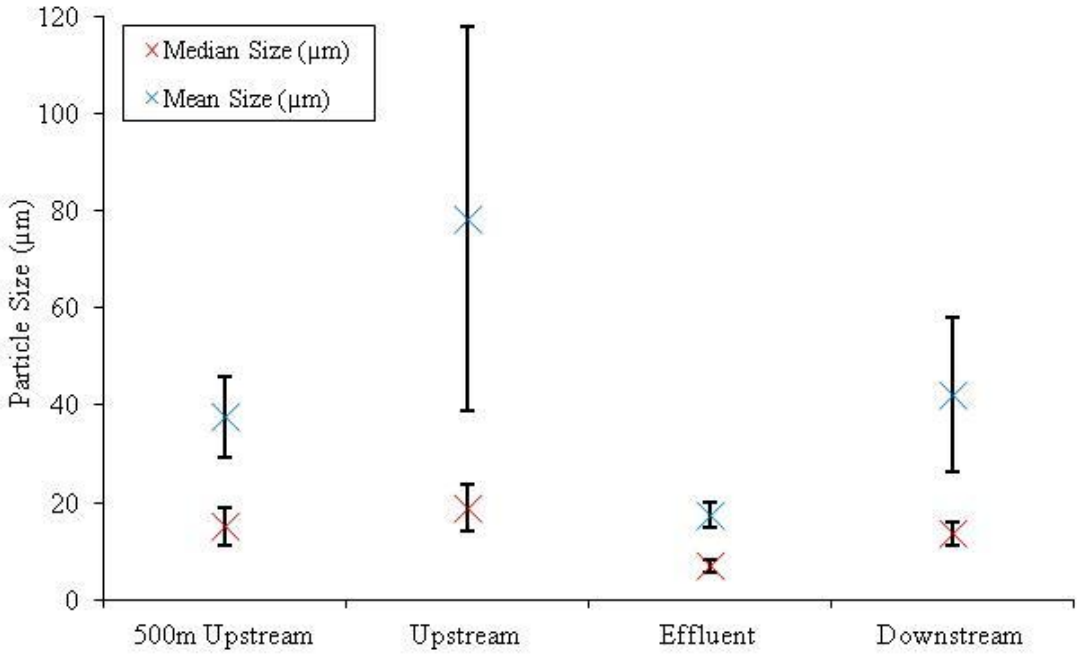

Figure 35 Analysis of paricle size statistics from the River Ouse (µm)

Particle size analysis was conducted using a HORIBA Partica LA-950 for Windows [Wet] Ver3.50 (Laser Scattering Particle Size Distribution Analyzer) for each sample, including replicates, for the River Ouse sediment analysis. The average median size of particles ranged between 6.95 – 18.86µm and the average mean size of particles ranged between 17.47 – 78.45µm (Table 13). Figure 35 clearly demonstrates that the Upstream location had the largest particle size, with a mean of 78.45µm. 500 meters upstream from the effluent size the average particle size was 37.48µm, effluent site was the lowest observed mean particle size of 17.47µm. Downstream from the effluent site, a mean particle size of 42.40µm was observed. Where the maximum particle size was observed, there was a high variability between sample replicates, with a standard deviation of 39.57. The effluent site had the best standard deviation of 2.68, which suggests that there are strict regulations made by the sewage treatment works at this location. There is an anomalous result the effluent site (effluent 3) which was not included in the analysis of results and has been discarded for further analysis. It is most likely a cause of larger particle filtrating to the sample on pre-treatment for the particle size analyzer.

Full particle size analysis data output from the HORIBA can be seen in Appendix 1.

3.5.8 Sample stability testing

Over a period of six weeks the stability of standard pharmaceutical stock solutions (200ppm) were monitored for loss of compounds and stability of samples. Stored under 4 different conditions (room temperature covered, room temperature uncovered, fridge temperature covered and freezer temperature covered), a triplicate sub sample was taken on a weekly basis and analyzed for concentration of pharmaceutical compounds (Figure 36, 37 and 38). Full data for these are found in Appendix 2 and 3.

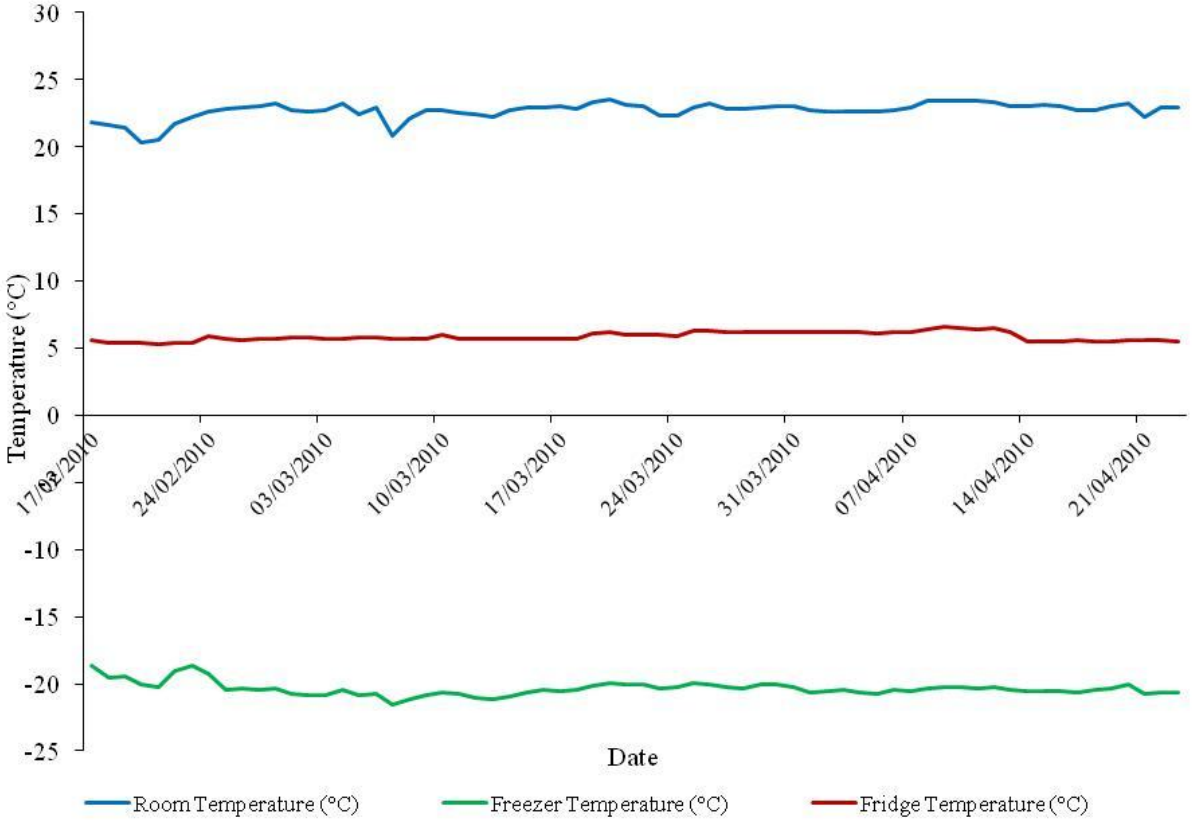

Figure 36 Temperature data for three different storage options

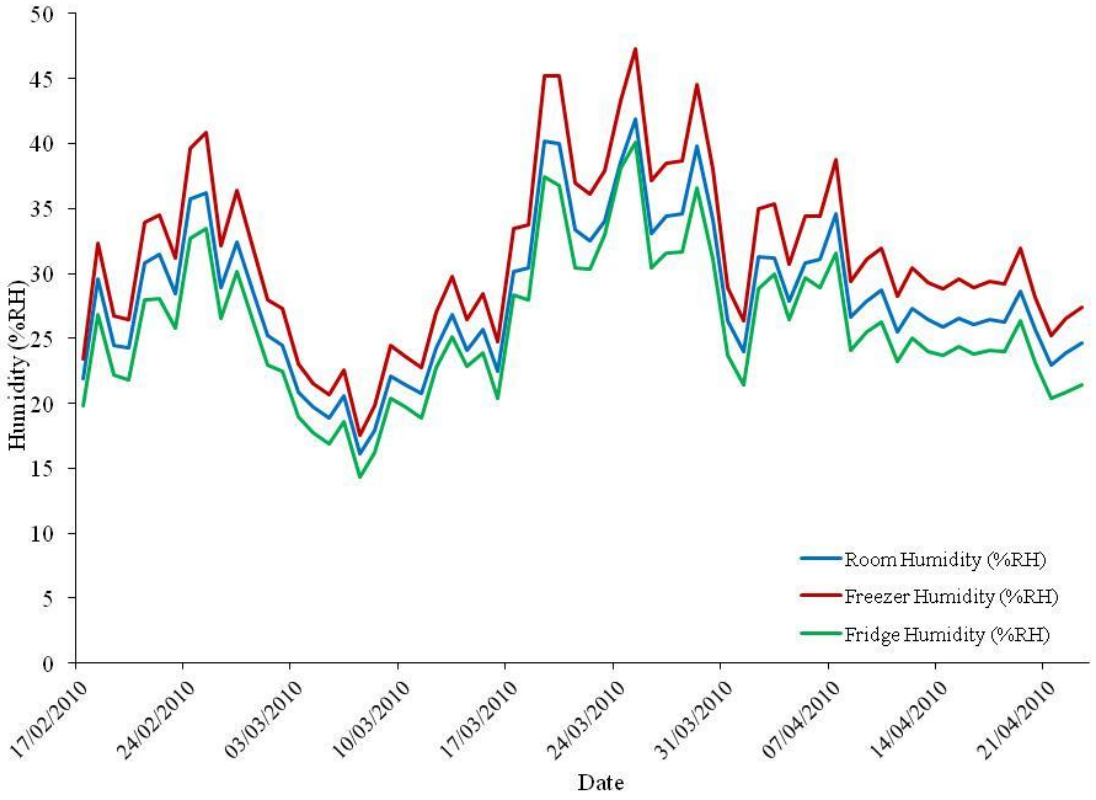

Figure 37 Relative Humidity (%RH) of the three storage solutions

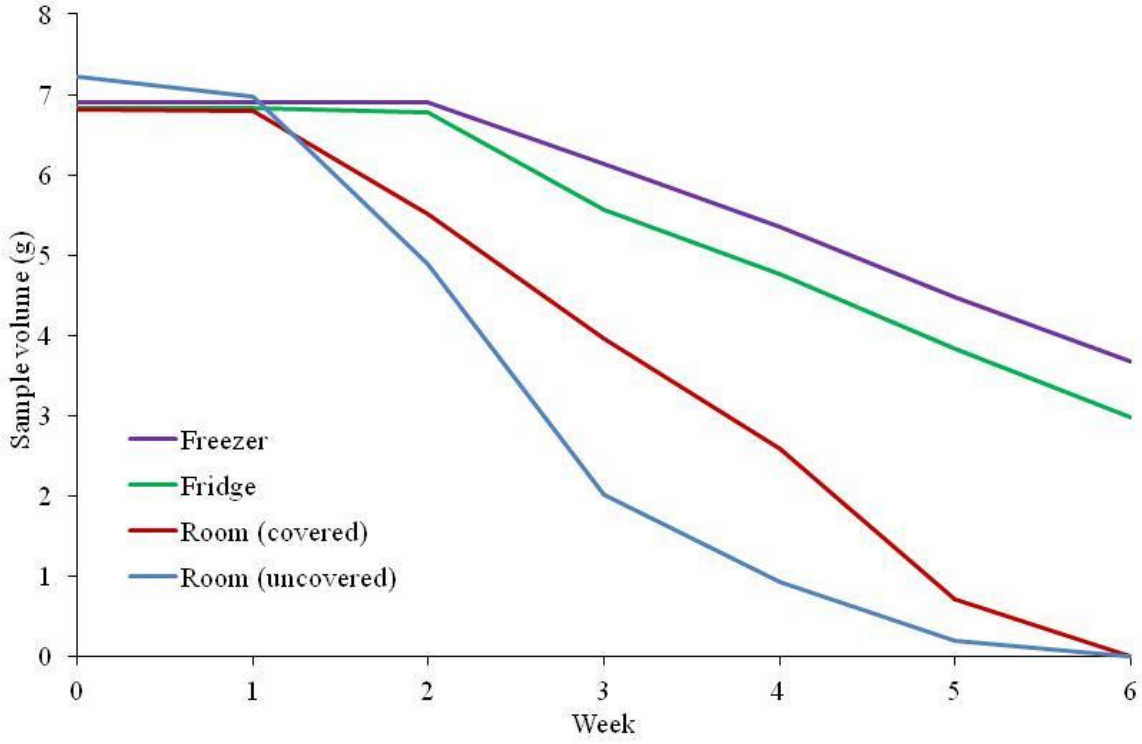

Figure 38 Sample volume losses (g) during stability testing

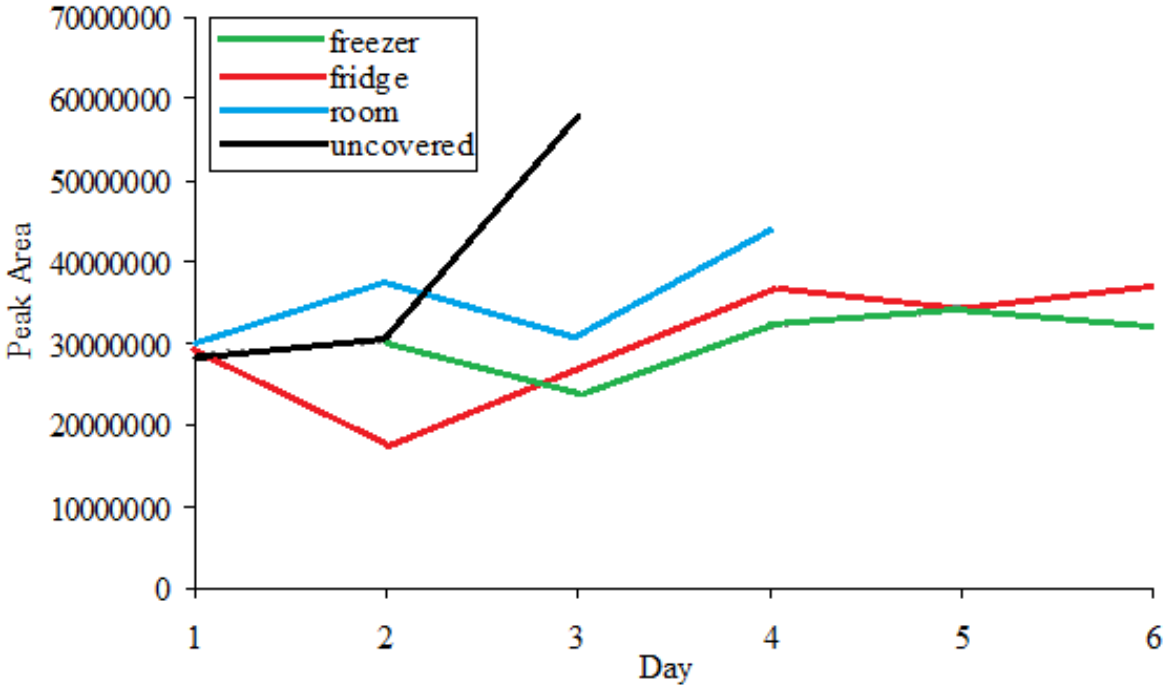

Figure 39 Stability of Propanolol over 10 weeks under different storage conditions

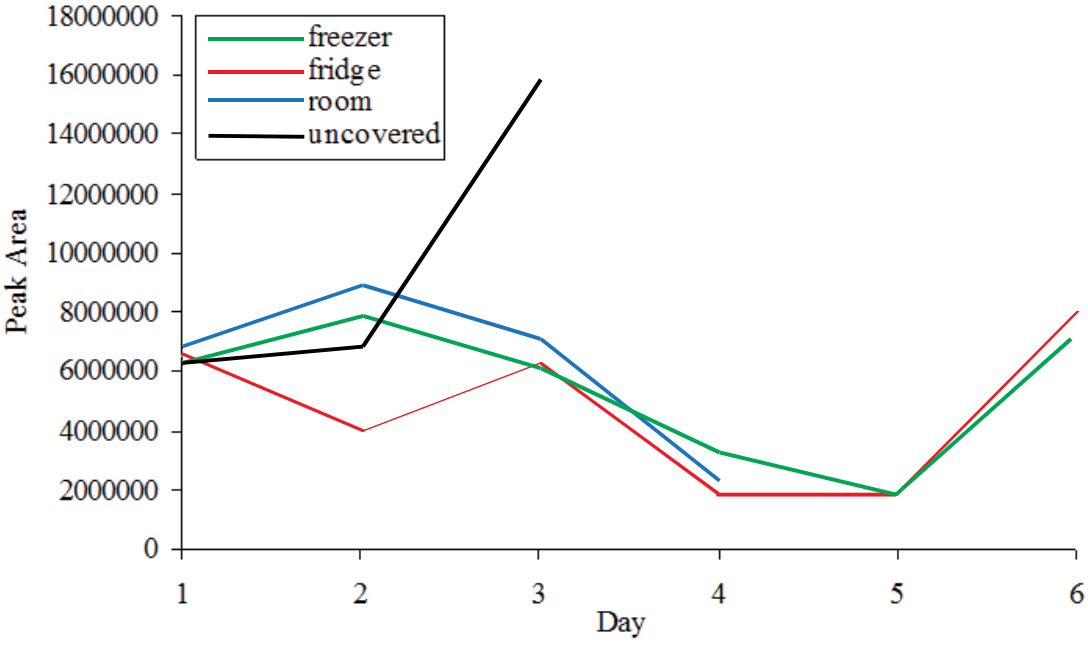

Figure 40 Stability of Sulfamethaxazole over 10 weeks under different storage conditions

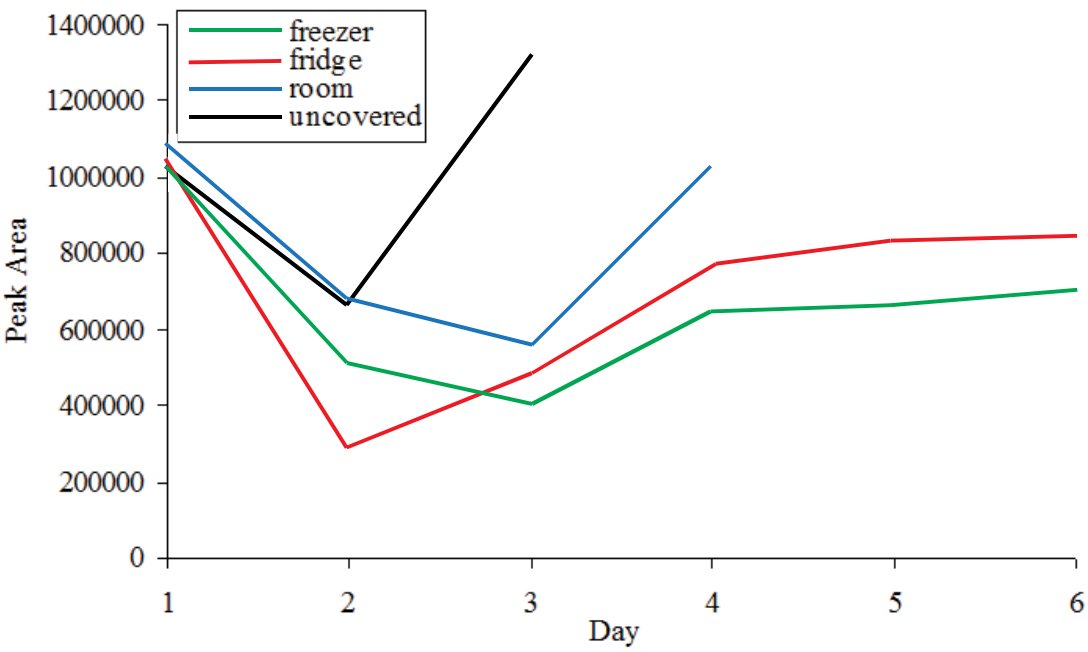

Figure 41 Stability of Mebeverine over 10 weeks under different storage conditions

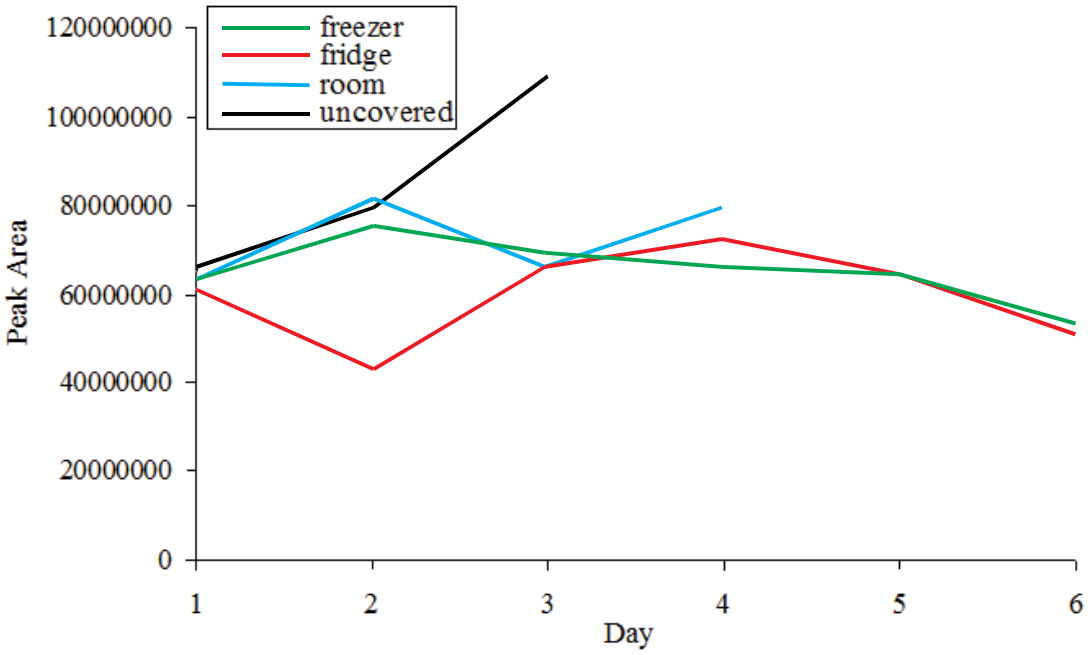

Figure 42 Stability of Thioridazine over 10 weeks under different storage conditions

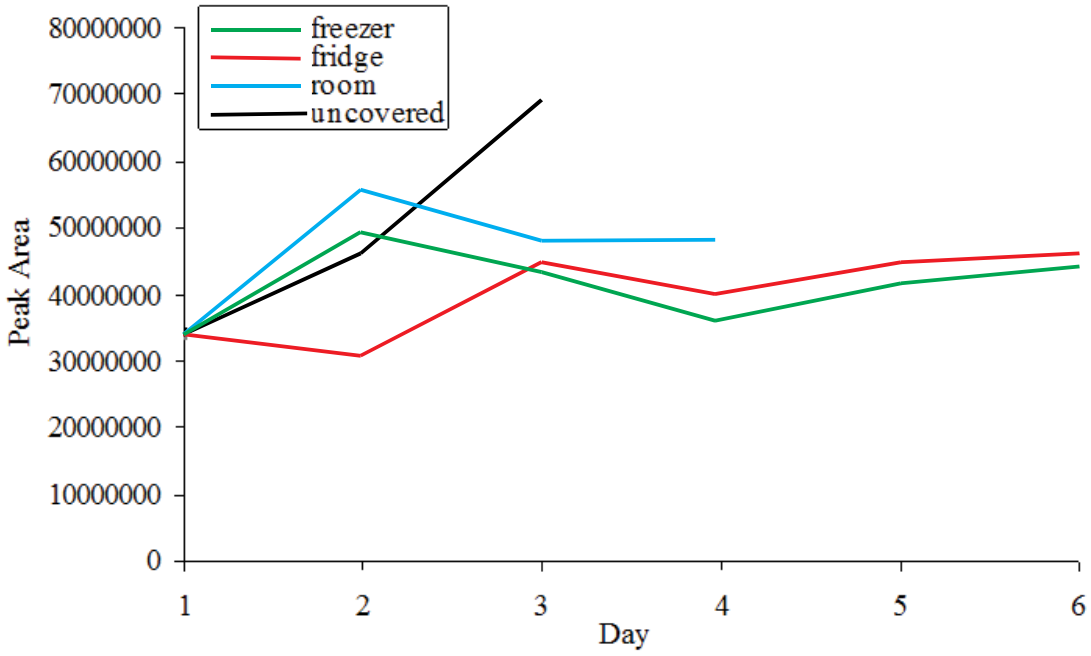

Figure 43 Stability of Carbemazepine over 10 weeks under different storage conditions

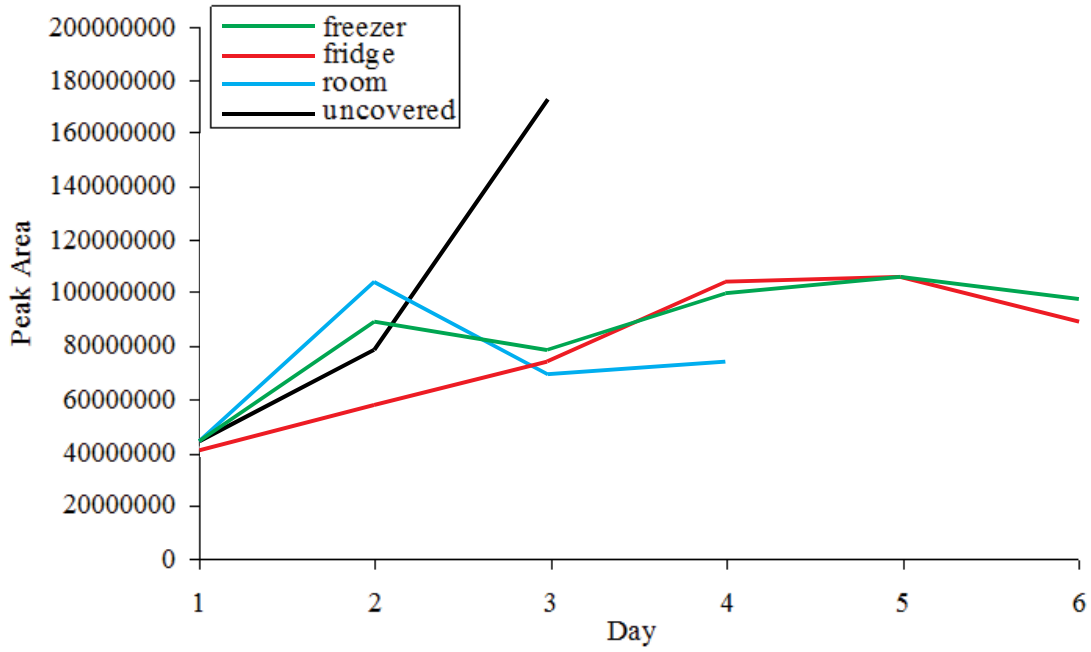

Figure 44 Stability of Tamoxifen over 10 weeks under different storage conditions

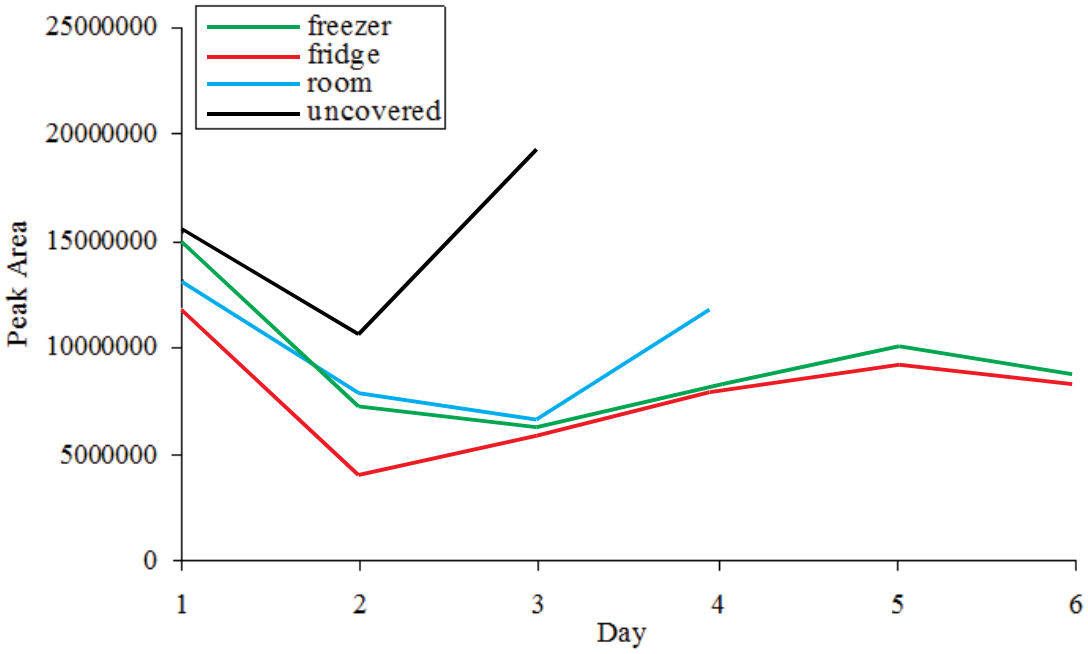

Figure 45 Stability of Indomethacine over 10 weeks under different storage conditions

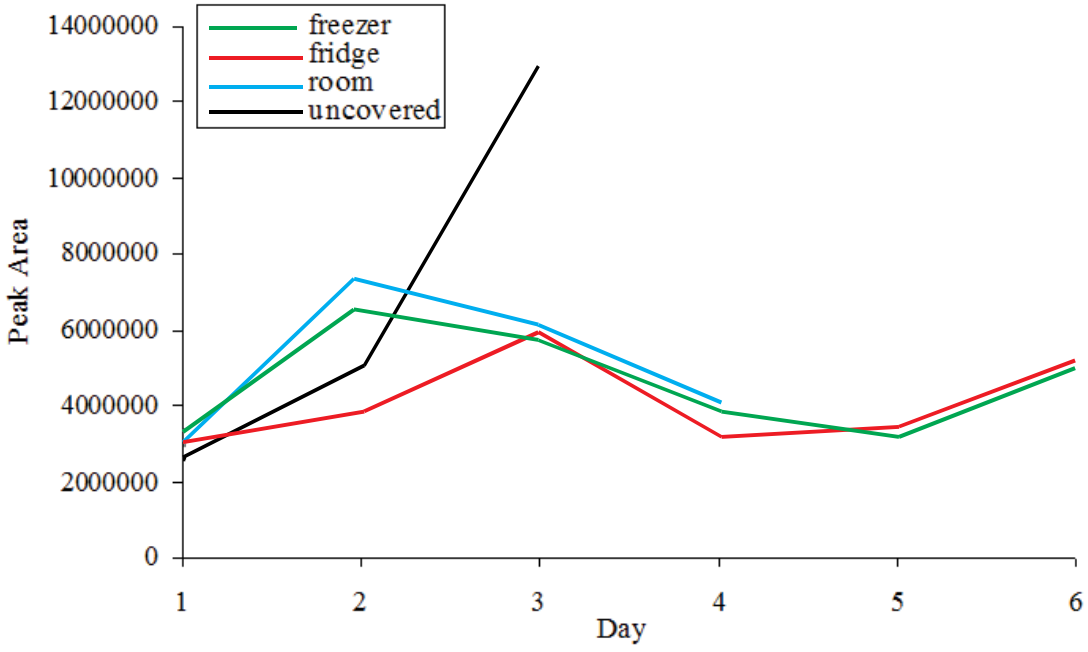

Figure 46 Stability of Diclofenac over 10 weeks under different storage conditions

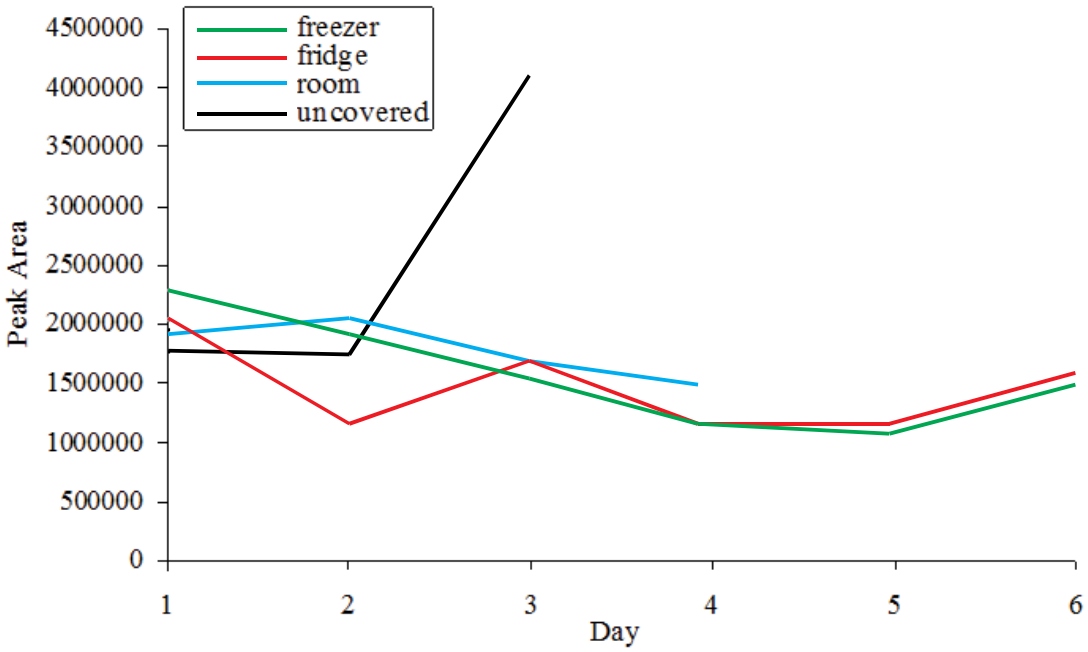

Figure 47 Stability of Meclofenamic Acid over 10 weeks under different storage conditions

After three weeks (day 3), all compounds in the 200ppm standard stock solutions had evaporated from uncovered samples at room temperature, as was the case after four weeks (day 4) for those stored at room temperature with a cover (Figure 39 - 47). The sample loss may have occurred between reading 2 and 3 or 3 and 4 for some compounds, however it is unknown the exact time due to weekly readings. To identify when exactly this happened, hourly readings would need to be considered. The covered sample may have allowed a small volume of the solvent matrix to be evaporated even though to the human eye a sufficient seal had been created. These samples were also exposed to light unlike the remaining two storage methods. All compounds in the uncovered sample increased after the second week of sample extraction. Exposure to surrounding air may have meant that the sample was contaminated with those compounds already present in the atmosphere which would increase existing levels in the sample. Target pharmaceutical compounds were also relatively volatile and so the uncovered sample rapidly lost those compounds under investigation.

It is observed that between the two remaining storage solutions in the fridge and freezer that overall, the fridge temperature sample produced the highest amount (peak area) of all target compounds excluding Indomethacine. As only one compound would be

compromised with this storage method, sample storage at fridge temperature is observed to be the most preferable. However it must be noted that this result is possibly due to errors in analytical protocols, despite the preferred storage conditions being given as a result. As there are no error bars provided in the discussed figures, it is difficult to comment on the results and so results are in this case assumptions based on available data.

3.5.9 Matrix Effect

It was important to consider the effect of the matrix on sample recovery and compound quantification as if a significant amount of known spiked compound was lost purely owing to a selected extraction method, it may affect results and give lower false amounts.

Firstly a sample containing no matrix, ie solvent and spiked compounds only, was analysed at 8 different concentrations on the LC-MS/MS to determine absolute optimum recovery (100%). The no matrix solution is simply a standard solution as a comparison to samples containing a matrix to assess the matrix effect. These 8 concentrations (10, 50, 100, 200, 500, 1000, 1500 and 2000ppm) were then analysed using three different extraction techniques; ultrasonication, microfugation and MAE. It was expected that with the addition of the matrix that compounds would be readily retained in the sediment sample. For these experiments a tested clean sediment sample was used to minimise background concentrations of target compounds. These concentrations were subtracted from the final result. Thioridazine, carbemazepine and Tamoxifen always obtained highest concentration recovery irrelevant of extraction technique. Results are displayed in Figure 48 – 51.

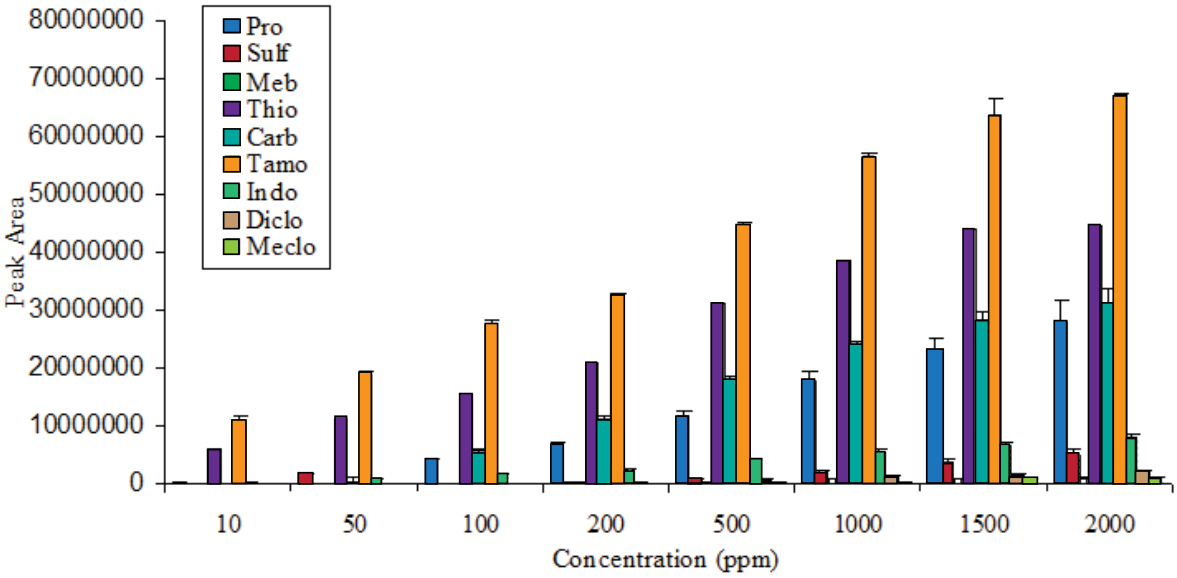

Figure 48 No matrix

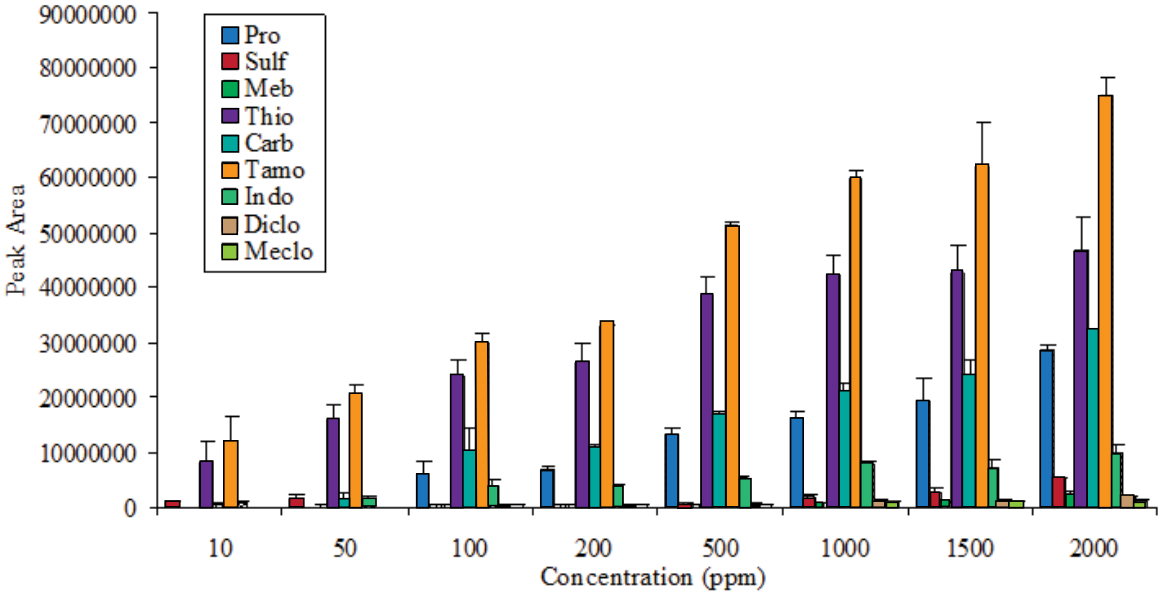

Figure 49 The effect of Ultrasonication on matrix effect

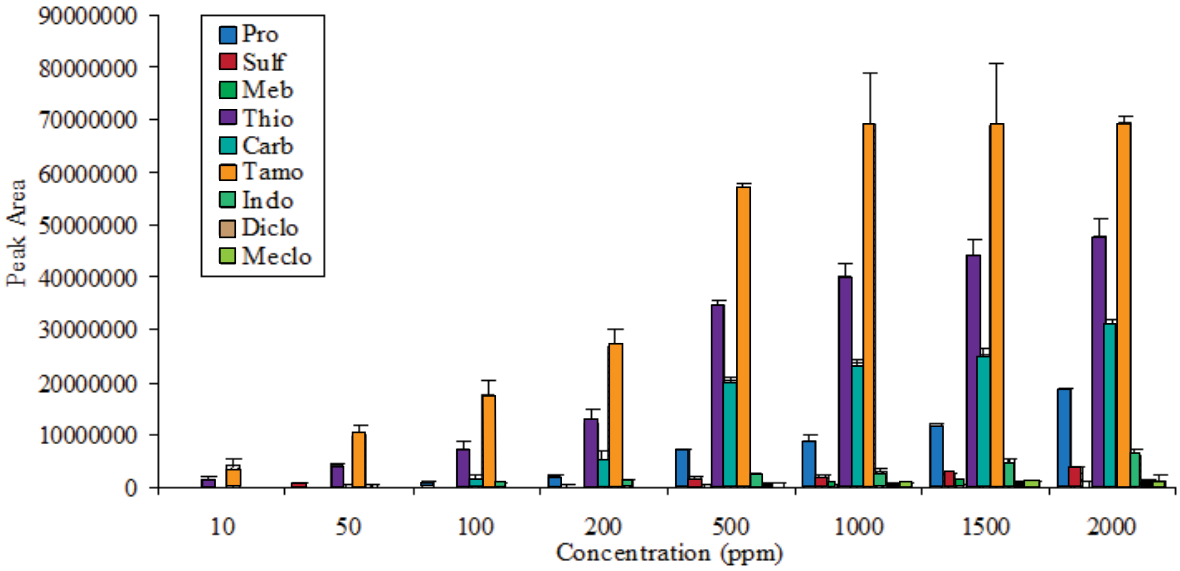

Figure 50 The effect of Microfugation on matrix effect

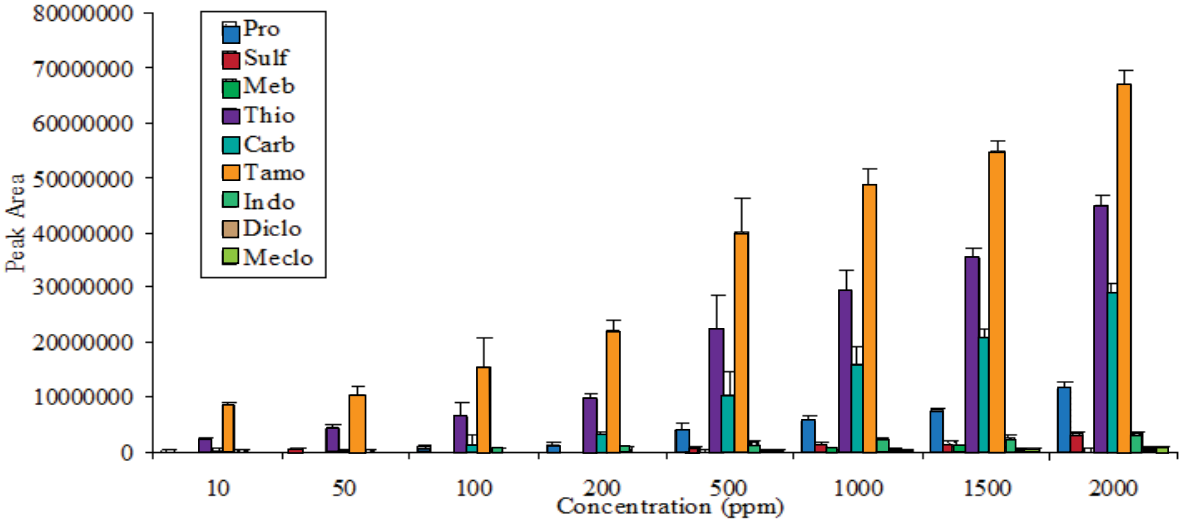

Figure 51 The effect of MAE on matrix effect

3.5.9.1 Compound by compound

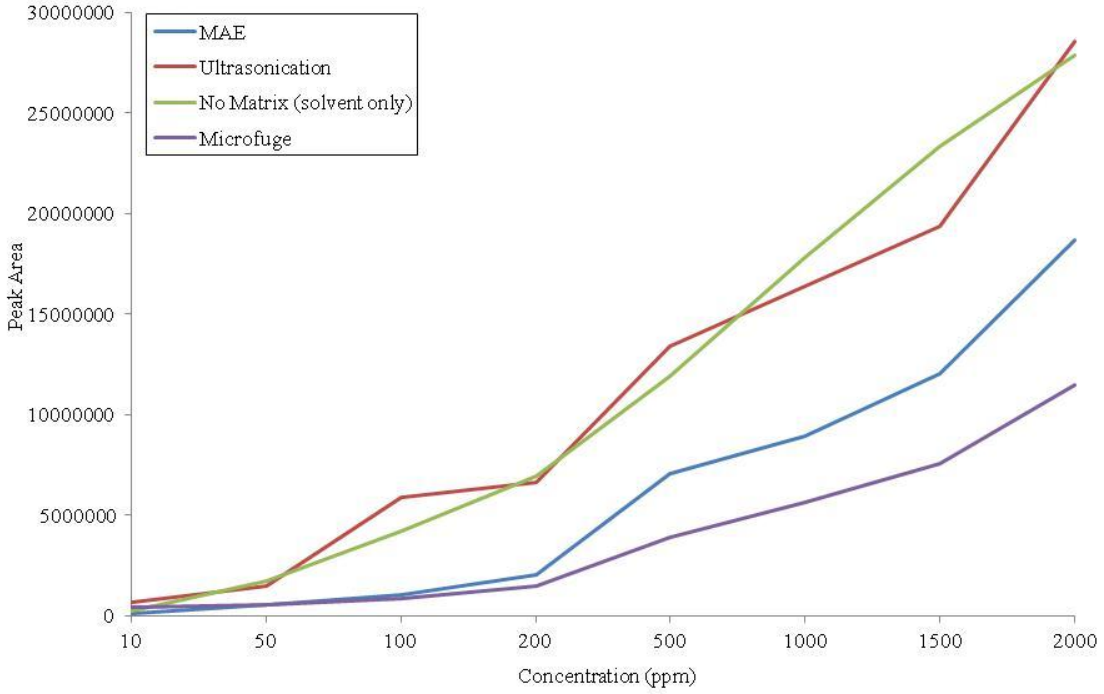

Figure 52 Different conditions affecting the matrix effect of Propanolol

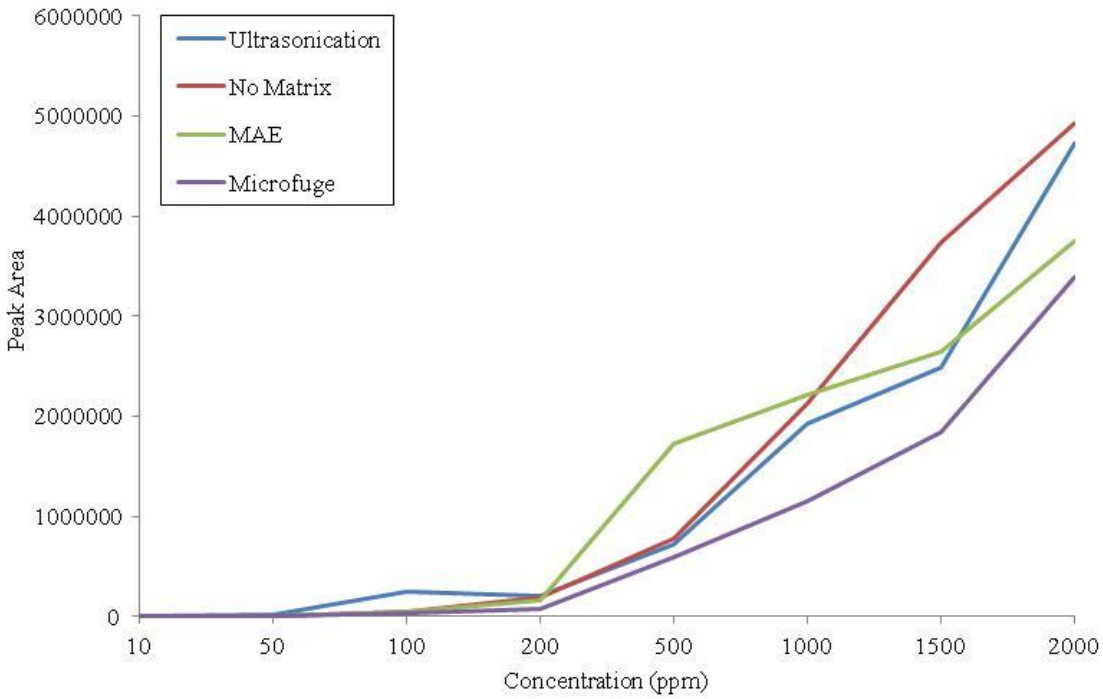

Figure 53 Different conditions affecting the matrix effect of Sulfamethaxazole

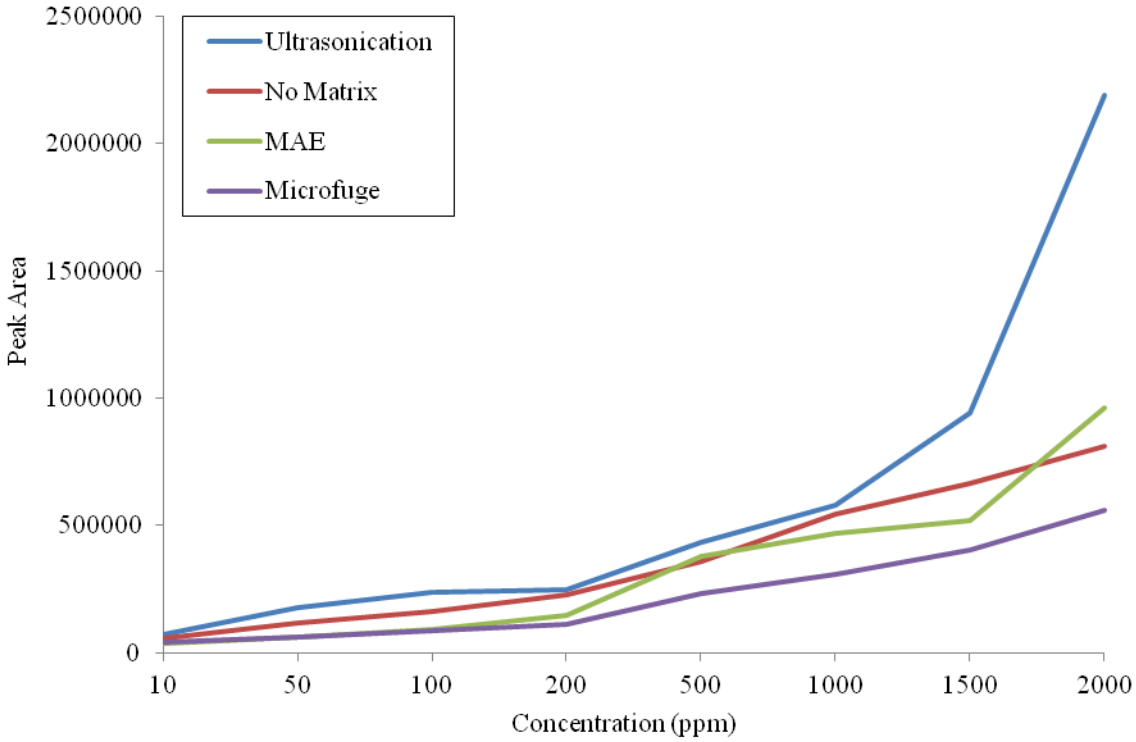

Figure 54 Different conditions affecting the matrix effect of Mebeverine

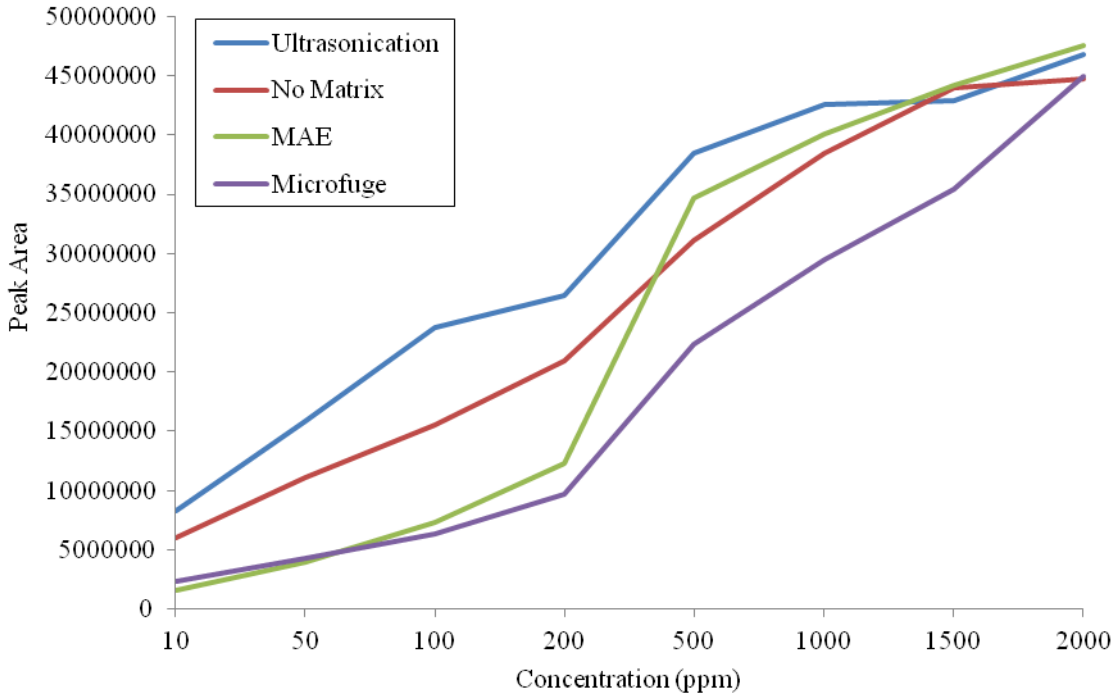

Figure 55 Different conditions affecting the matrix effect of Thioridazine

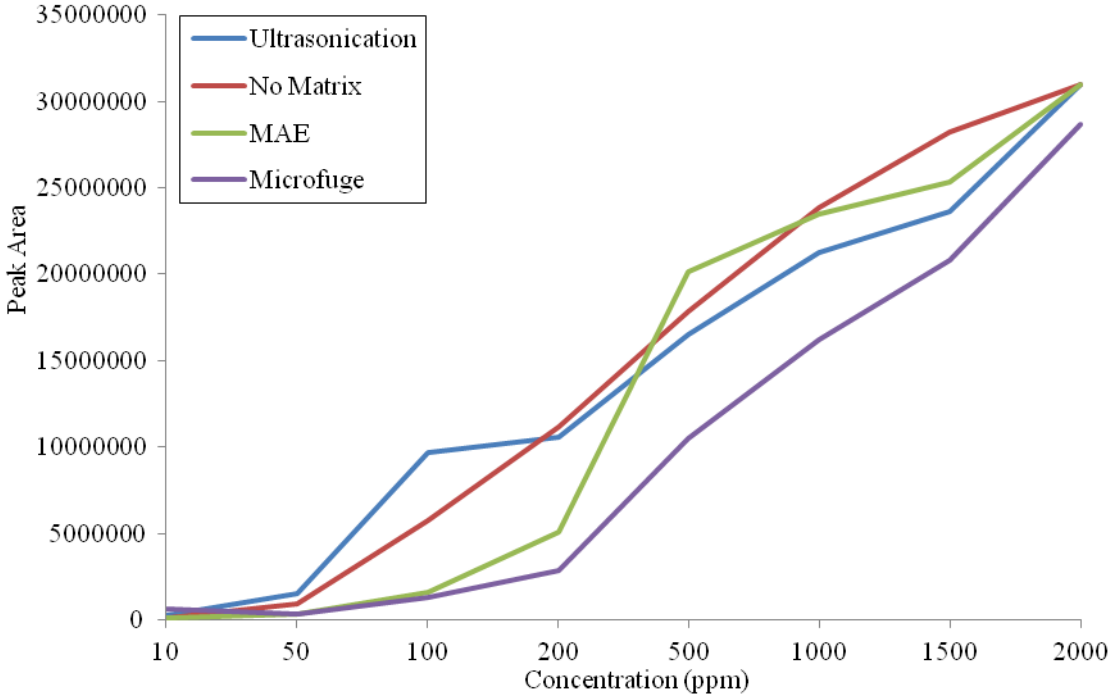

Figure 56 Different conditions affecting the matrix effect of Carbemazepine

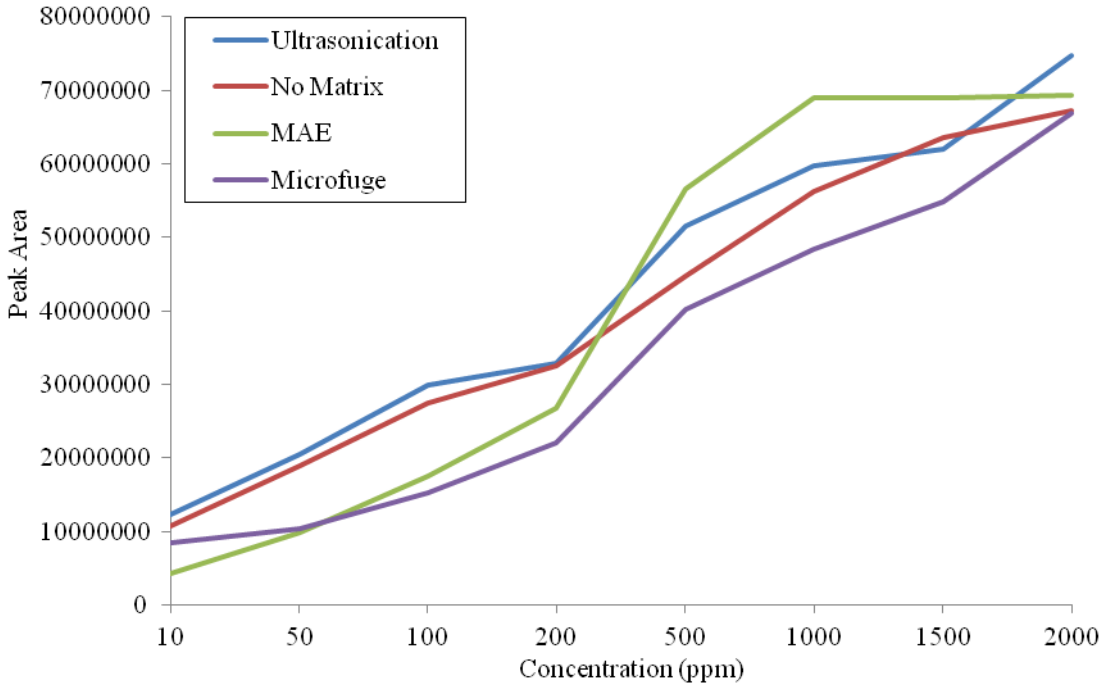

Figure 57 Different conditions affecting the matrix effect of Tamoxifen

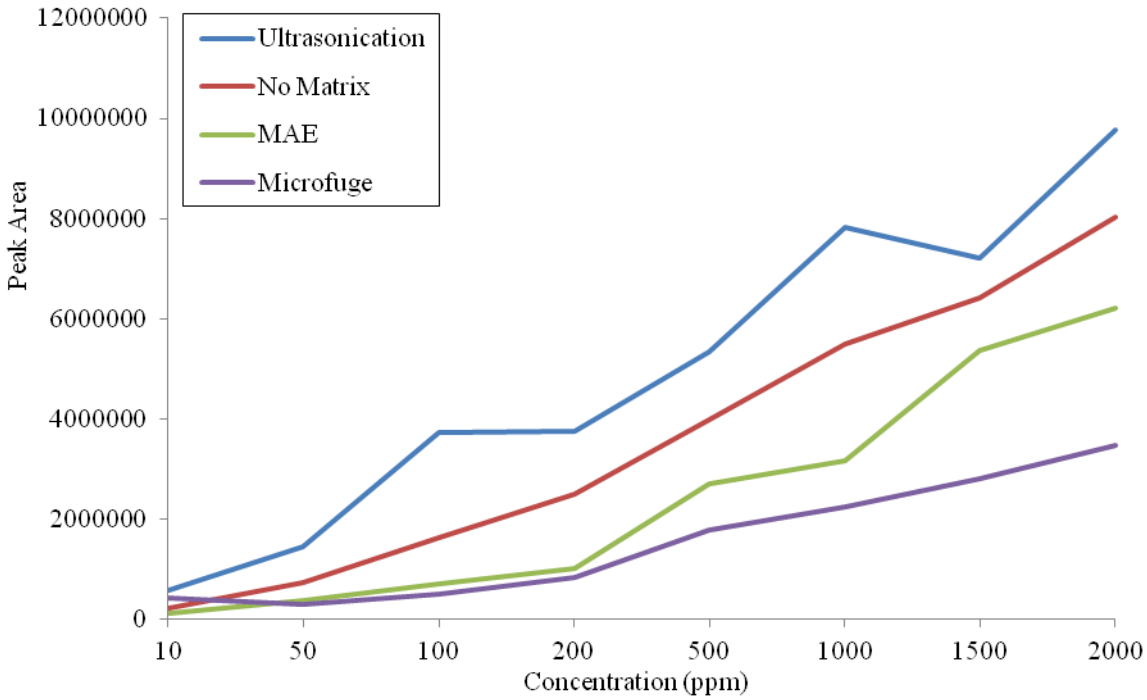

Figure 58 Different conditions affecting the matrix effect of Indomethacine

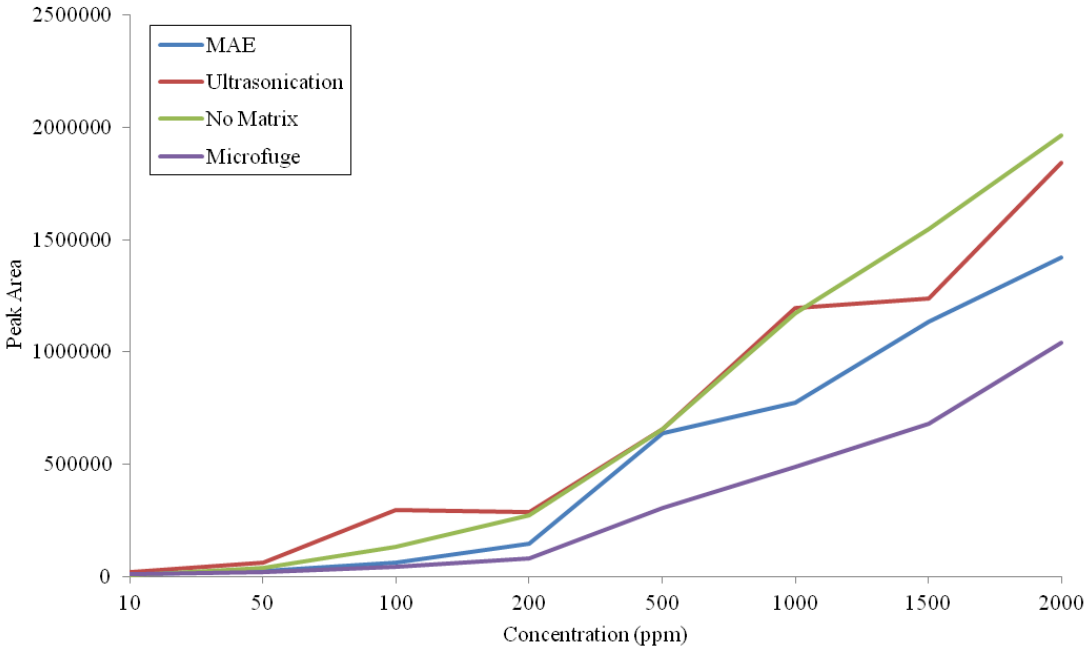

Figure 59 Different conditions affecting the matrix effect of Diclofenac

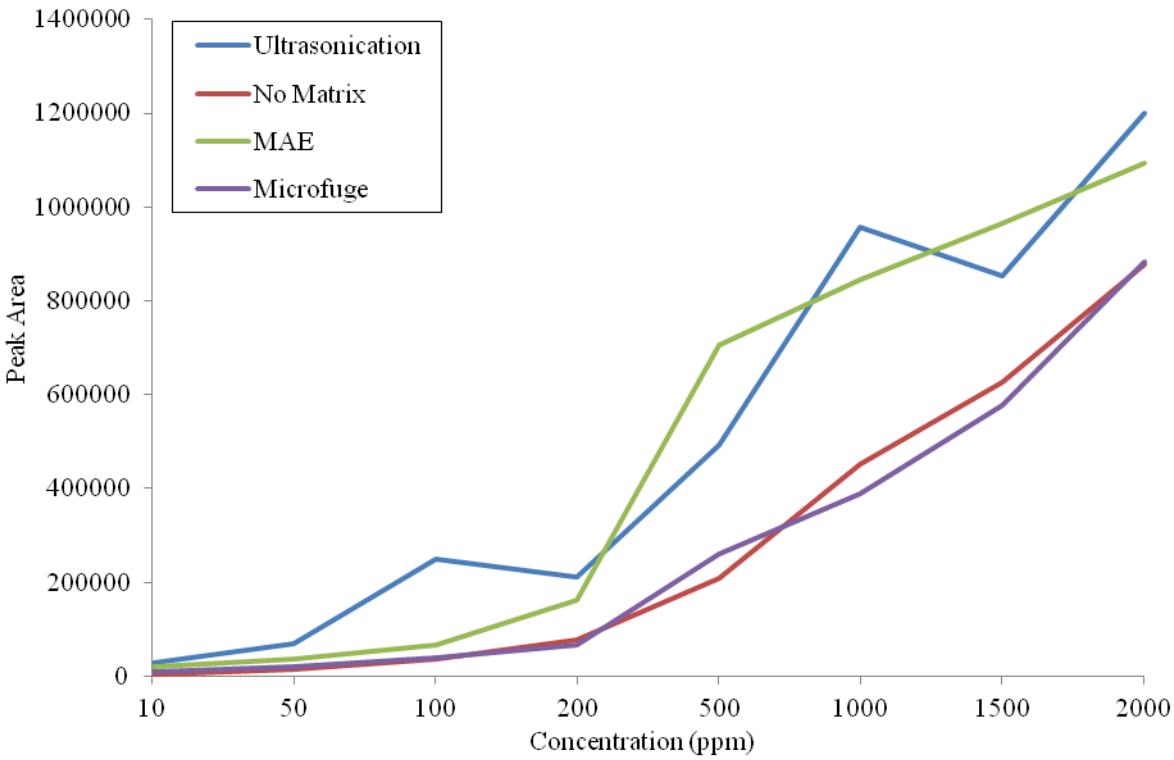

Figure 60 Different conditions affecting the matrix effect of Meclofenamic Acid

Compound	Extraction Method	10	50	100	200	500	1000	1500	2000
Pro	Ultrasonication	654335.6	1505998.2	5889040	6633493	13389999	16373719	19363265	28562990
	No Matrix	232897	1704087.2	4187361	6959406	11912919	17796931	23346081	27885177
	MAE	130457.9	531191.56	1057884	2014050	7068718	8917420	12047628	18682930
	Microfuge	426531	534418.29	881446.2	1462755	3892074	5628995	7562895	11450468
Sulf	Ultrasonication	5197.603	17669.843	257285.8	203343.1	726103.5	1933092	2486482	4730446
	No Matrix	1944.691	10061.485	52170.57	189069.7	786275.8	2135699	3738977	4924089
	MAE	4232.557	11476.187	43593.06	163576.6	1726292	2221110	2651249	3751246
	Microfuge	3264	11210.225	35792.23	71008.34	588137.4	1155725	1842576	3386314
Meb	Ultrasonication	72168.05	176037.09	237341	246683.1	433032.4	578793.5	940594.9	2188634
	No Matrix	57292.04	116283.1	161590.5	228208	360381.2	546589.5	665326.6	812134.3
	MAE	34500.58	62481.677	94360.88	149192.6	377699.9	467568.3	521684	962154
	Microfuge	43625	63155.348	85260.18	110009	233736.6	308309.5	403465.3	558672.5
Thio	Ultrasonication	8243437	15856814	23788117	26497016	38449646	42536067	42904816	46771376
	No Matrix	5967521	11131669	15515082	20886195	31113333	38515228	43945808	44721469
	MAE	1549628	4007290.1	7291371	12331959	34702108	40081180	44213268	47512365
	Microfuge	2315486	4329846.5	6302520	9708877	22338814	29461855	35407181	44917979
Carb	Ultrasonication	261148.1	1532813.3	9703134	10604853	16529519	21288420	23650402	30955826
	No Matrix	81596.27	923798.21	5749273	11182770	17866317	23839597	28201629	30984838
	MAE	75977.76	360266.33	1624761	5060420	20117209	23493485	25315492	30945321
	Microfuge	645213	351220.45	1291165	2870050	10500765	16187895	20827381	28704063
Tamo	Ultrasonication	12252385	20517798	29966033	32948120	51565039	59727233	62011755	74778432
	No Matrix	10760782	18882765	27413484	32580304	44814924	56274605	63656660	67214287
	MAE	4273851	9910785.2	17501031	26789627	56645448	68933518	68952199	69314258
	Microfuge	8512341	10349461	15238911	22072875	40246548	48371731	54830700	66821109
Indo	Ultrasonication	589547.2	1457637	3728475	3745315	5327826	7817558	7213059	9778408
	No Matrix	224917.5	736715.27	1632753	2484964	3989310	5495329	6416809	8017631
	MAE	112593.1	370805.11	699251.5	1012457	2713417	3151472	5362147	6214587
	Microfuge	425319	282549	492561.1	842324.2	1769779	2250975	2792071	3461670
Diclo	Ultrasonication	18313.98	59845.363	294989.8	287179.7	657118.1	1197502	1237739	1842226
	No Matrix	5867.163	37318.9	133899.8	272285.9	657010.8	1172072	1546987	1964949
	MAE	10309.16	25176.426	61212.13	144486.3	638343.9	772802.9	1136895	1421579
	Microfuge	10945	19565.45	41395.58	83029.79	304912.7	490082.4	682596.3	1042737
Meclo	Ultrasonication	29025.32	69533.376	250866.1	211276.1	493061.1	957174.6	852063	1199917
	No Matrix	4363.477	15225.52	37073.96	77932.36	207728.9	452223.8	627758.4	878195.3
	MAE	19618.83	35832.637	67618.57	162974	704383.4	844494.4	965472	1092444
	Microfuge	10374	19994.517	38105.65	66458	260820.6	389566.5	576845.3	883452.7

Table 14 Summary of Matrix Effect data on all target compounds

It was observed from figures 52 to 60 that compound recovery had been significantly diminished under all conditions, with the smallest matrix effect observed using U extraction. For compounds Meb, Thio, Tamo, Indo and Meclo, ultrasonication significantly increased observed peak area for all concentrations possibly owing to agitation releasing a higher concentration of the target compound in the sediment sample. It must be noted that the sediment sample used may have already contained an unknown amount (although minimal) of target compounds. This was also the case for Meb tested using MAE, due to other physiochemical properties such as boiling and melting temperature. Table 14 gives full data information.

Microfugation caused the lowest compound peak area observations. During the micro filtration process compounds may become trapped in the sediment left behind, leaving only a small amount to pass through the filter film for analysis. For this reason microfugation was not tested at any further stage during method developent and analysis.

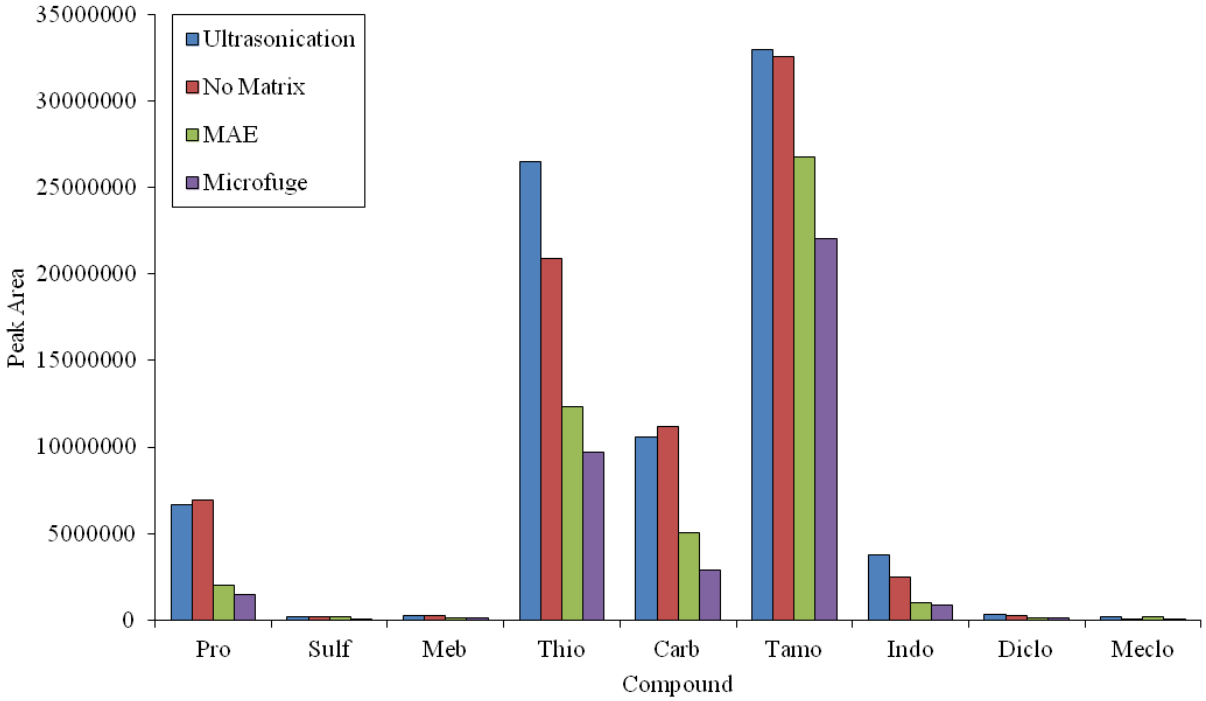

Figure 61 Matrix of all compounds at 200ppm

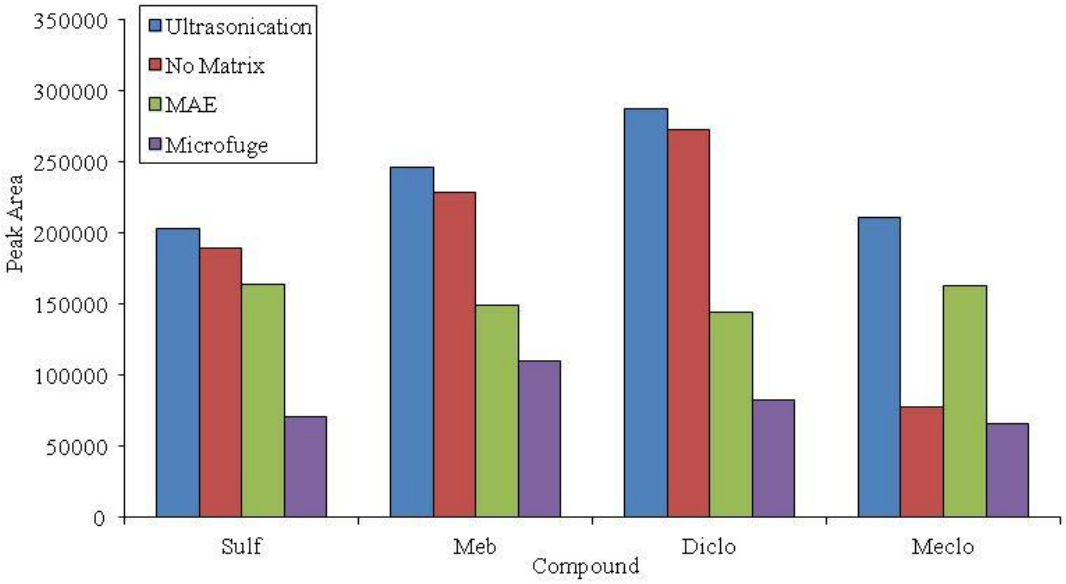

Figure 62 Normalization of 4 compounds at 200ppm

A concentration of 200ppm stock solution of pharmaceuticals was identified to be the most effective for sample testing and analysis. Illustrated in figure 61, it is evident that U produces overall best compound recovery, and outperforms a solvent only sample tested to illustrate the effect of no matrix. Sulf, Meb Indo, Diclo and Meclo although all producing conclusive results, were on a smaller scale and so a normalised results graph illustrates again how U outperforms any other extraction method (Figure 62), with microfugation, as for all compounds, producing poorest results. All compounds are successfully recovered at a good volume using 200ppm compound solutions with U, therefore the IS stock solution of Diuron was produces at a concentration of 200ppm and used to spike all subsequent experiments. U was the extraction method of choice. Table 15 gives the peak area for the matrix effect of the method.

	Ultrasonication	No Matrix	MAE	Microfuge
Pro	6633493.0	6959405.7	2014049.7	1462755.3
Sulf	203343.1	189069.7	163576.6	71008.3
Meb	246683.1	228208.0	149192.6	110009.0
Thio	26497015.5	20886195.3	12331959.0	9708877.0
Carb	10604853.0	11182770.0	5060419.9	2870050.0
Tamo	32948119.5	32580304.0	26789626.8	22072875.0
Indo	3745314.8	2484963.9	1012456.6	842324.2
Diclo	287179.7	272285.9	144486.3	83029.8
Meclo	211276.1	77932.4	162974.0	66458.0

Table 15 Peak area for matrix effect of developed analytical method

3.5.10 Inter-day variability

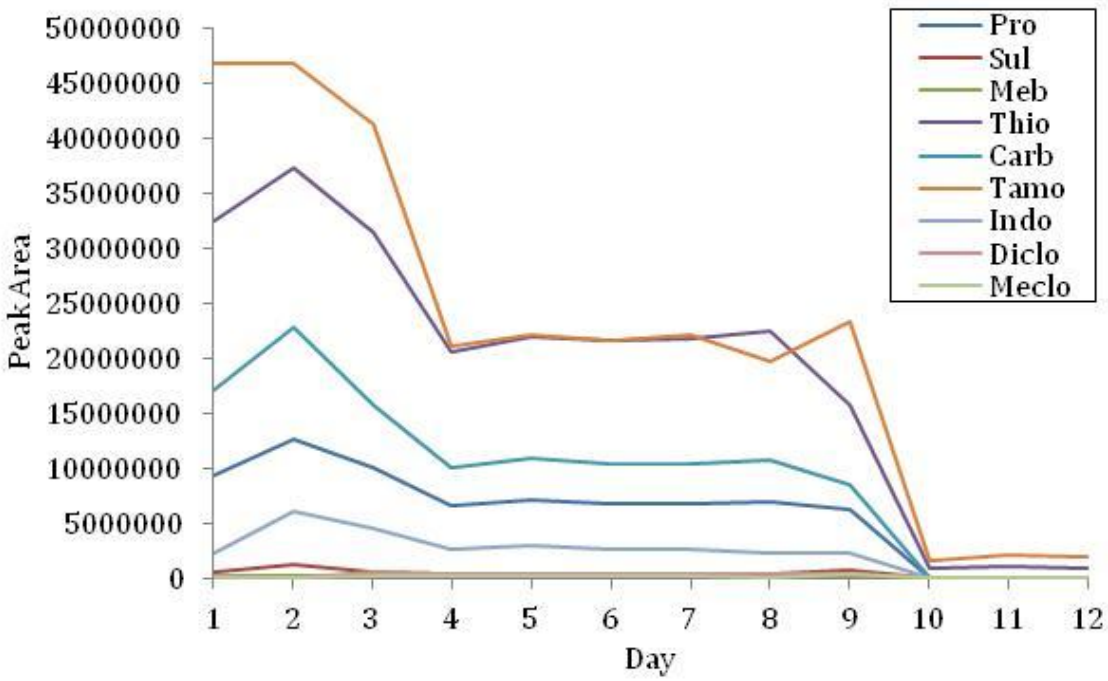

Figure 63 Inter-day variability over twelve days of all compounds

A stock solution sample of 200ppm was run once a day for twelve days on the LC-MS/MS to test variability of the equipment used, using a no matrix sample. In theory, the sample should remain at a constant level until there is none left. It can be seen in figure 45 that this is not true. Tamo, Carb and Thio recorded the highest peak areas, and the lowest recorded by Meb, Sulf and Meclo. Tamo showed the highest degradation between day 1 and 10, at which point the sample data reduces to a level below quantification due to degradation and evaporation.

Degradation of samples plays a contributing factor in testing inter-day variability of the LC-MS/MS. Figure 63 clearly indicates that after day 10 the sample had degraded to such an extent that all compounds became almost undetectable, and the reality was that the sample had dried up after being stored at room temperature (or warmer) inside the LC-MS/MS. Storage under investigated optimum conditions of fridge temperature may significantly restore peak area and sample usefulness to a normal level and last a lot longer. It can be concluded here that samples stored at room temperature are only effective up to ten days before the peak area recorded is too low. Although there is preferred detection of up to 10 days, the sample is at its optimum for use up until day 4.

3.5.11 Intra-day variability

Over a period of twelve days, intra-day variability was tested for LC-MS/MS equipment to analyse stability of the equipment. Results day by day are illustrated in Figure 64 to Figure 75 and a sample was taken and analysed over a three hour period on each day at the same time each day. It is noted that all results for compounds and days are stable and so as long as validation is carried out at the beginning of a set of samples this should be adequate. To ensure the highest accuracy, calibration was carried out once every 20 samples using the same stock standard solution each time to identify if there was and discrepancy and variability in results.

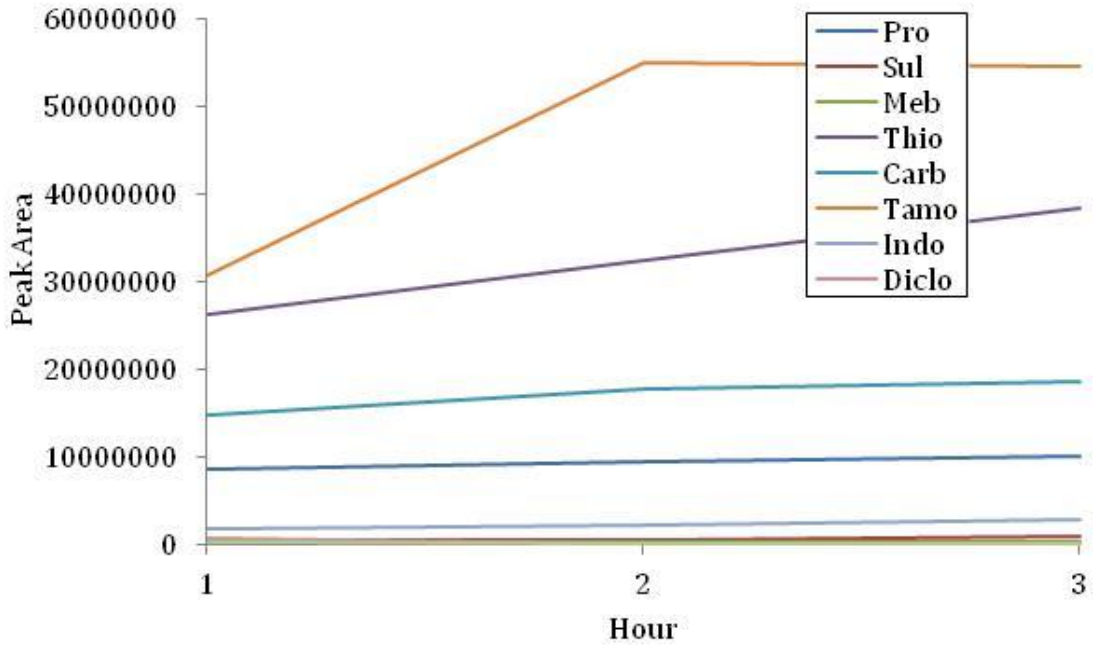

Figure 64 Intra-day variability day 1

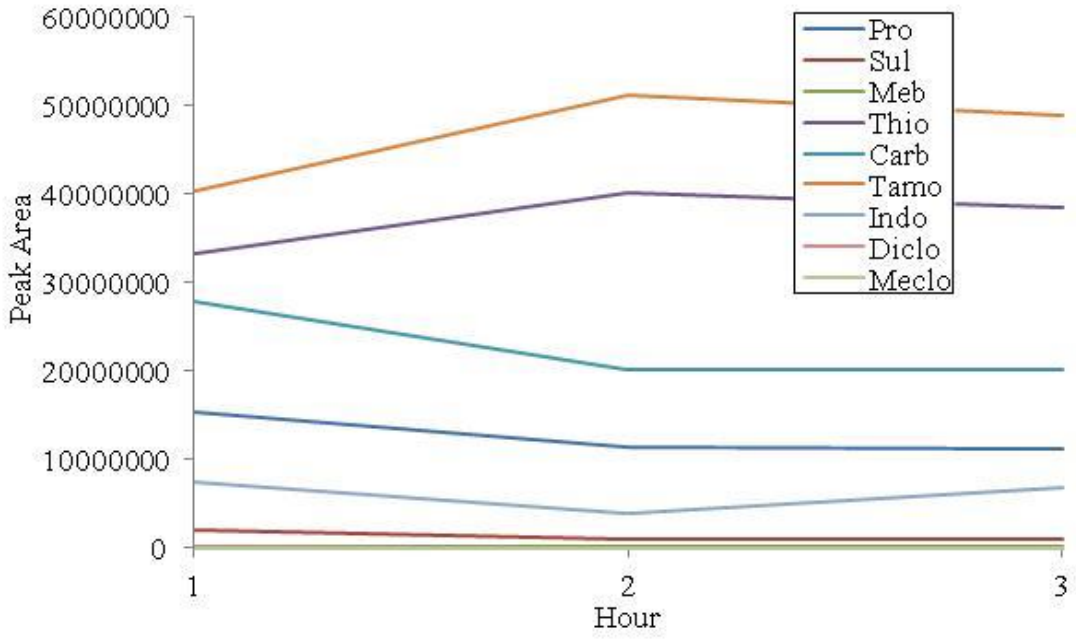

Figure 65 Intra-day variability day 2

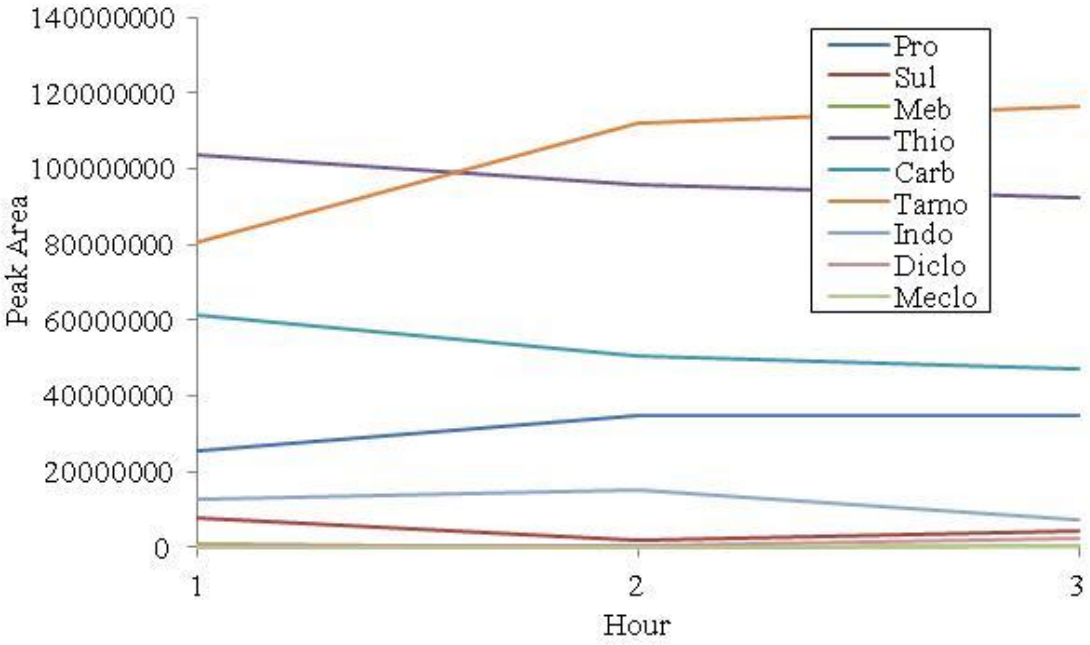

Figure 66 Intra-day variability day 3

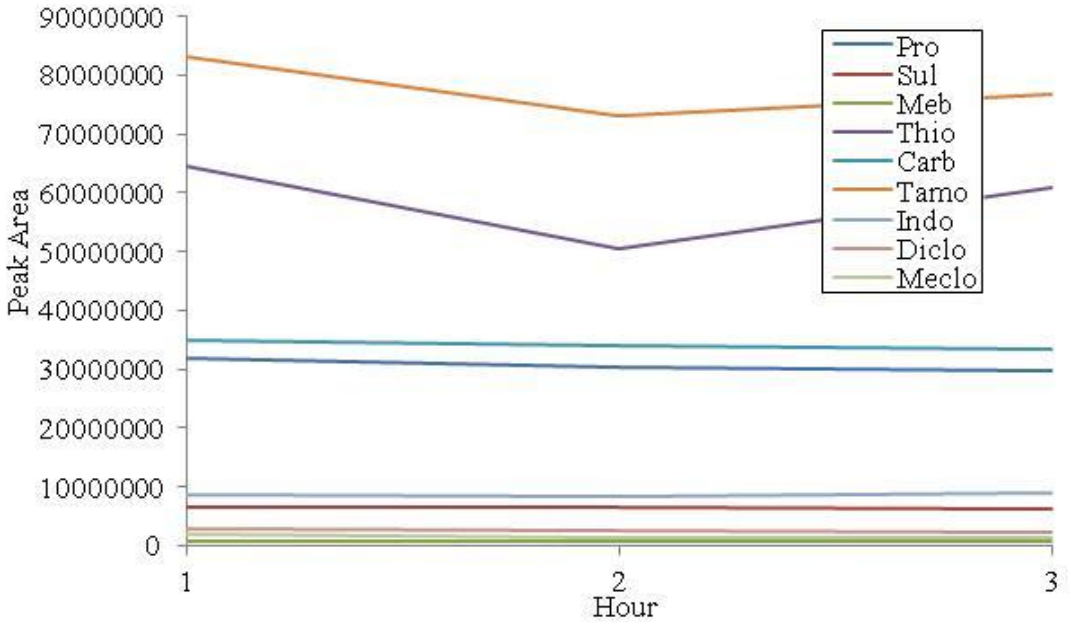

Figure 67 Intra-day variability day 4

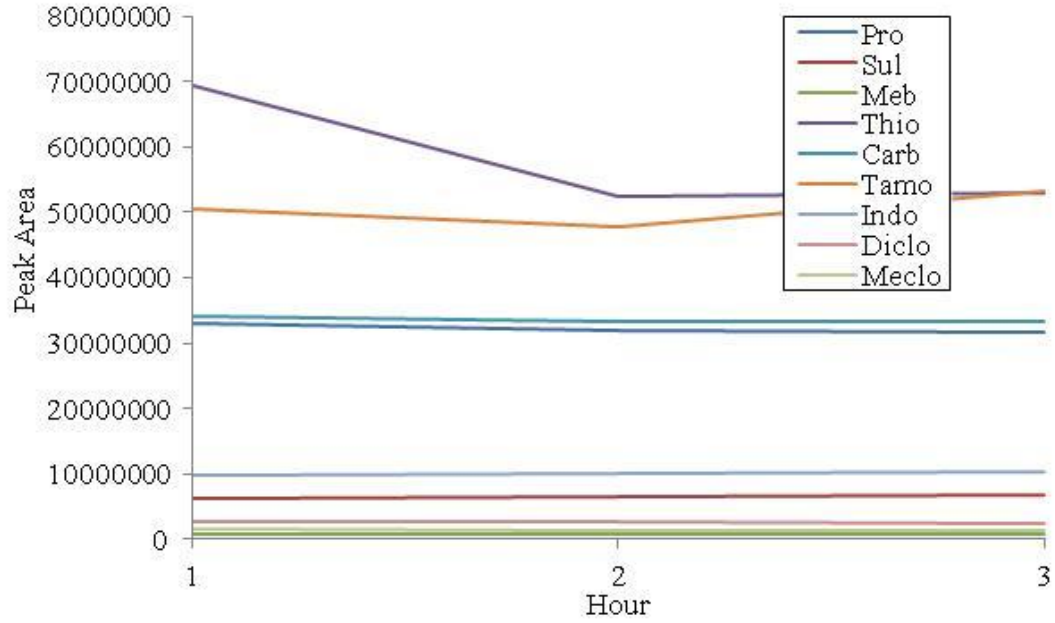

Figure 68 Intra-day variability day 5

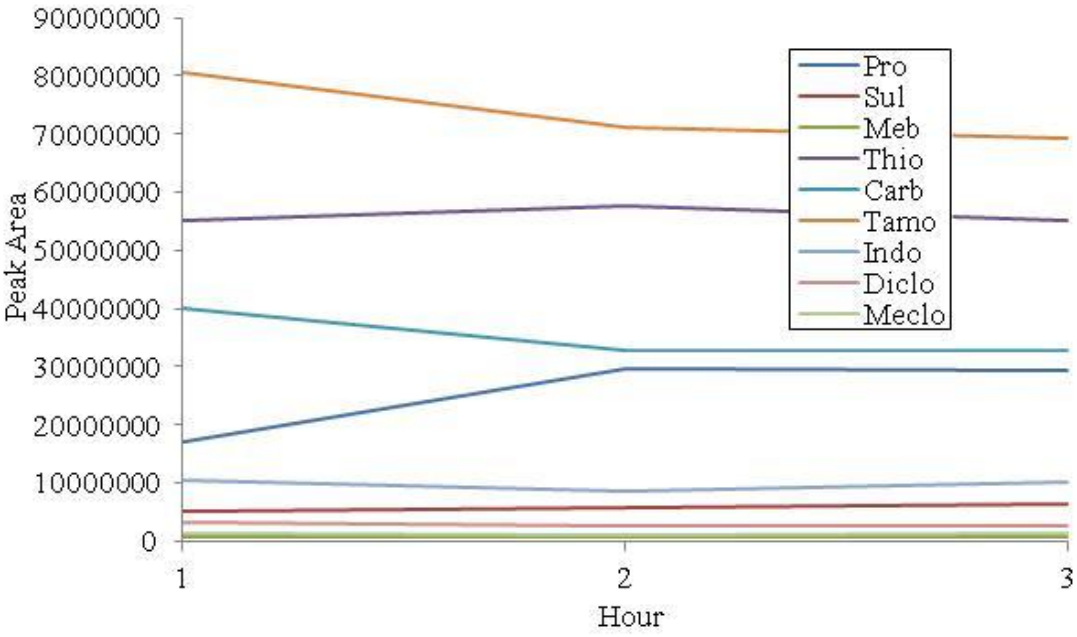

Figure 69 Intra-day variability day 6

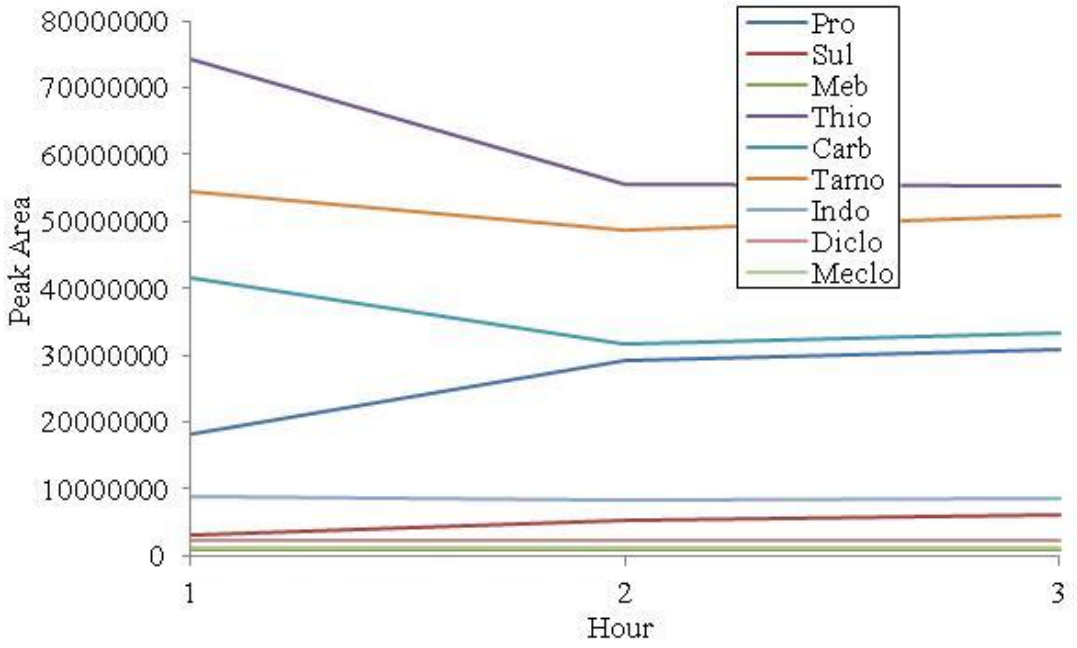

Figure 70 Intra-day variability day 7

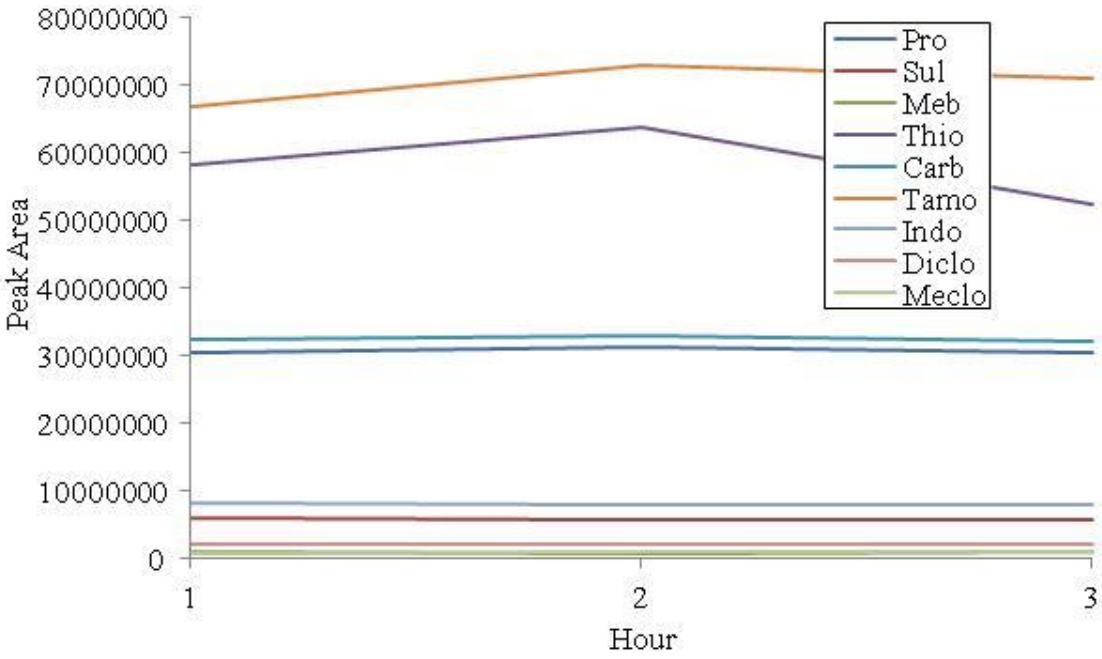

Figure 71 Intra-day variability day 8

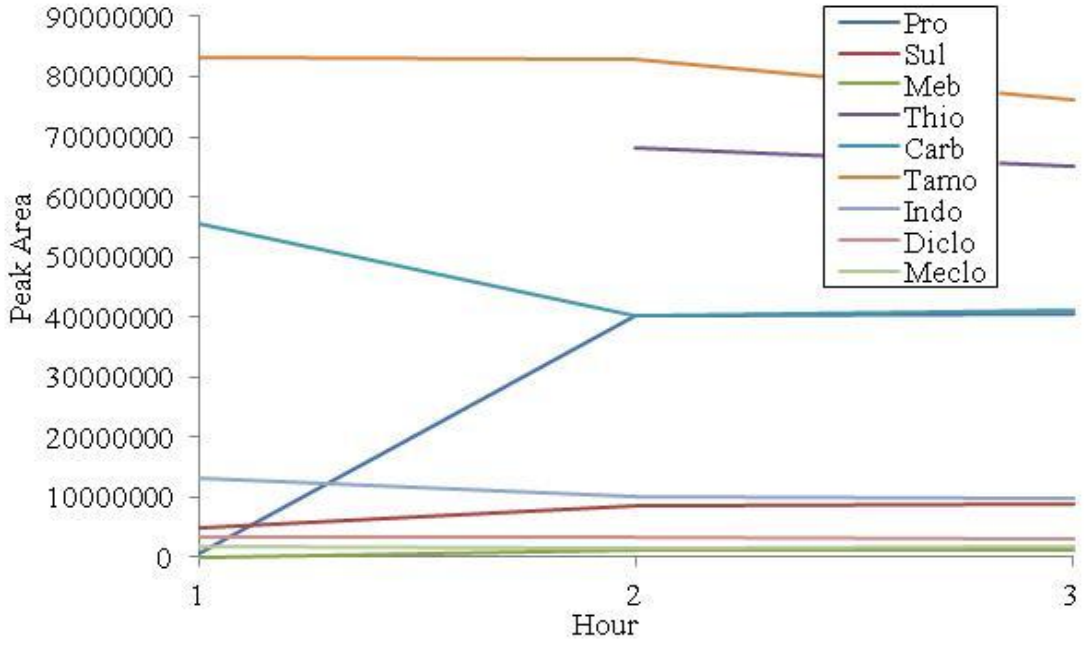

Figure 72 Intra-day variability day 9

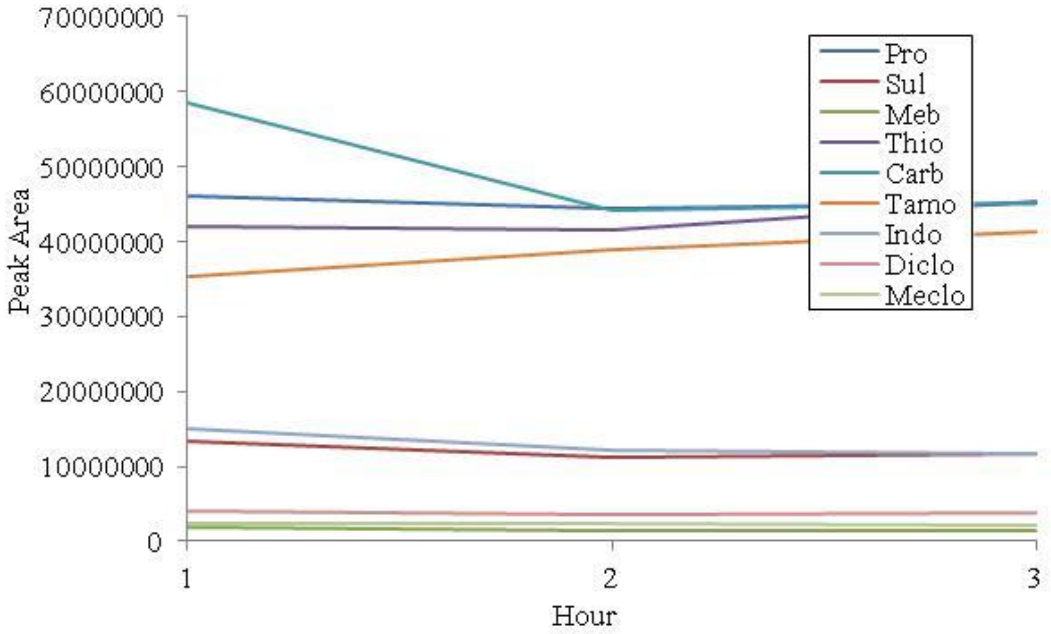

Figure 73 Intra-day variability day 10

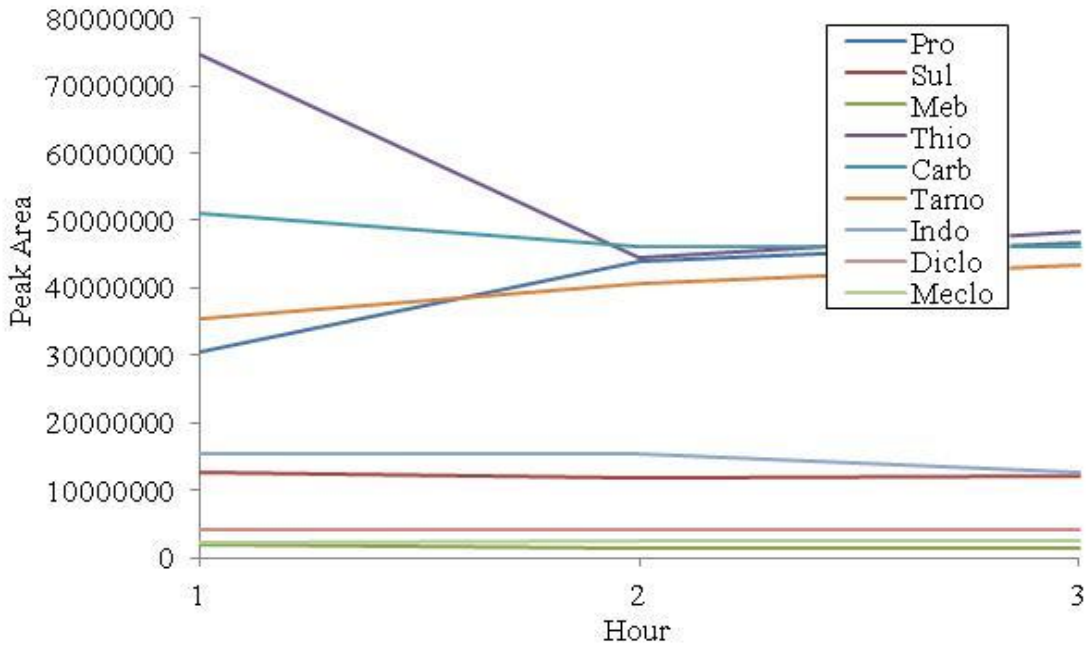

Figure 74 Intra-day variability day 11

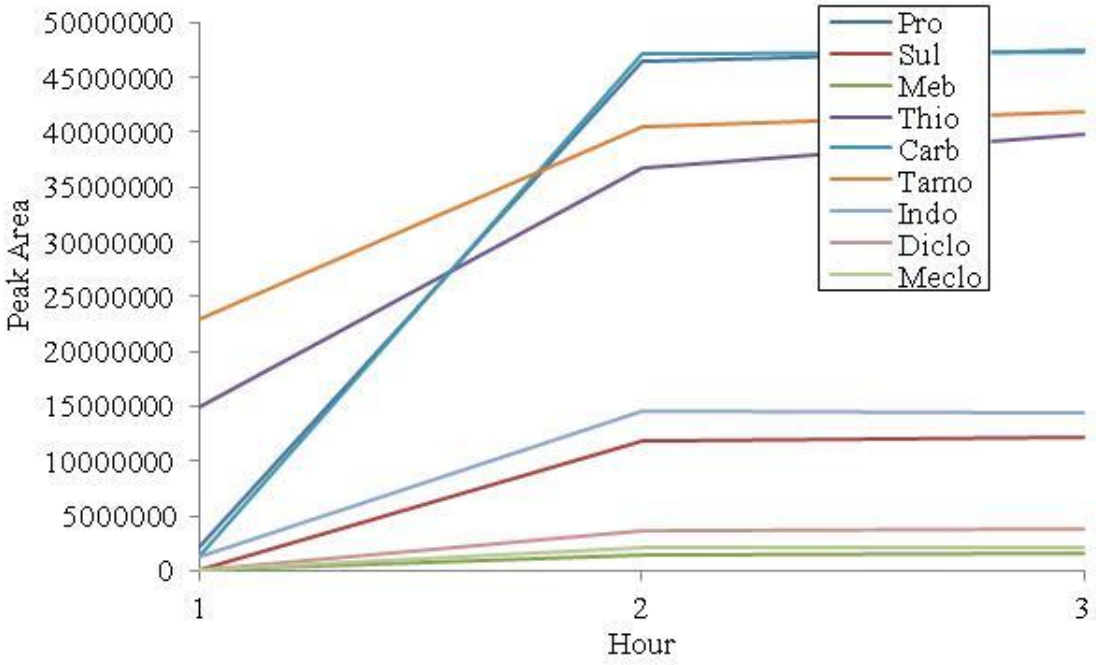

Figure 75 Intra-day variability day 12

Chapter 4 - Validation of the developed method by application to environmental samples

4.1 Introduction

It is important to assess quality assurance (QA) at every step of method development, as highlighted in chapter 3. Following these, this chapter is a natural progression; method validation by application to real environmental samples. The analytical method developed based on ultrasonication was validated by the linear range, sensitivity, accuracy and precision as detailed in chapter 3. A series of injections of the target compounds in the concentration range from 1 μg/ml to 200 μg/ml and 1 μg/ml of internal standards were used to determine the linear concentration range of LC-MS/MS instrumentation. Repeated injections confirmed the linear range being between 1-100 μg/ml with values of $r^2 > 0.92$.

The limits of detection (LOD), calculated as the concentration of three-times the standard deviation in 10 independent blank performance (Zhang and Zhou, 2007), are again given in chapter 3.5.5. The limits of quantification (LOQ) are the minimum concentrations of quantitative analysis, and determined as the analyte amount related to a signal/noise ratio of 10. For all the compounds being analysed, their LOD values fell between 0.01 and 0.71 ng/g, and their LOQ values varied from 0.03 to 2.10 ng/g, all on dry weight basis.

Full procedure recovery tests were performed for the validation of this method by spiking three different levels of standard mixture in sediment samples, and results presented in Table 3. Mean recoveries of all analytes except Meb and Thio in sediments ranged from 61.7 to 93.2 % at the spiking level of 5-50 ng/g, with RSD less than 22 %. Therefore, the results confirm that the method developed exhibits a satisfactory precision and reproducibility for the separation and determination of pharmaceutical compounds from sediment samples.

Figure 76 gives a full process flow of experimentation and validation for this thesis.

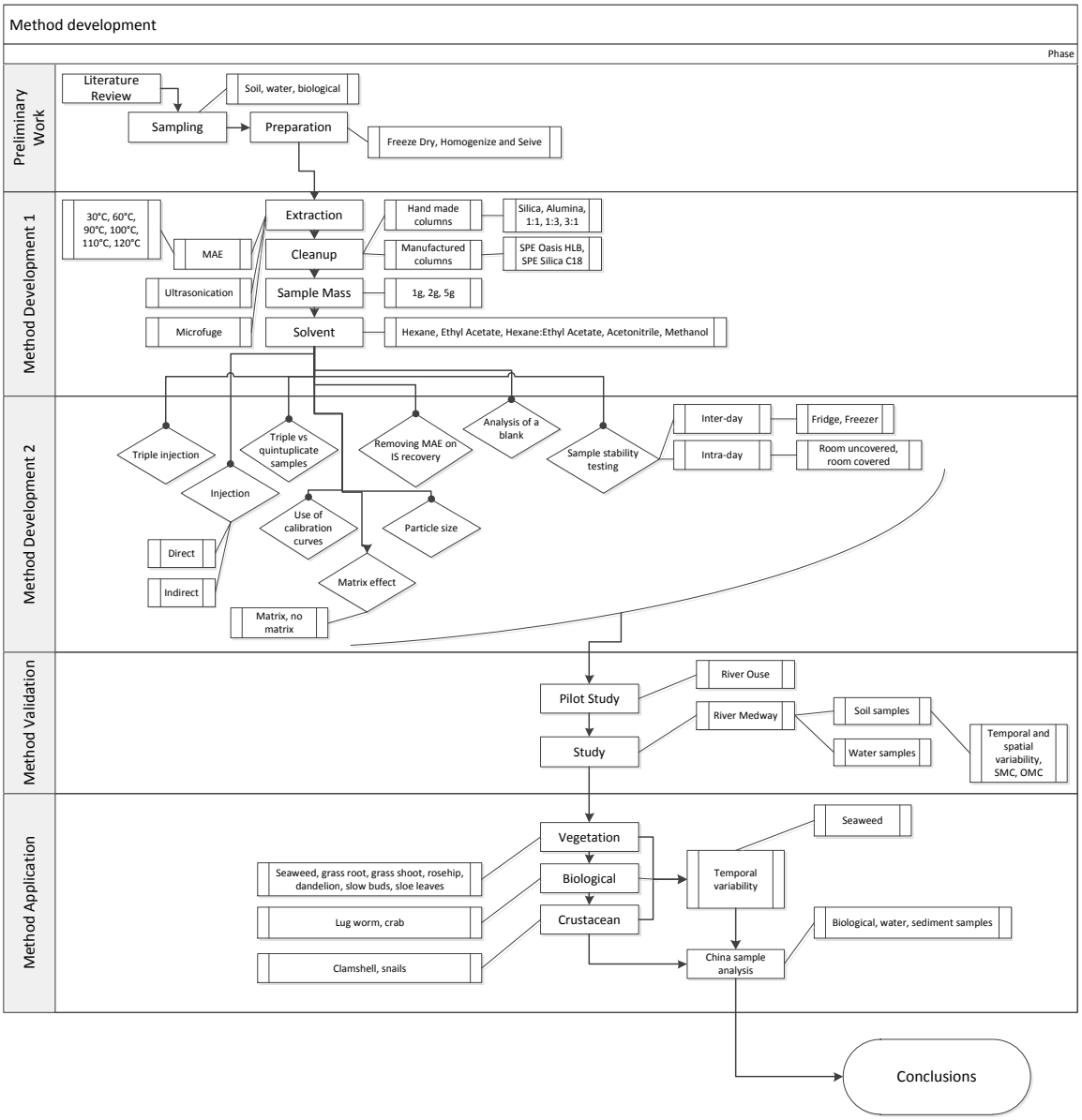

Figure 76 Process flow of thesis and experimentation

4.2 Pilot Study - Analysis of environmental sediment samples in the River Ouse

To test the analytical method developed in chapter 3 for the analysis of a range of PPCPs in sediment samples, this was applied to real environmental samples. Prior to the final sampling location being assessed, a small pilot study was conducted at a different location to further ensure that the method was applicable to real environmental samples rather than in a laboratory without using a real sample. However following matrix effect consideration experiments there should be no reason that the method would be any less effective.

A small stretch of the River Ouse in East Sussex, UK, was selected for this purpose. This was chosen as a sampling location due to ease and close proximity to the laboratory, but more importantly the effluent site which could act as a source of the target pharmaceuticals into the river. Four sampling locations were selected; the effluent site, 100 m downstream from this, 10 m upstream, and 500 m upstream. This is proposed to give a good indication of how the effluent site affects the concentration of named PPCPs up and downstream from this.

Figure 77 is a satellite image of the River Ouse which is clearly seen in the middle of the image. The sewage treatment works are also clearly identified just below the centre of the image. Figure 78 is a simple road map of the same location with the four sampling sites indicated by a circle at each. From left to right the sampling sites are as follows; 100 m downstream, effluent site, 10 m upstream, 500 m upstream.

Figure 77 Satellite image of sampling location at River Ouse (www.google.com, viewed on 14.04.2010). The outline of the river is seen in Figure 2.

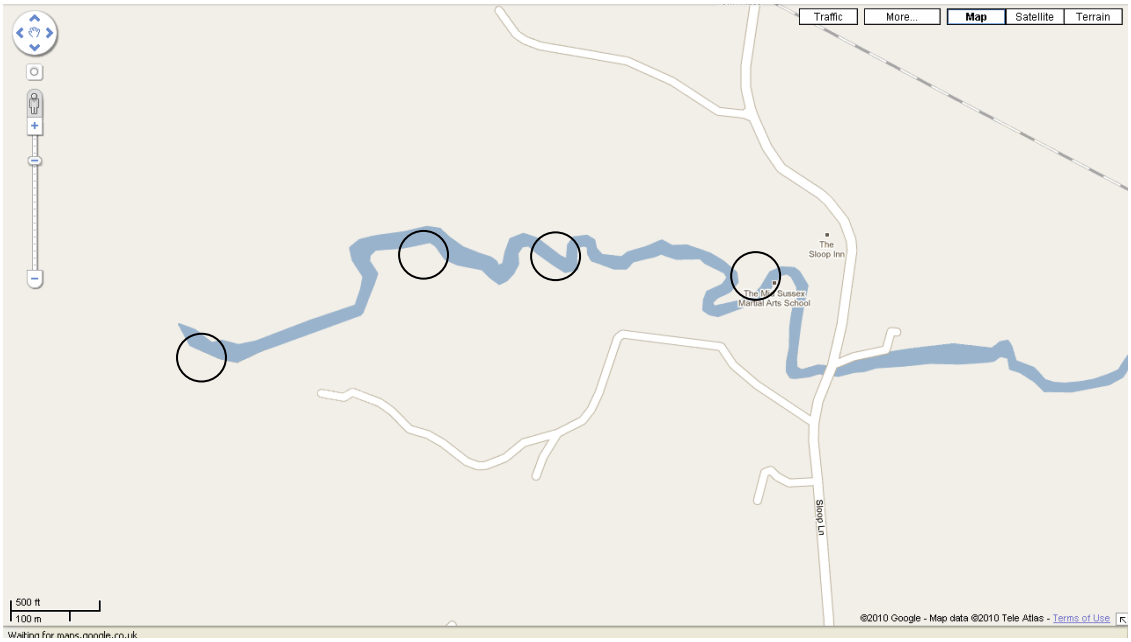

Figure 78 Four sampling sites at the River Ouse (www.google.com, viewed on 14.04.2010)

4.2.1 River Ouse pilot study report

After the preparation of samples using the Ultrasonication (U) method, samples were analysed using LC-MS/MS. It can be concluded from Figure 5 that the mean concentrations of pharmaceutical compounds are most dominant at the sewage effluent site (1.5 - 213.3 ng/g dry weight), as also seen in Table 16. This is all except Meb which had the highest concentration at the upstream site (23.59 ng/g). The concentrations of the target compounds became elevated at the effluent site, and also at downstream due to output at the effluent site. The most abundant and potentially persistent pharmaceutical compound identified was Tamo with concentrations between 63.9 ng/g and 213.3 ng/g, and the least being Thio with concentrations between < LOD and 1.5 ng/g.

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
500m Upstream	1.43	0.64	7.42	96.33	7.66	103.27	18.16	11.43	17.10
Upstream	4.24	0.30	23.59	100.67	18.27	117.31	19.83	11.15	20.55
Effluent Site	9.15	5.90	20.89	100.04	53.08	271.28	69.42	35.77	54.81
Downstream	4.38	2.49	3.72	96.05	18.00	192.39	62.40	31.50	38.60

Table 16 Concentration (ng/g) of pharmaceutical compounds found at the River Ouse (Sussex) in June 2009.

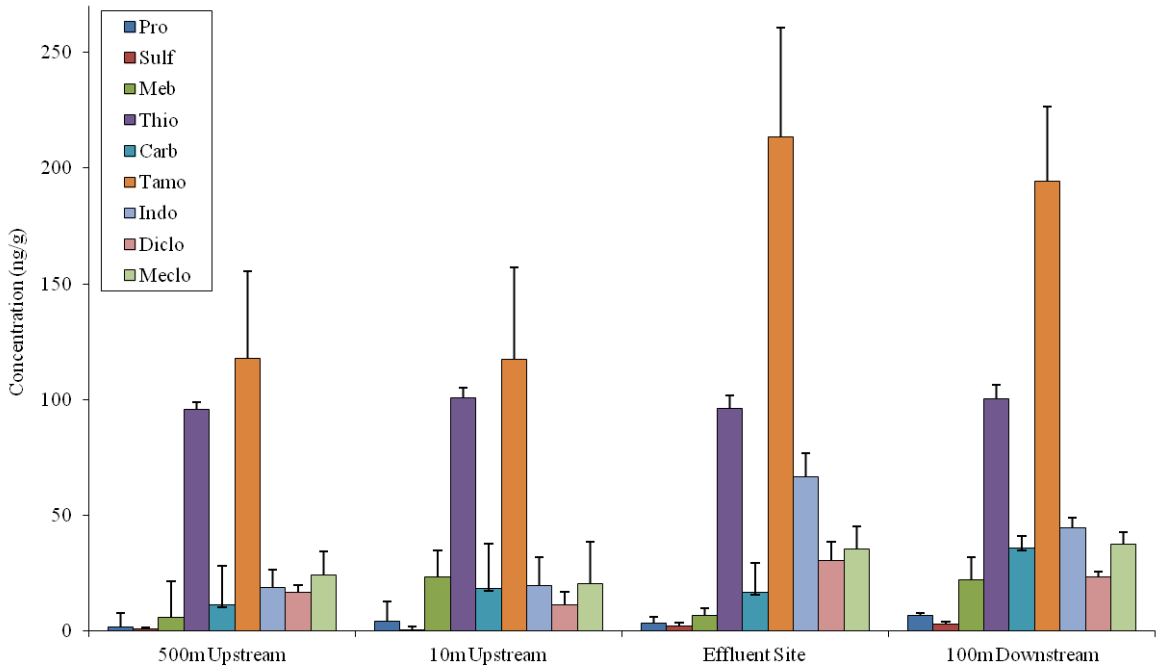

Figure 79 Concentration (ng/g) of pharmaceutical concentrations at sampling sites along the River Ouse (Sussex) in June 2009.

When displayed graphically, it is apparent that the highest concentration of target PPCPs is identified at the effluent site (Figure 79). As expected, the concentrations of pharmaceuticals downstream from the effluent site are considerably raised to what were considered the normal (background) concentrations upstream. It can be argued that the concentration 10 m upstream may have some backflow PPCPs from the effluent site due to the natural tide flow of the river, however this does not appear to be significant statistically in this pilot study. As highlighted previously, Tamo not only has the highest concentration of all PPCPs analysed, but also the highest variability, shown in Figure 79 by error bars.

Compounds became increasingly concentrated as from 500 meters upstream to the effluent site, after which downstream the concentration remains significantly higher than before due to output at the effluent site. The most persistent pharmaceutical compound identified was Tamo with concentrations between 103.27 ng/g and 271.3 ng/g, and the least being Sulf with concentrations between 0.30 ng/g and 5.90 ng/g. The concentrations of other compounds were as follows; Pro 1.43 – 9.15 ng/g, Meb 3.72 –

23.59 ng/g, Thio 96.05 – 100.67 ng/g, Carb 7.66 – 53.08 ng/g, Indo 18.16 – 69.24 ng/g, Diclo 11.15 – 35.77 ng/g and Meclo 17.10 – 54.81 ng/g.

Analysis of a blank concluded no significant background concentration of target compounds (Table 17). Compounds Sulf, Thio, Indo, Diclo and Meclo produced zero concentrations in the blank sample. Only four compounds identified background concentrations, all below 0.80 ng/l; Pro 0.04 – 0.11 ng/l, Meb 0.26 – 0.79 ng/l, Carb 0.02 – 0.05 ng/l and Tamo 0.01 – 0.03 ng/l. Average and variability values are also calculated. Although minimal background contamination, average blank values were deducted from sample validation analysis.

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Blank1	0.11	0.01	0.26	0.01	0.05	0.01	0.01	0.01	0.01
Blank2	0.04	0.01	0.79	0.01	0.02	0.01	0.01	0.01	0.01
Blank3	0.07	0.01	0.34	0.01	0.02	0.03	0.01	0.01	0.01
Average	0.07	0.01	0.46	0.01	0.03	0.02	0.01	0.01	0.01
Stdev	0.04	1.69	0.29	1.69	0.02	0.01	1.69	1.69	1.69
LOD	0.11	5.10	0.86	5.10	0.05	0.03	5.10	5.10	5.10

Table 17 Analysis of a blank during River Ouse experiments (ng/l)

The limit of detection (LOD) defined as the concentration that corresponds to three times the standard deviation of blanks (Zhang and Zhou, 2007), was measured by integrating blank peak area for each analyte in three independent performances with ultrapure water as a blank. LOD varied from 0.01 – 5.10 ng/l (Table 17).

In comparison to data from other studies, the pharmaceutical concentrations in the River Ouse are similar in magnitude. For examples, 1.45 - 137 ng/g of Carb and 4.56 - 128 ng/g of Diclo were detected in sediment samples from rivers and groundwater in the UK (Maskaoui and Zhou, 2010). Similar levels of Carb (49 ng/g) and Sulf (6.8 ng/g) have been reported by Stein et al. (Stein et al., 2008). Sanchez-Prado et al. recently collated information from different sources on the analysis of emerging pollutants in solid samples, including Pro in sediment samples (Sanchez-Prado et al., 2010), Carb in mollusk (Cueva-mestanza et al., 2008b) and Diclo in river sediment (Antonic and

Heath, 2007) (Dobor et al., 2010). Cueva-Mestanza et al. concluded and evaluated that no real contaminated samples although recoveries of over 70 % were identified (Cueva-mestanza et al., 2008a). Cueva-Mestanza et al. also could not determine the concentrations of pharmaceuticals in the mollusk samples, but reported a recovery of >85 % (Cueva-mestanza et al., 2008b). Although Diclo was being analysed in river sediments by Anatonic and Heath (2007), they did not report any concentration data but only a recovery of 46 %. Dobor et al. (2010) reported Diclo concentrations of 23 – 138 ng/g in activated sludge samples and a recovery of 83 %, both of which are similar to what is being found in River Ouse sediment samples.

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
500m Upstream	5.76	0.66	15.58	3.21	16.80	37.59	7.75	3.12	10.07
Upstream	8.61	1.74	11.16	4.64	19.29	39.64	12.18	5.78	17.84
Effluent Site	2.82	1.13	2.73	5.45	12.39	47.44	10.20	7.80	9.95
Downstream	1.18	0.95	9.86	6.03	5.38	32.47	4.25	2.18	4.85

Table 18 Standard deviation of pharmaceutical compounds found at the River Ouse (Sussex) in June 2009.

It is interesting to assess the variability between replicate samples taken at the same location at the same sampling time. Table 18 highlights the standard deviation (variability) of replicate samples taken from River Ouse analysis. The lowest overall sample variability was identified from Sulf, with values between 0.66 to 1.74, compared to Tamo which displayed the highest variability between replicate samples between 32.47 and 47.44. However, even Tamo has acceptable levels of variability for environmental samples.

Table 19 details how in the laboratory (ex situ), subsamples vary between one another and how variable their retrievals are. There were 4 groups of samples (5up, up, eff and down) with three subsamples taken in the laboratory (ca, cb, cc etc). Most samples have a good precision of samples during the River Ouse analysis. Others showed a high variability, for example; Pro for 5up has results of 0.28, 1.53 and 15.47 and Tamo for eff has results of 4.36, 38.89 and 99.08.

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
% recovery	500m Upstream	83.00	50.00	53.00	51.33	63.00	72.00	88.00	87.33	93.67
	10m Upstream	62.00	43.00	55.00	52.00	64.00	60.67	78.33	80.00	83.67
	Effluent Site	56.33	55.67	51.00	60.67	63.67	61.00	54.00	87.00	90.00
	100m Downstream	65.67	39.00	60.00	53.67	51.67	67.00	84.67	88.67	76.00
RSD	500m Upstream	1629.27	435.10	132.10	6.37	2606.53	117.59	78.18	464.32	428.34
	10m Upstream	1736.93	1318.20	72.59	9.28	3289.90	29.23	107.84	21.62	172.18
	Effluent Site	128.75	435.75	147.15	11.34	137.74	267.67	206.89	221.58	118.67
	100m Downstream	164.99	297.64	265.86	12.53	279.43	115.69	46.86	132.23	165.08

Table 19 Precision of samples during River Ouse analysis

Bias	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
500m Upstream	6.14	0.24	9.65	2.41	29.42	93.06	18.99	16.98	27.46
10m Upstream	14.15	9.76	18.04	2.78	89.91	206.81	81.93	39.22	62.00
Effluent	1.81	3.42	3.41	0.43	15.76	272.90	91.15	42.52	38.11
100m Down	0.85	0.50	3.14	2.90	7.52	83.61	15.29	14.35	19.73

Table 20 Bias (+) of samples during River Ouse analysis.

Between the same sites, Table 20 highlights how between the same sites, how variable the retrievals are in the form of bias. Overall, Tamo has the highest bias with results between 83.61 and 272.90, and Thio the lowest with a variability of 0.43 to 2.90. This means that Thio is by far the most stable (less varying between sites) compound. Pro and Sulf also have a good low bias.

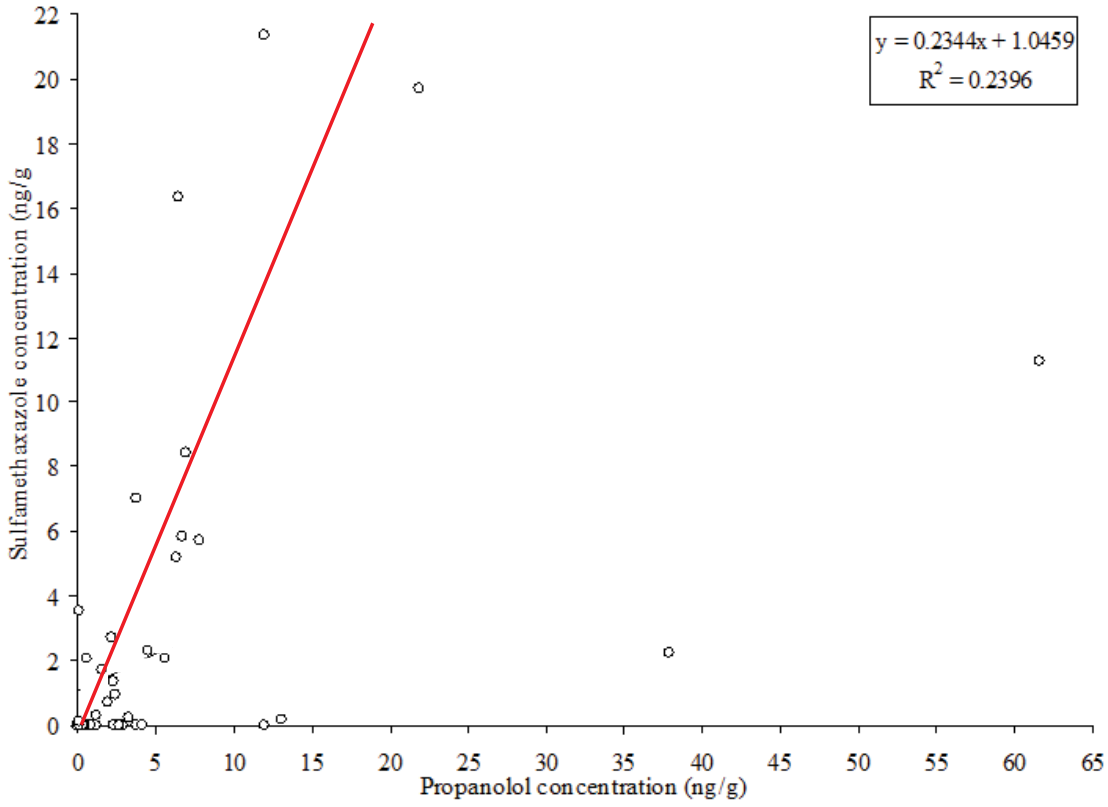

Figure 80 R squared value of Sulf and Pro concentrations at the River Ouse

To identify whether there is a relationship between compounds, a graph displaying the r squared value of one example is displayed in Figure 80 between Sulf and Pro, ie as one compound increases/decreases so does the other. Results for all compounds are given in Table 21. Pro and Sulf were chosen as they have the highest correlation coefficient of 0.2396 %, which is still not overly significant so r^2 was disregarded for any further analysis and assumed that compounds are independent of one another.

Rsquared	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Pro	-	0.2396	0.2189	0.0056	0.0057	0.0076	0.013	0.0254	0.0059
	Sulf	-	0.2189	0.0056	0.0057	0.0076	0.013	0.0254	0.0059
		Meb	-	0.0056	0.0057	0.0076	0.013	0.0254	0.0059
			Thio	-	0.0057	0.0076	0.013	0.0254	0.0059
				Carb	-	0.0076	0.013	0.0254	0.0059
					Tamo	-	0.013	0.0254	0.0059
						Indo	-	0.0254	0.0059
							Diclo	-	0.0059
								Meclo	-

Table 21 All R squared values from the River Ouse (%)

4.3 Further Method Validation and Temporal Variability; Report of the River Medway Sewage treatment works.

4.3.1 Introduction

A small part of the River Medway in North Kent, UK, was selected for the environmental analysis of sediment samples using the developed and tested method (Figure 81). Figure 81 is a satellite image of the specific part of the River Medway used for these sampling locations. The River Medway is a large tidal river and begins at the West Sussex border and continues until it ends in the Thames Estuary; stretching for over 70 miles. Man has had an increasing effect on this river for centuries. In recent years, activity surrounding the river on the banks includes two industrial plants, gas towers, a paper mill and many STWs and WTWs. Run off from these activities poses an increased risk of contamination into the river. Due to the previously highlighted occurrence of PPCPs through excretion by humans, particular focus was drawn to one particular STW located on Motney Hill, located towards the end of Motney Hill Road (Figure 82). Figure 82 is a simple road map of the same location with the three sampling sites indicated by a circle at each. Three sampling locations were selected; upstream from the effluent site, at the effluent site, and downstream from this (left to right on Figure 82). This is proposed to give a good indication of how the effluent site affects the concentration of named PPCPs up and downstream.

Figure 81 Satellite image of sampling site at the River Medway (www.google.com, viewed on 10.12.2009)

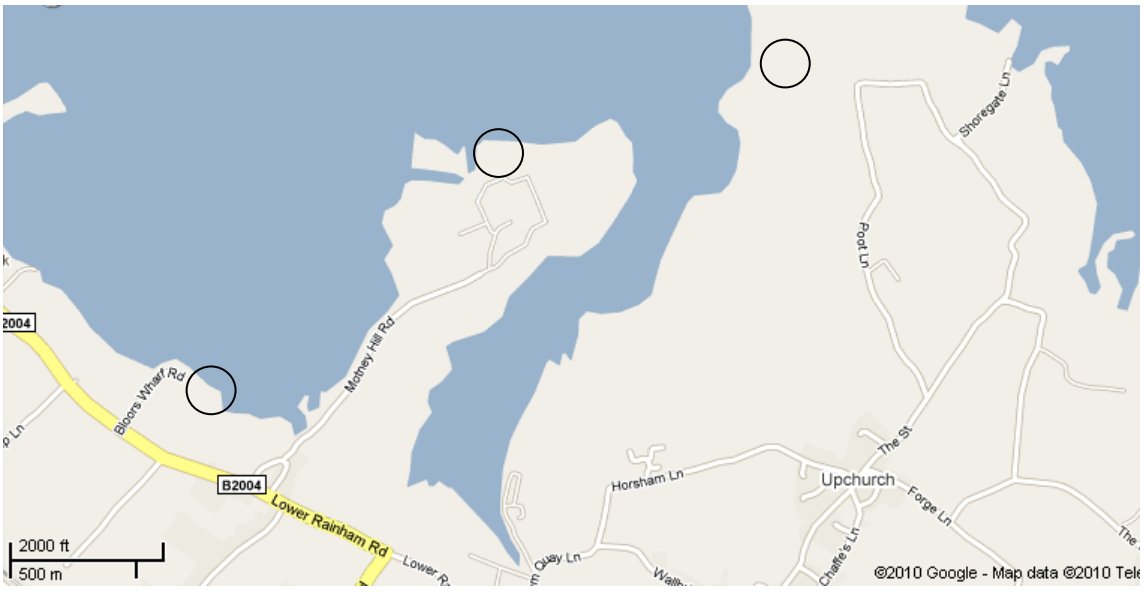

Figure 82 Sampling locations at the River Medway (www.google.com, viewed on 19.01.2010)

4.3.2 Spatial Variability

4.3.2.1 December 2009

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	18.53±32.04	0.64±4.79	13.56±10.20	101.30±96.56	54.04±64.05	283.68±1132.70	46.69±90.87	16.47±74.70	31.83±65.36
Effluent	21.94±44.09	2.10±9.06	6.50±9.20	95.63±99.69	39.29±83.66	1194.24±727.98	82.20±70.50	57.98±75.29	55.95±51.83
Downstream	43.94±14.34	8.95±0.78	11.22±3.73	99.78±3.50	89.00±22.85	715.57±20.36	70.59±14.27	73.56±4.96	52.90±7.21

Table 22 Concentration (ng/g) and standard deviation of target pharmaceuticals from River Medway samples from December 2009

Concentrations of pharmaceuticals at the River Medway in December ranged from 0.6 ng/g to 1194.2 ng/g for Sulf upstream and Tamo at the effluent site respectively (Table 22).

Figure 82 clearly illustrates Tamo having the highest concentration at all three sample sites, with 283.7 ng/g upstream, 1194.2 ng/g at the effluent site and 715.6 ng/g downstream.

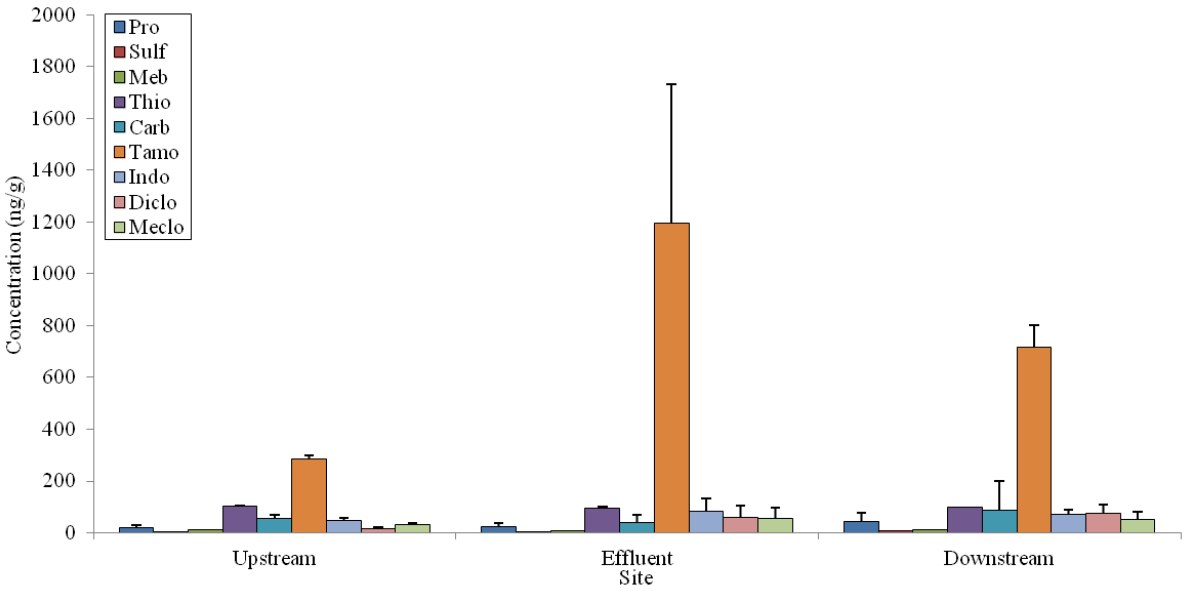

Figure 83 displays the data as a bar chart where Tamo is clearly identified as the highest detected concentration of compound across all sampling locations. Standard deviation indicates that Sulf is the most reliable detected compound with results closest to the mean value (0.8 – 9.1), compared to Tamo which alongside its highest concentration values also has the highest standard deviation ranging from 20.4 to 1132.7. This is not surprising as for higher values there is the possibility of more room of variability. Although the concentration was high, the values have been averaged from replicated in and ex situ. Overall, variability is lowest downstream from the STW, with standard deviation ranging only from 0.8 to 22.8.

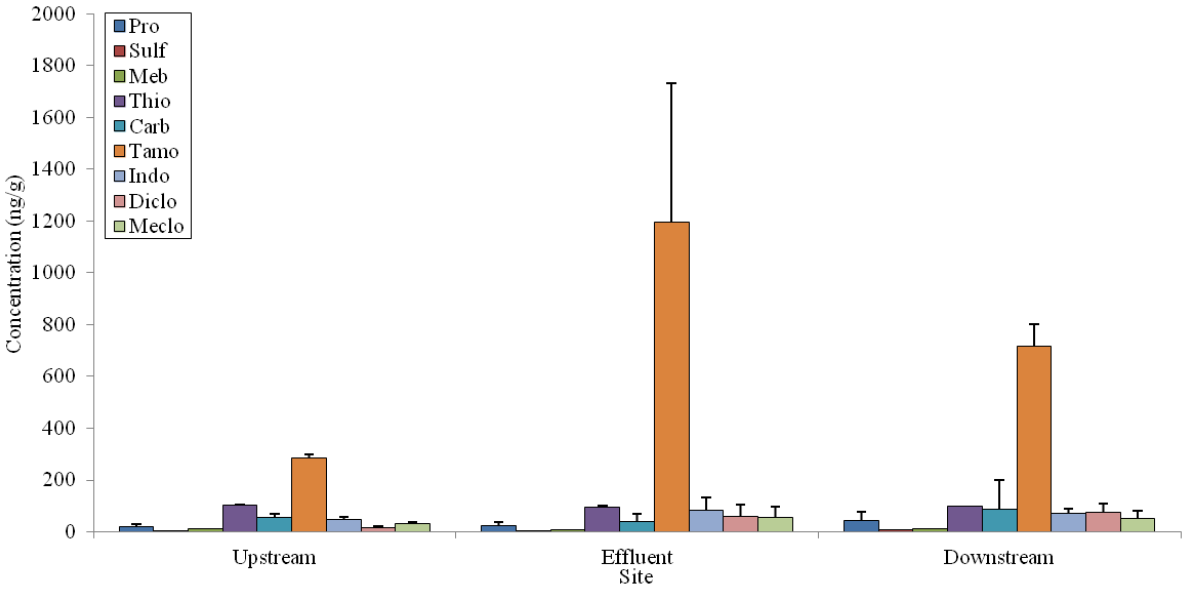

Figure 83 Total concentration of pharmaceuticals at River Medway sample locations for December 2009

All compounds excluding Meb, Thio and Carb increase from the upstream to effluent sampling site, the largest increased being Diclo with an increase of 41.5 ng/g from 16.5 – 58 ng/g. All compounds excluding Tamo, Indo and Meclo remain high or at increased levels after the effluent site, downstream. The largest increase from effluent to downstream sampling site being Carb (49.7 ng/g), from 39.3 – 89.0 ng/g.

Due to the high concentration of Tamo at all sites, all compounds appear to recover small concentrations, which is not the case as normalization of results demonstrates (Figure 84). Aside from Thio, all compounds are highest at the sampling location downstream from the effluent site, which were significantly higher than concentrations observed upstream. Therefore as you travel downstream the concentration of target pharmaceutical compounds increases and remains this way due to the effluent site of the sewage treatment plant. This indicates that compounds released from the effluent site accumulate downstream as sediment containing target compounds settles on the river bed.

Table 22 concludes individual data points used to create Figure 82 and Figure 83. Concentration of target compounds were as follows; Pro 18.5 – 43.9 ng/g, Sulf 0.6 – 9.0 ng/g, Meb 6.5 – 13.6 ng/g, Thio 95.6 – 101.3 ng/g, Carb 39.3 – 89.0 ng/g, Tamo 283.7 – 1194.2 ng/g, Indo 46.7 – 82.2 ng/g, Diclo 16.5 – 73.6 ng/g and Meclo 31.8 – 56.0 ng/g.

It is sometimes not considered good practice to normalize results but however removing one to three compounds from the group on graphical analysis simply enhances those with lower concentrations and makes them visibly easier to analyze. Absolute concentrations are shown in the data tables and first graphical representations. This is seen in Figure 8.

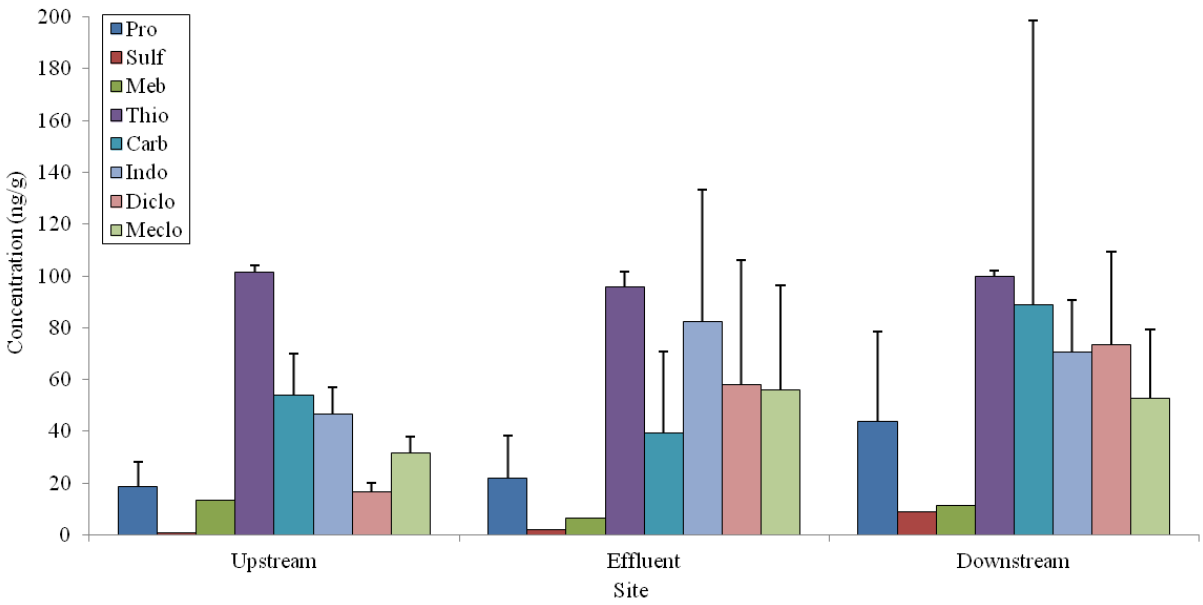

Figure 84 Total concentration of pharmaceuticals at River Medway sample locations for December 2009. Normalization of results.

4.3.2.2 February 2010

Pharmaceutical compounds at the chosen sampling locations in February ranged from 0.47 ng/g for Sulf at the effluent site to 239.42 ng/g for Tamo downstream from the effluent site. Overall, Sulf once again had the lowest observed concentrations from 0.47 to 1.06 ng/g, and Tamo the highest from 144.73 to 239.42 ng/g (Figure 85). There was an overall low variability (standard deviation) excluding one value for Tamp of 160.28. All others ranged from 0.25 for Pro to 63.33 for Carb. This suggests that the levels of pharmaceutical concentration during February were relatively stable.

It is obvious that from upstream to effluent to downstream sampling sites the concentration overall for target pharmaceutical compounds increases Figure 85. This is also true for the precision of sampling, with the highest standard deviation results observed at the downstream site (0.30 – 160.28), in Table 23. For all compounds excluding Thio and Diclo, the downstream sampling location produced the highest concentration. Those to the exception were only lower by a small amount.

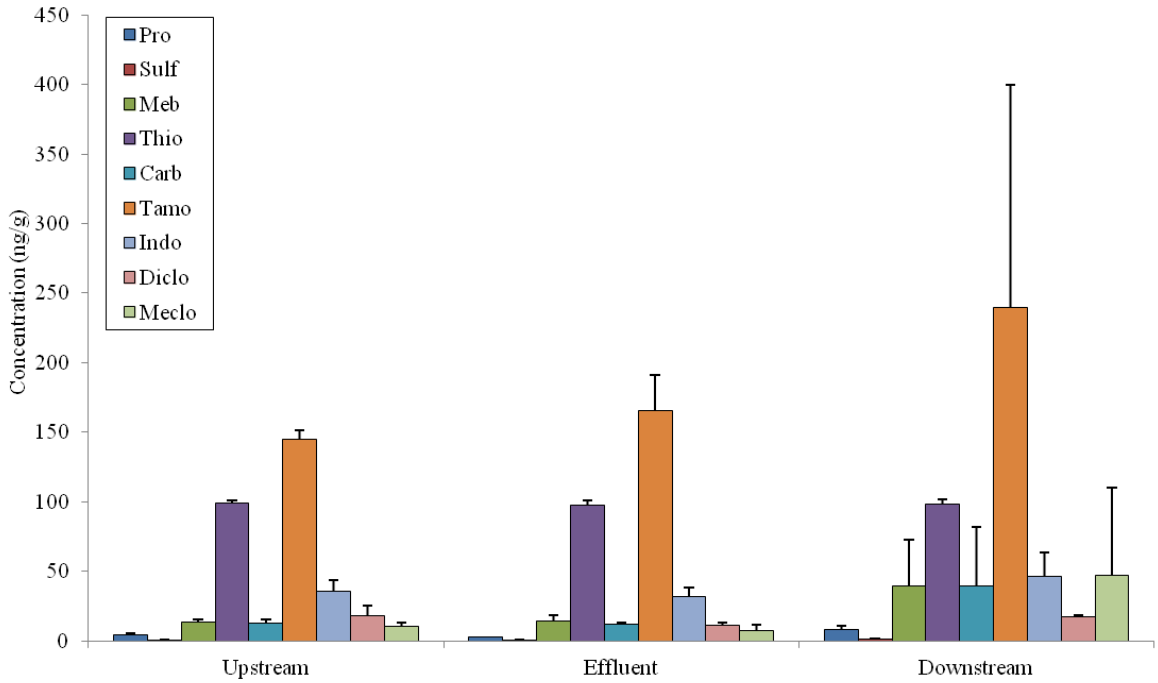

Figure 85 Total concentration of pharmaceuticals at River Medway sample locations for sediment samples in February 2010.

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	4.60±0.70	0.51±0.55	13.75±1.41	98.95±2.39	12.71±2.94	144.73±6.42	35.84±7.71	17.85±7.25	10.29±2.65
Effluent	3.16±0.25	0.47±0.29	14.27±4.04	97.66±3.25	11.84±1.38	165.33±25.98	32.08±6.13	11.23±1.64	7.33±4.61
Downstream	8.38±2.64	1.06±0.30	39.23±33.26	98.33±3.61	39.49±42.38	239.42±160.28	46.23±17.29	17.03±1.48	46.92±63.33

Table 23 Sediment sample data for February 2010 at the River Medway

4.3.2.3 April 2010

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	32.57±24.35	14.83±13.65	93.33±61.17	99.97±1.61	57.35±17.70	175.84±60.01	135.03±29.49	250.37±13.28	109.79±65.71
Effluent	39.22±33.65	11.03±0.64	213.28±45.81	99.73±2.69	65.44±46.31	710.54±35.09	96.15±11.51	68.47±34.16	76.24±84.70
Downstream	303.37±38.60	32.92±18.16	413.83±99.20	98.01±2.60	149.30±39.98	2080.75±68.29	533.89±23.19	401.64±52.23	459.90±115.98

Table 24 Concentration (ng/g) and standard deviation of target pharmaceuticals from River Medway samples from April 2010

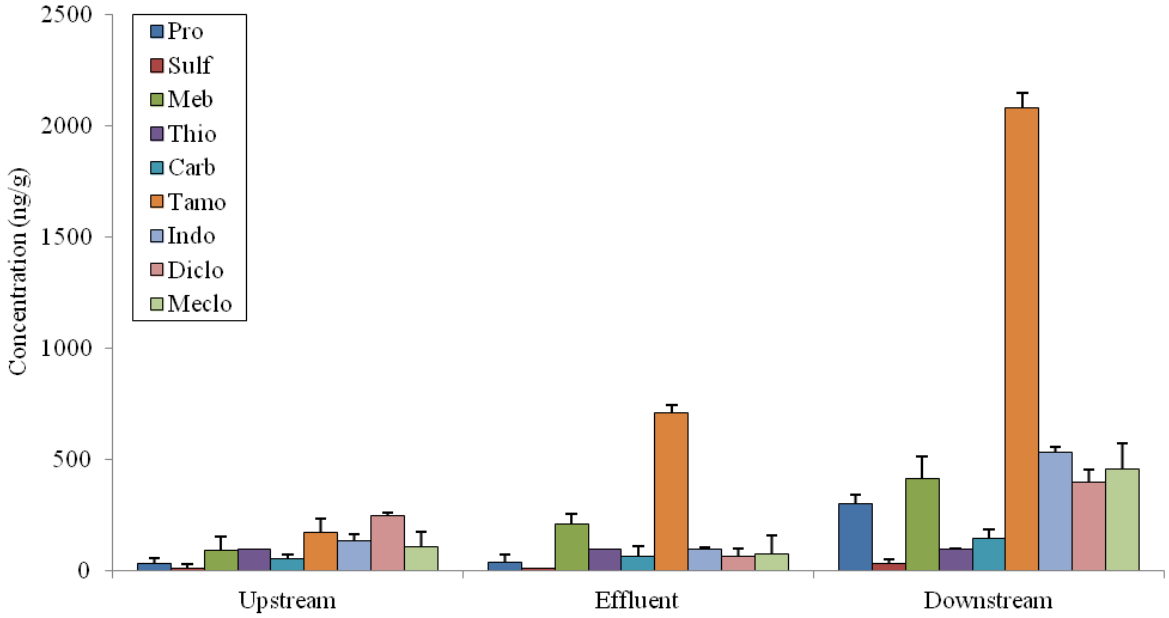

Figure 86 Total concentration of pharmaceuticals at River Medway sample locations for April 2010

Concentration of pharmaceutical compounds ranged from 11.03 ng/g (Sulf at the effluent site) to 2080.75 ng/g (Tamo at the downstream site) (Table 24). All compounds had a good recovery, in particular Tamo which had a range between 175.84 ng/g to 2080.75 ng/g. The compound with the least concentration in sediment samples was Sulf, which had a maximum value of only 32.92 ng/g at the downstream sampling location. All target pharmaceutical compounds were recovered from all sampling sites. The site and compound with the best precision was Sulf at the effluent site, with a statistic of 0.64. The most precise overall compound for recovery was Thio with values in the range of 1.60 to 2.69, with recoveries between 98.01 ng/g (downstream), 99.73 ng/g

(effluent) and 99.97 ng/g (upstream), in ascending order,

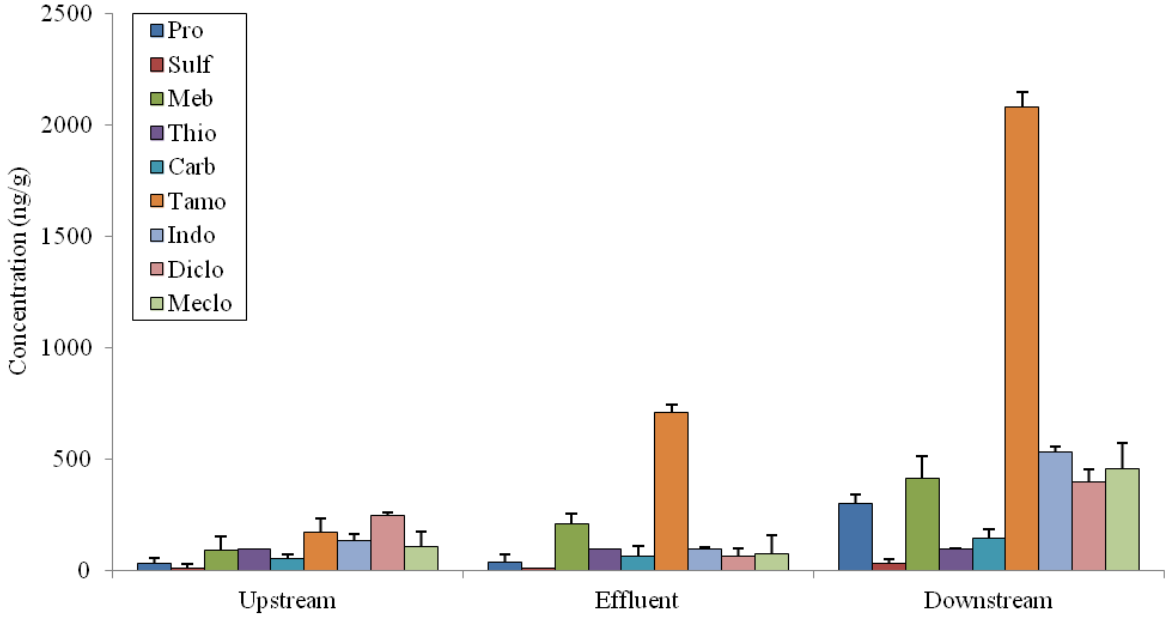

Figure 86. Overall, compounds were identified with highest concentrations at the site downstream from the effluent outflow path, which is the case for all compounds except Thio, with a very slight increase of only 1.72 ng/g between the downstream and effluent site.

The variability between samples is generally small for those taken in April, with the lowest being 0.64 for Sulf at the effluent site and highest for Meclo with 115.98 downstream (Table 24). Minimum and maximum values of all target pharmaceutical compounds are as follows; 32.57 – 303.27 ng/g for Pro, 11.03 – 32.92 ng/g for Sulf, 93.33 – 413.83 ng/g for Meb, 98.01 – 99.97 ng/g for Thio, 175.84 – 2080.75 ng/g for Tamo, 96.15 – 533.89 ng/g for Indo, 68.47 – 401.64 ng/g for Diclo and lastly 76.24 – 459.90 ng/g for Meclo.

4.3.2.4 June 2010

Concentrations of pharmaceuticals at the River Medway in June ranged from 11.57 ng/g to 6156 ng/g for Sulf downstream and Diclo at the effluent site respectively (Table 25). Concentration of target compounds were as follows; Pro 328.42 – 590.05 ng/g, Sulf 11.57 – 25.53 ng/g, Meb 51.96 – 136.93 ng/g, Thio 98.82 – 100.30 ng/g, Carb 251.50 – 547.33 ng/g, Tamo 1058.48 – 1742.55 ng/g, Indo 176.47 – 425.51 ng/g, Diclo 2228.56 – 5295.62 ng/g and Meclo 1599.31 – 6156.95 ng/g.

Figure 86 clearly illustrates that both Diclo and Meclo have the highest concentration at all three sample sites, with Diclo ranging from 2228.56 to 5295.62 ng/g, and Meclo ranging from 1599.31 to 6156.95 ng/g. Figure 87 displays the data as a bar chart where Meclo is identified as the highest detected concentration of compound at the sampling locations upstream and at the effluent site, whereas Diclo is the highest at the downstream sampling site. Standard deviation indicates that Thio is the most reliable detected compound with results closest to the mean value (2.88 – 5.01), compared to others including Tamo with up to 1507.94, Meclo with 1090.25 and Diclo with 1008.41. These also have high initial detected concentrations; this is not surprising as for higher values there is the possibility of more room of variability. Although the concentration was high, the values have been averaged from replicated in and ex situ.

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	526.26±54.25	25.53±7.55	69.98±42.57	98.82±5.01	410.97±93.74	1060.07±442.73	425.51±243.96	2995.13±689.50	4490.11±1090.25
Effluent	590.05±392.86	21.50±17.50	136.93±105.07	99.16±2.88	547.33±450.04	1058.48±609.14	413.38±304.70	5295.62±1008.41	6156.95±745.54
Downstream	328.42±68.40	11.57±2.84	51.96±31.14	100.30±3.16	251.50±99.02	1742.55±1507.94	176.47±55.28	2228.56±443.07	1599.31±493.45

Table 25 Concentration (ng/g) and standard deviation of target pharmaceuticals from River Medway samples from June 2010

Almost all compounds increase from the upstream to effluent sampling site, the largest increased being Diclo with an increase from 2995.14 ng/g to 5295.62 ng/g. Those which do not only have a very small increase, for example Tamo decreases from 1060.07 ng/g to 1058.48 ng/g. All compounds excluding Thio and Tamo decreased in concentration after the effluent site to downstream. The largest decrease from effluent to downstream sampling site being Meclo from 6156.95 – 1599.31 ng/g.

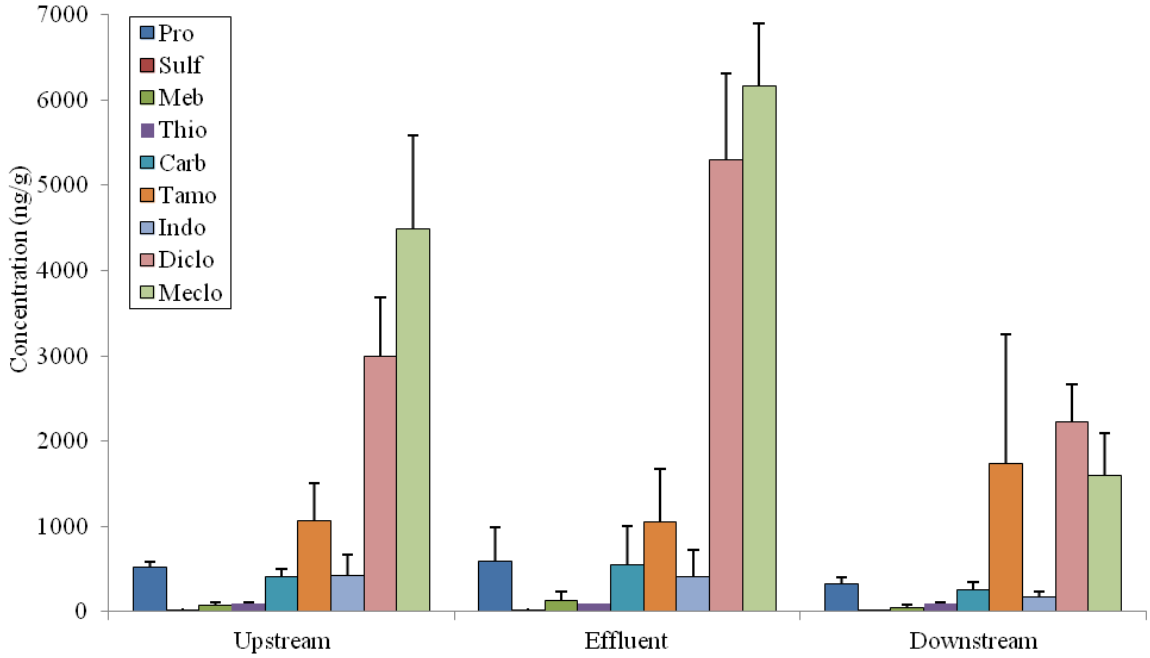

Figure 87 Total concentration of pharmaceuticals at River Medway sample locations for June 2010

4.3.3 Soil Moisture Content (SMC) and Organic Matter Content (OMC)

Table 26 highlights representative data collected when three samples were tested to analyze for SMC and OMC of the samples. The SMC ranged from 30.41 % to 31.08 % with an average of 30.79 %, and an excellent standard deviation of 0.35. The OMC ranged from 1.64 % to 2.43 %, with an average of 2.08 % and once again excellent standard deviation of 0.40 %. To conclude, approximately one third of the samples were soil moisture, and only an average of 2.08 % organic matter. This suggests that there may be a considerable concentration of pharmaceutical compounds present in the water part of the sample which could be lost during sample preparation (drying), which may pose an important significance when considering analysis. The organic matter however only makes up an average of 2.08 % of the sample and is not lost during sample preparation, simply dried as a solid part of the matrix, and subsequently included in analysis for target pharmaceutical compounds. The precision of both the SMC (2.26) and OMC (38.38) were within very good (<10%) and acceptable (<60%) limits.

Sample	Crucible weight	Crucible plus wet sediment	After drying	After combustion	Net Weight (wet)	Net Weight (dry)	Net Weight (combust)	SMC (%)	OMC (%)
1	47.50	61.34	57.06	56.86	13.84	9.56	9.36	30.90	2.16
2	27.58	39.61	35.95	35.81	12.03	8.37	8.24	30.41	1.64
3	43.61	54.60	51.18	51.00	10.99	7.57	7.39	31.08	2.43
							Mean	30.79	2.08
							Stdev	0.35	0.40
							Precision	2.26	38.38

Table 26 SMC (%) and OMC (%) of three samples taken from the River Medway during sample anaysis

4.3.4 Water samples

Total target compound concentration in water sampling varied between 10.40 ng/l for Sulf downstream to 14573.12 ng/l for Diclo at the effluent sampling location (Table 27). Remaining concentrations were as follows; 61.81 - 261.63 for Pro, 10.40 - 31.54 ng/l for Sulf, 79.07 – 1583.28 ng/l for Meb, 70.50 – 140.90 ng/l for Thio, 850.82 – 4821.10 ng/l for Carb, 57.93 – 260.21 ng/l for Tamo, 94.90 – 569.82 ng/l for Indo, 1131.45 – 14573.12 ng/l for Diclo and finally 1743.84 – 3404.38 ng/l for Meclo. Seven out of the nine compounds were higher at the effluent site than downstream, but interestingly two compounds were higher at the upstream sampling location, before the effluent site. Also, seven of the nine compounds were found in a smaller concentration at the downstream site compared to the effluent site.

With regards to the standard deviation (variability) of results, again some of the statistics are high, however when compared to the high concentration values of corresponding concentration data, are well within the acceptable limit (Table 27). This is displayed more clearly graphically in Figure 88 Total concentration (ng/l) of pharmaceuticals at River Medway sample locations for water samples in June

In comparison to the sediment samples taken at the same time (June), there can be no assumption of a direct relationship, other than that if there is a high concentration detected in the sediment samples, there may be a higher concentration in the water due to agitation due to water movement, remobilizing PPCP compounds back into the water.

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	61.81±175.13	11.06±11.20	224.28±521.77	70.50±2.96	850.82±380.88	260.21±217.35	94.90±67.91	1131.45±928.83	1743.84±1206.68
Effluent	252.26±109.86	31.54±46.46	79.07±244.44	140.97±0.85	4821.10±1726.67	101.34±328.65	569.82±261.39	14573.12±4869.24	3404.38±2824.70
Downstream	261.63±219.94	10.40±1.50	1583.28±1589.67	104.37±16.36	4173.48±577.96	57.93±128.09	372.96±54.46	3795.09±1524.57	2265.43±2115.73

Table 27 Concentration (ng/l) and standard deviation of target pharmaceuticals from River Medway water samples from June 2010

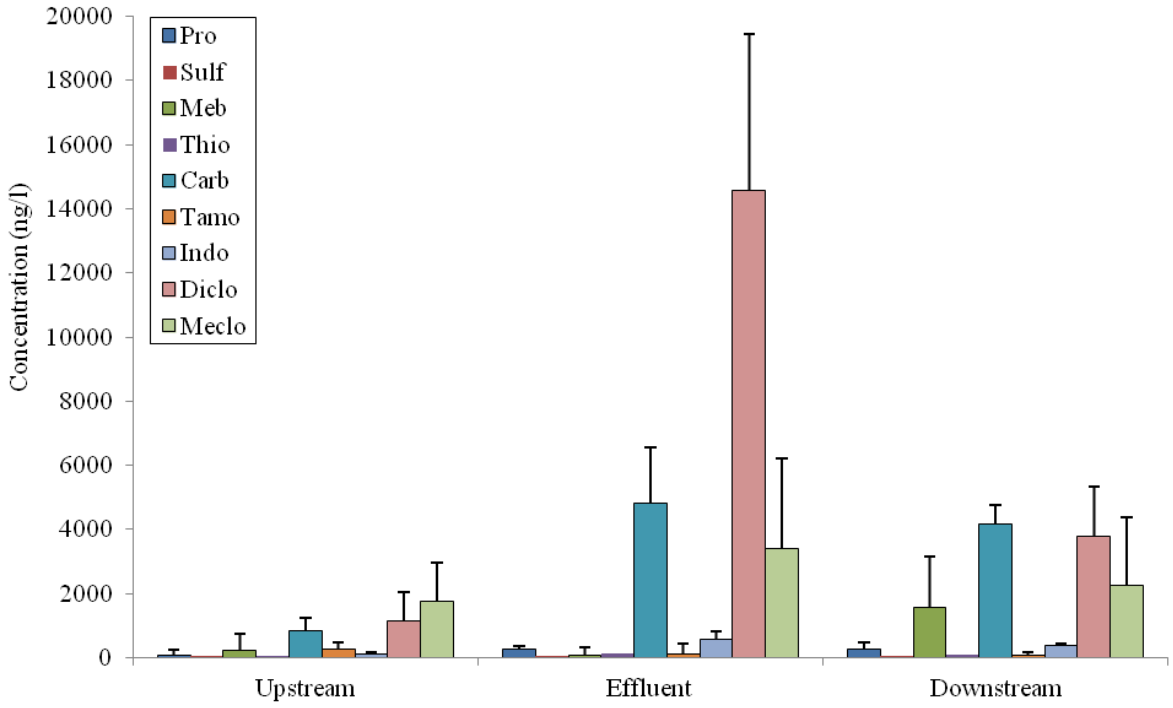

Figure 88 Total concentration (ng/l) of pharmaceuticals at River Medway sample locations for water samples in June 2010

4.3.5 Temporal Variability

Data collected for the analysis of pharmaceutical compounds in the River Medway allowed for a second data set to be produced for temporal variability. This was conducted every other month for eight months between December to June (December, February, April and June). From this, a snapshot into variability of pharmaceuticals at the same site can be drawn for a period of eight months and spanning three seasons (Winter, Spring and Summer). Due to limitations of this project, an analysis for Autumn was unable to be conducted along with any further sampling.

4.3.5.1 Upstream Site

During the first three sampling instances, the concentration of target pharmaceutical compounds were significantly lower than the final in June, when there was a sudden increase; namely for Meclo (4490.11ng/g), Diclo (2995.14 ng/g), Tamo (1060.07 ng/g) and Pro (526.26 ng/g) (Figure 89). Whether or not there is a relation to it being the coldest month during which samples were taken, February was noticeably the time when target PPCP compounds were at their lowest concentration, ranging from 0.51

ng/g for Sulf to 144.73 ng.g for Tamo. December and April were also fairly low in terms of concentration observed at the upstream site across all compounds, with December results ranging from 0.64 ng/g for Sulf to 283.68 ng/g for Tamo, and April ranging from 14.83 ng/g for Sulf and 250.37 ng/g for Diclo (Table 28). There may have been a slight overall increase in compound concentration in April due to a slightly warmer temperature, however this is only an assumption. Another contributing factor to consider is rainfall, which I would typically expect to increase in autumn and winter months. This is not reflected at all sites tested for temporal variability.

Upstream	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Dec	18.54	0.64	13.56	101.30	54.04	283.68	46.69	16.47	31.83
Feb	4.60	0.51	13.75	98.95	12.71	144.73	35.84	17.85	10.29
Apr	32.57	14.83	93.33	99.97	57.35	175.84	135.03	250.37	109.79
Jun	526.26	25.53	69.98	98.82	410.97	1060.07	425.51	2995.14	4490.11

Table 28 Concentration (ng/g) of target pharmaceuticals from River Medway water samples between December 2009 to June 2010 for the upstream sampling location.

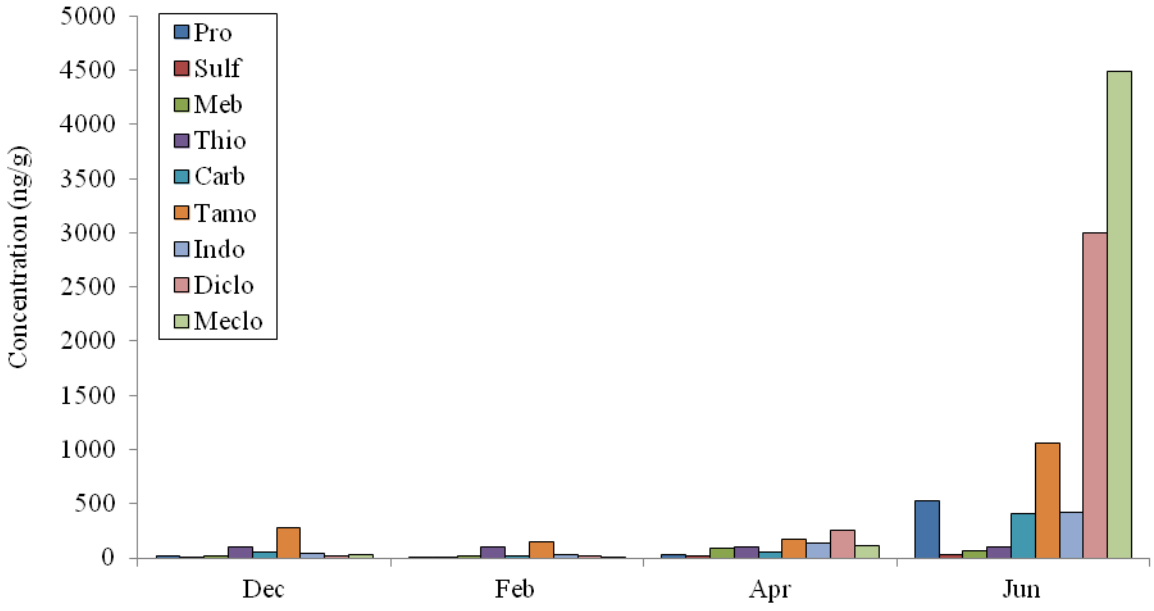

Figure 89 Temporal variability of all nine target pharmaceutical over seven month sampling period at upstream sampling location (Dec 2009 to Jun 2010)

4.3.5.2 Effluent Site

Compared to the upstream sampling site, the effluent site has an increased concentration of target PPCP compounds, which is to be expected (Figure 90). In fact, the effluent sampling location had the highest of all three, which is again expected. Concentrations were the highest during June, with a range between 21.50 ng/g for Sulf to 6156.95 ng/g for Meclo (Table 29). Sampling during this month had the highest identified concentrations for eight of the nine compounds. The only exception being Meb, which had a concentration of 136.93 ng/g in June compared to 213.28 ng/g in April. As with the upstream sampling location, February had the overall lowest concentrations when compared to the others in December, April and June. Concentrations only ranged from 0.47 ng/g for Sulf (also the lowest overall detected concentration at the effluent site) to 165.33 ng/g for Tamo. Surprisingly, Tamo has the highest observed concentration at the effluent site during December, with a concentration of 1194.24 ng/g. April once again had a higher concentration than both December and February for all compounds except one (Tamo in Dec, previously discussed).

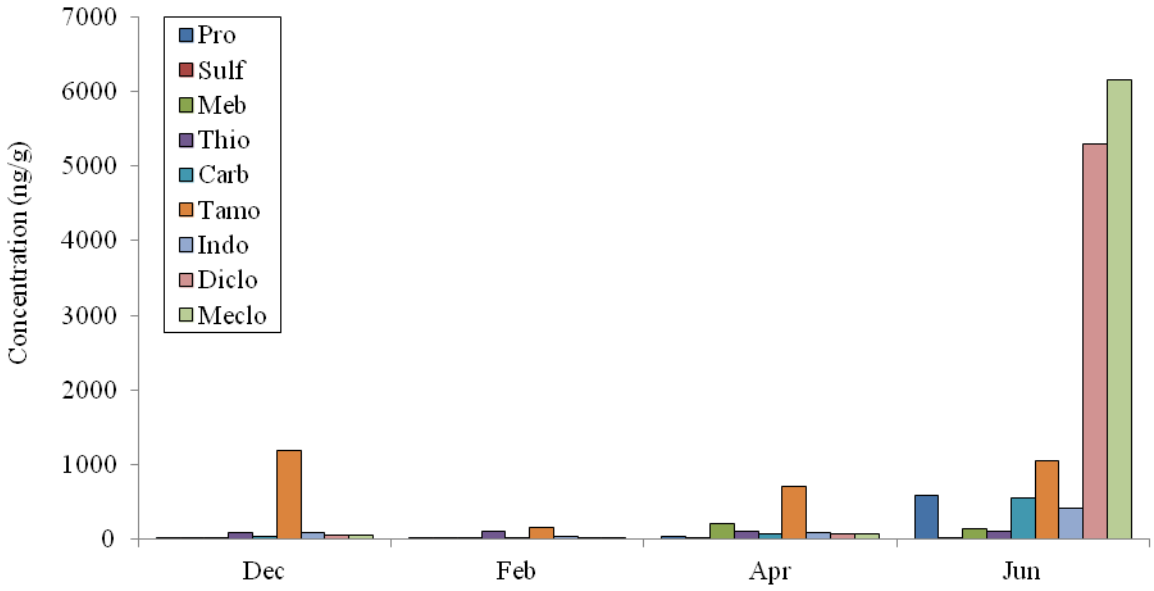

Figure 90 Temporal variability of all nine target pharmaceutical over seven month sampling period between December 2009 to June 2010 at effluent sampling location

Effluent	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Dec	21.94	2.10	6.50	95.63	39.29	1194.24	82.20	57.98	55.95
Feb	3.164	0.47	14.27	97.66	11.84	165.33	32.08	11.23	7.33
Apr	39.22	11.03	213.28	99.73	65.44	710.54	96.15	68.47	76.24
Jun	590.05	21.50	136.93	99.16	547.32	1058.48	413.38	5295.62	6156.95

Table 29 Concentration (ng/g) of target pharmaceuticals from River Medway water samples across all months for the effluent site sampling location (Dec 2009 to Jun 2010).

4.3.5.3 Downstream Site

At the downstream sampling location, concentrations were higher than at the upstream site, but lower than at the effluent site suggesting an increase following the effluent site discharge. As seen in Figure 91, there is a higher variability of target PPCP concentrations across all sites. In particular, Tamo has a noticeably higher concentration at the downstream site (following discharge from the STW at the effluent site), with a range between 690.04 ng/g to 2080.75 ng/g, also the highest observed concentration at the downstream location across all months (Table 30). During June, concentrations were again higher than the remaining times sampled. Aside from Tamo (1742.55 ng/g), the other two high compound concentrations observed were for Diclo and Meclo (2006.34 ng/g and 1599.31 ng/g respectively) (Table 30). Disregarding one compound (Meb), February has the lowest observed concentrations compared to the other three months.

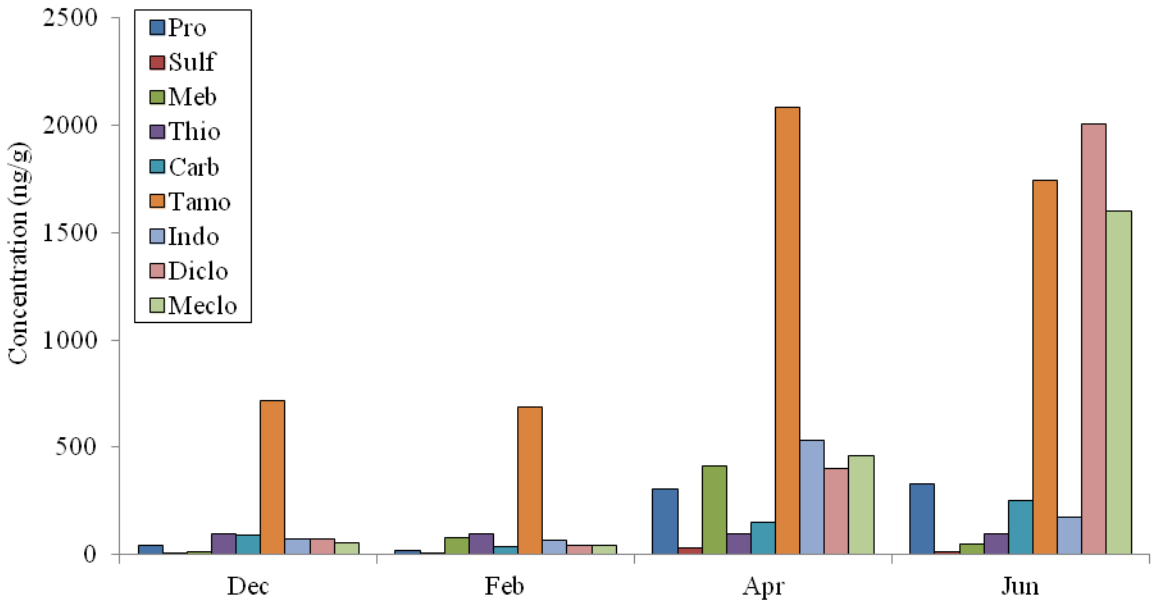

Figure 91 Temporal variability of all nine target pharmaceutical over seven month sampling period between December 2009 to June 2010 at downstream sampling location

Downstream	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Dec	43.94	8.95	11.22	99.78	89.00	715.57	70.59	73.56	52.90
Feb	21.44	4.53	78.02	97.67	38.86	690.04	70.15	45.90	46.51
Apr	303.37	32.92	413.83	98.01	149.30	2080.75	533.90	401.64	459.90
Jun	328.42	11.57	51.96	100.31	251.50	1742.55	176.47	2006.34	1599.31

Table 30 Concentration (ng/g) of target pharmaceuticals from River Medway water samples across all months for the downstream sampling location (Dec 2009 to Jun 2010).

4.4 Applications of the developed and validated method to different environmental matrices

Following both successful development and validation, the methods were applied to different environmental sample matrices to further assess the fate of pharmaceutical compounds in the aquatic environment. A selection of different samples were taken on site at the River Medway during the final sampling period. They were then stored appropriately and prepared as the river sediment samples were, to ensure that the method was applied in the same way as to reduce bias.

Eleven different sample matrices were tested to identify if the developed method could be applied to different samples other than sediment. These samples included seaweed, rosehip, grass root, lugworm, clamshell, crab, snails, grass shoots, buds, dandelions and leaves. These experiments are different to method validation in that it is applied to different matrices other than simply sediment from the River Medway. It is important to assess if the method has a wider use and variety of applications. Ultimately it would be preferable if the developed and validated method was applicable to different matrices (vegetation and biological) without sacrificing recovery or reliability levels. Recovery was calculated as a percentage, and reproducibility was analysed by taking replicates in and ex situ.

Following collection, the samples were processed as in 3.2.6; stored at -18 °C, freeze dried and homogenized. The samples were then subjected to the same process as the sediment samples and analysed for the same selected PPCPs using LC-MS/MS. Each sample collected was spiked with 5 ng of 20 ppm pharmaceutical standard solution. The data in Figure 92 indicates that aside from this 100 ng/l spiked to each sample, there was already a high volume of existing pharmaceuticals in each sample. However it must be noted that the water compounds input may enhance the signal of the LCMS.

All applications, proved to have both high recoveries and concentrations of PPCPs tested, with a range between 2.51 – 536.44 %. In particular Meb, with recoveries between 40.58 % and 520.37 %; the highest was found in the crab sample (Table 31). This would cause significant effects on the crustacean as detailed in the review of relevant literature. The minimum was observed for Sulf in the rosehip sample and the maximum for Tamo in dandelions. The concentration data in the samples is not given due to the recovery being so high; this is more significant as it shows the viability and

success of the method. The actual PPCP content itself is not so important as recovery in this instance.

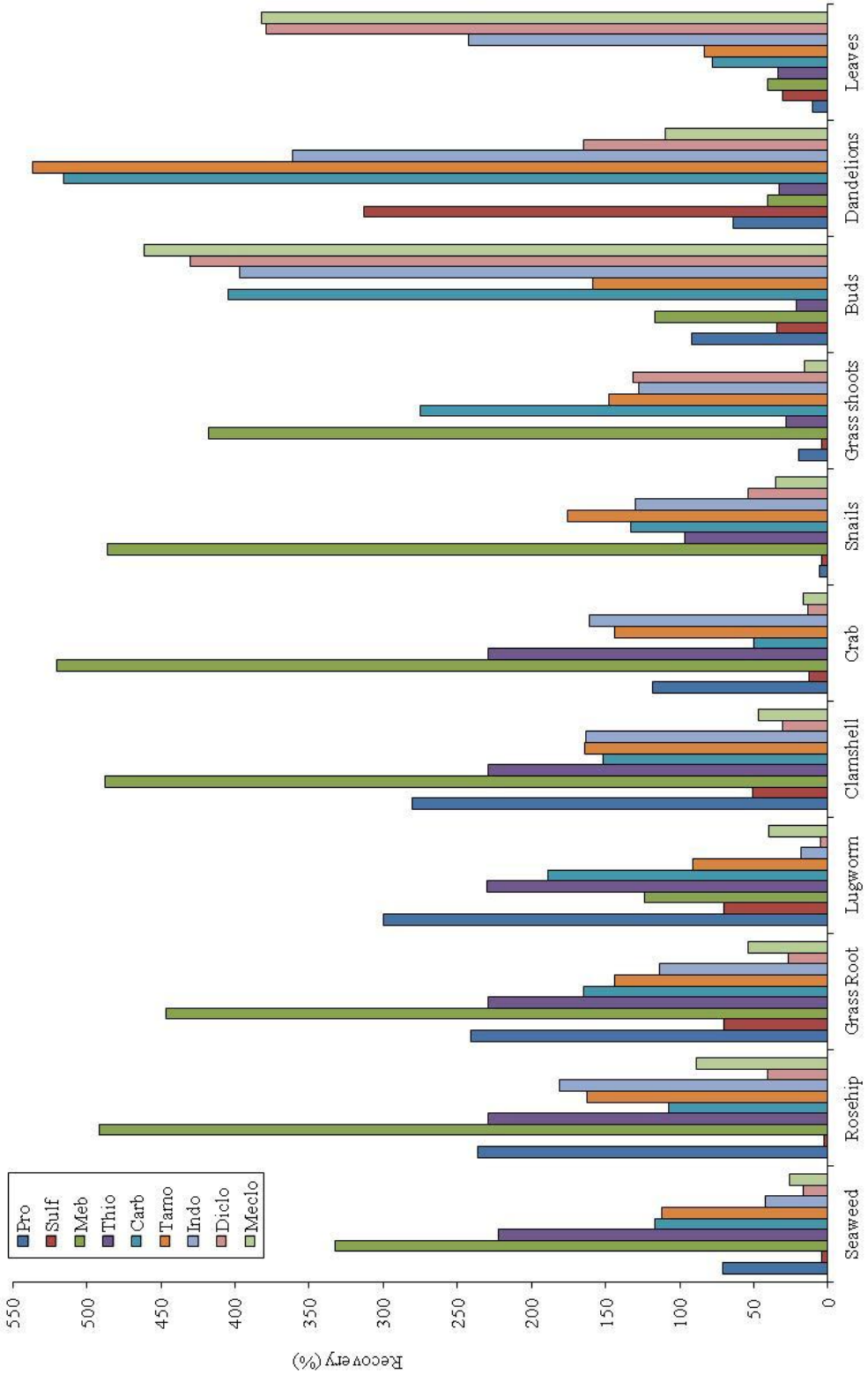

Figure 92 Application data from method development and validation for different matrices in 2009

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Seaweed	70.58	4.22	332.14	221.93	116.99	112.03	42.49	16.93	25.87
Rosehip	236.53	2.51	491.69	229.35	107.10	162.22	181.42	40.77	88.88
Grass Root	240.86	69.88	446.62	229.35	164.53	144.14	113.41	26.73	53.64
Lugworm	299.86	69.74	123.42	229.63	188.86	91.10	18.12	4.92	39.84
Clamshell	280.28	51.02	487.32	229.35	151.57	164.09	163.14	30.18	46.75
Crab	118.53	13.03	520.37	229.35	50.15	144.20	160.98	13.25	16.23
Snails	5.86	4.23	486.35	96.44	133.10	175.78	129.65	53.76	34.85
Grass shoots	19.88	4.52	417.65	27.85	274.85	147.67	127.52	131.73	16.07
Buds	92.16	34.15	116.38	21.03	404.33	158.33	396.55	430.11	461.22
Dandelions	64.13	312.78	40.93	32.97	515.34	536.44	361.22	164.67	109.55
Leaves	10.32	30.61	40.58	33.62	77.84	83.11	242.67	379.38	381.80

Table 31 Recovery values for different sample matricies for the application of the developed pharmaceutical method

4.4.1 Vegetation samples

4.4.1.1 Seaweed

Seaweed (*Ascophyllum nodosum*) samples produced recoveries between 4.22 % and 332.14 % (Sulf and Meb respectively), with five out of nine target compounds over satisfactory recoveries (≥50 %); Pro (70.58 %), Meb (332.14 %), Thio (221.93 %), Carb (116.99 %) and Tamo (112.03 %).

4.4.1.2 Grass root

Grassroot sample followed trend with the highest recovery for Meb (446.62 %). The lowest observed recovery was 26.73 % for Diclo. All other eight compounds excluding Diclo produced acceptible recoveries above and beyond the accepted recovery value. Recoveries observed were as follows; Pro 240.86 %, Sulf 69.88 %, Thio 229.35 %, Carb 164.53 %, Tamo 144.14 %, Indo 113.41 % and Meclo 53.64 %.

4.4.1.3 Grass shoot

Meb far outperformed any other compound recovery on grass shoot matrix analysis with 417.65 %. The next highest recovery was for the analysis of Carb with 274.85 % recovery; the highest of all matrices analysed. The lowest compound recovery for grass

shoots was unsurprisingly Sulf (4.52 %). The next lowest recoveries were Meclo and Pro (19.88 % and 16.07 %). Other compounds analysed produced significant recoveries of 147.67 % (Tamo), 127.52 % (Indo) and 131.73 % (Diclo). The remaining compound below the acceptible recovery of ≥50 % and not yet mentioned was Thio, with 27.85 %.

4.4.1.4 Rosehip

The rosehip sample was taken from a rose bush on the side of the effluent site and so was in prime position to take up any contaminants into its system, providing the method application was sucessful to show this. Recoveries ranged from 2.51 % to 491.69 % (Sulf and Meb respectively), with seven out of nine target compounds producing acceptible recoveries over 50 %. Those which did not were Sulf (previously mentioned) and Diclo which gave a recovery of 40.77 %.

4.4.1.5 Dandelion

The dandelion sample (*taraxacum*) had a maximum recovery of 536.44 % as previously mentioned as the highest overall result for Tamo. This was closely followed by Carb (515.34 %). The lowest observed contaminant recovery for the dandelion samples was 32.97 % and 40.93 % for Thio and Meb respectively. In ascending order, remaining compound recoveries were as follows; Pro (64.13 %), Meclo (109.55 %), Diclo (164.67 %), Sulf (312.78 %), Indo (361.22 %).

4.4.1.6 Sloe buds

Sloe buds (*Prunus spinosa*), also commonly known as ‘blackthorn’, had significantly high recoveries for all compounds, in particular Indo, Diclo and Meclo which were the highest observed recoveries for all samples of 396.55, 430.11 and 461.22 %. Carb also had a high recovery of 404.33 %. The lowest recovery of 21.03 % was observed by Thio.

4.4.1.7 Sloe leaves

The target compound which obtained the highest recovery for slow leaves was Meclo, with 381.80 %, very closely followed by Diclo with 379.38 %. The minimum recovery identified was for Pro of only 10.32 %. Remaining compounds, in ascending order, obtained recoveries of 30.61 % (Sulf), 33.62 % (Thio), 40.58 % (Meb), 77.84 % (Carb), 83.11 % (Tamo) and finally 242.67 % (Indo).

4.4.2 Biological (tissue) samples

4.4.2.1 LugWorm

Interestingly the lugworm (*Arenicola marina*) samples gave a highest recovery for the compound Prol (299.89 %), which suggests an interesting conclusion for biological uptake of this compound. The compound with the lowest observed recovery was 4.92 % for Diclo. Excluding this, Indo (18.12 %) and Meclo (39.84 %), all other compounds produced recoveries for target compounds far in excess of the minimum acceptible recovery.

4.4.2.2 Crab

The highest overall observed recovery in these sets of observations was in the crab; 520.38 % for Meb. The lowest observed recovery for the crab was 13.03 % for Sulf, as with many other matrices for having low recorded recovery. Other recoveries below the accepted 50 % value were Diclo (13.25 %) and Meclo (16.23 %). All other compounds exceeded the minimum accepted value with recoveries of 118.53 % for Pro, 229.35 % for Thio, 50.15 % for Carb, 144.20 % for Tamo and 160.98 % for Indo.

4.4.3 Crustacean samples

4.4.3.1 Clamshell (bivalve mollusk)

The clamshell again had a high recovery for Pro (280.28 %), but the highest recovery was afor Meb (487.32 %). The lowest recovery was observed for Diclo (30.18 %). Seven out of nine compounds produced recoveries above the threshold; Pro (280.28 %), Sulf (51.02 %), Meb (487.32 %), Thio (299.35%), Carb (151.57 %), Tamo (164.09 %) and Indo (163.14 %). Meclo had an observed recovery of 46.75 %, just below the accepted recovery value.

4.4.3.2 Snails

Following trend, Meb was the compound identified with the highest recovery of 486.35 %. The next highest recovery was Tamo (175.78 %), closely followed by Carb and Indo (133.10 % and 129.65 % respectively). The lowest recovery of all compounds was Sulf with 4.23 % closely followed by Pro with 5.86 %. Remaining compounds were Thio, Diclo and Meclo with recoveries of 96.44 %, 53.76 % and 34.85 % in descending order.

4.5 Report of the River Medway Sewage treatment works – seaweed samples, following successful method application to different sample matrices.

4.5.1 February 2010

It is immediately obvious that concentration of pharmaceutical compounds in seaweed samples in the River Medway sampling sites increases as the samples proceed downstream from the upstream, effluent to downstream sampling location (Figure 93).

The upstream sampling site has a range between 0.06 ng/g for Pro to 4.84 ng/g for Diclo. Remaining target compounds in ascending order were 0.11 ng/g for Sulf, 0.43 ng/g for Thio, 1.02 ng/g for Meb, 3.11 ng/g for Indo, 3.15 ng/g for Carb, 3.99 ng/g for Meclo and 4.53 ng/g for Tamo (Table 32).

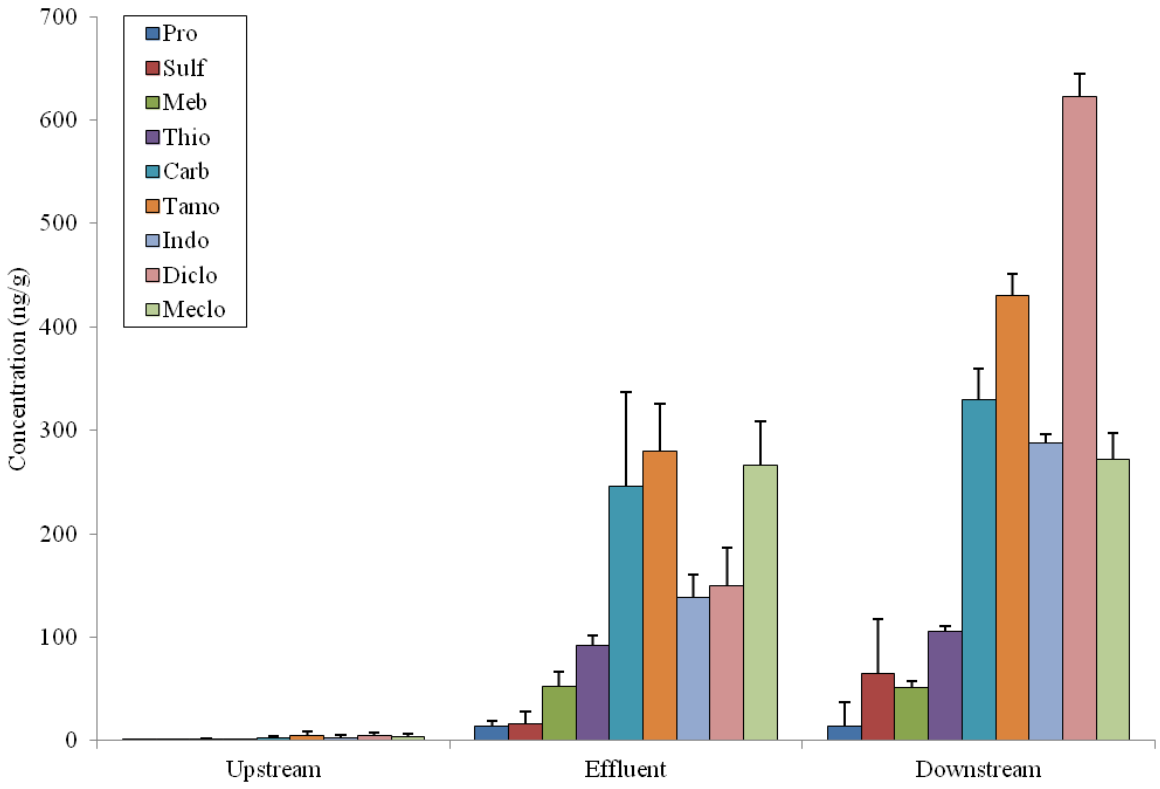

Figure 93 Total concentration of pharmaceuticals at River Medway sample locations for seaweed samples in February 2010

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	0.06±0.07	0.11±0.07	1.02±0.92	0.43±0.37	3.15±1.71	4.53±4.31	3.11±2.20	4.84±3.07	3.99±2.86
Effluent	13.94±5.19	16.35±11.95	52.85±14.31	92.64±9.40	245.52±91.72	280.23±45.32	138.70±21.48	149.47±37.32	266.22±42.32
Downstream	14.14±23.45	64.98±52.18	51.21±6.55	105.62±4.98	329.62±29.82	430.76±20.53	287.62±8.30	622.22±22.39	272.10±25.71

Table 32 Seaweed sample data for February 2010 at the River Medway

Results for seaweed analysed from the downstream site at the River Medway sampling location were the highest observed in comparison to the other two sites. The maximum concentration observed was Diclo with 622.22 ng/g, also the highest overall result for seaweed in February. Lowest observed concentration of a pharmaceutical compound here was 14.14 ng/g for Pro. The standard deviation of results demonstrates that precision is fairly high for all compounds (between 4.98 and 29.82) with an exception of Sulf (52.18), demonstrated in Table 323. Concentrations for remaining compounds were as follows; Sulf 64.98 ng/g, Meb 51.21 ng/g, Thio 105.62 ng/g, Carb 329.62 ng/g, Tamo 430.76 ng/g, Indo 287.62 ng/g and Meclo 272.10 ng/g.

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	50.46±30.91	46.92±16.13	78.44±62.29	95.32±3.92	77.63±32.15	432.92±31.05	20.55±5.74	72.64±53.87	122.40±51.90
Effluent	132.58±25.93	162.33±12.96	735.10±17.99	111.73±19.05	77.00±61.54	125.72±3.10	135.22±23.39	266.64±103.12	806.08±630.17
Downstream	1400.66±191.07	366.18±48.02	280.70±47.57	98.59±7.92	2337.89±170.02	2089.51±94.81	4712.55±126.48	646.50±32.91	2956.73±62.77

Table 33 Concentration (ng/g) and standard deviation of target pharmaceuticals from River Medway seaweed samples from April 2010

4.5.2 April 2010

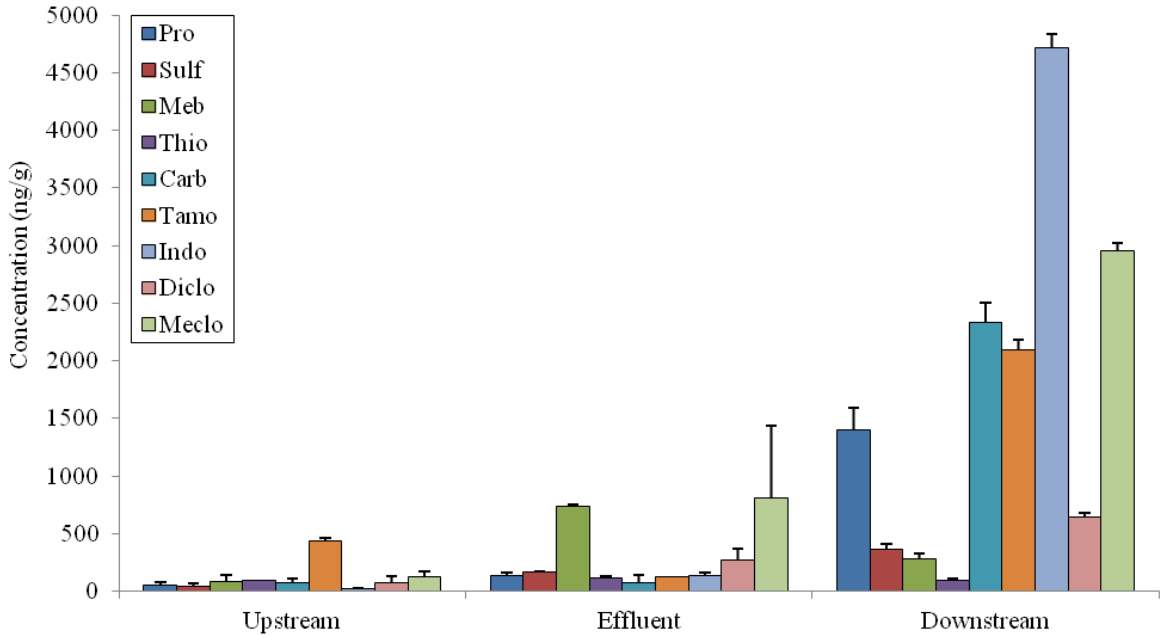

Figure 94 Total concentration of pharmaceuticals at River Medway sample locations for seaweed samples in April 2010

It is clear that concentration of target pharmaceutical compounds increases as you travel downstream, with the overall lowest observations at the upstream sampling location and the highest downstream (Figure 94). This may be because the outfall may be a little further downstream than where samples were collected at the 'effluent' site. Also compounds may be carried in water samples and deposited further downstream, to then be taken up by other matrices as seaweed for an example here. Compound concentration ranged from 20.55 ng/g to 4712 ng/g at upstream and downstream sites respectively both for Indo. Therefore this compound has the highest variability in results. Excluding two compounds, Carb and Tamo, all sites observed an increase from upstream to effluent sampling sites. This is again the case between effluent and downstream sampling sites, with the exception of Meb and Thio. Figure 94 suggests that compounds Carb, Tamo, Indo and Meclo increased as a direct result of the effluent outfall.

At the upstream sampling site, the lowest observed concentration was for Indo with 20.55 ng/g and the highest for Tamo with 432.92 ng/g. At the effluent site, minimum concentration was observed for Carb of 77.00 ng/g and the maximum of 806.08 ng/g

for. At the downstream sampling location, the highest concentration calculated was for 4712.55 ng/g for Indo and the lowest 98.59 ng/g for Thio.

Range of concentration for each compound were as follows (4.5.2 April 2010

); 50.46 – 1400.66 ng/g for Pro, 46.92 – 366.18 ng/g for Sulf, 78.44 – 735.10 ng/g for Meb, 95.32 – 111.73 ng/g for Thio, 77.00 – 2337.89 ng/g for Carb, 125.72 – 2089.51 ng/g for Tamo, 20.55 – 4712.55 ng/g for Indo, 72.64 – 646.50 ng/g for Diclo and finally 122.40 – 2956.7 ng/g for Meclo.

The standard deviation for seaweed samples in April ranged from very good (low variability) of 3.10 for Tamo at the effluent site, to extremely poor (high variability) of 630.17 for Meclo at the effluent site.

4.5.3 June 2010

It is immediately obvious that concentrations of pharmaceutical compounds in seaweed samples in the River Medway sampling sites from June are very high compared to previous analyses (Table 34). The upstream sampling site has a range between 102.51 ng/g for Thio to 14206.81 ng/g for Meclo. At the effluent and downstream sampling sites the ranges were from 109.41 ng/g for Thio to 17704.05 ng/g for Diclo and 108.36 to 43987.78 ng/g for Meclo respectively. Individual target compound ranges across the three sites were as follows; 1440.12 – 5739.89 ng/g for Pro, 366.97 – 450.47 ng/g for Sulf, 239.53 – 1296.50 ng/g for Meb, 102.51 – 109.41 ng/g for Thio, 4260.02 – 5241.70 ng/g for Carb, 1155.20 – 8180.56 ng/g for Tamo, 698.39 – 3283.66 ng/g for Indo, 12361.19 – 43866.78 ng/g for Diclo and lastly 14206.81 – 43987.78 ng/g for Meclo (Table 34).

Results for seaweed analysed from the downstream site at the River Medway sampling location in June were the highest observed in comparison to the other two sites. The maximum concentration observed was Meclo with 43987.78 ng/g, also the highest overall result for seaweed in June. Lowest observed concentration of a pharmaceutical compound in June was 239.53 ng/g for Meb, which was surprisingly at the effluent site. The standard deviation of results demonstrates that precision is varied for all compounds, between a very good 4.53 for Tamo downstream to 5428.97 for Carb also at the downstream site, demonstrated in Table 34. A graphical analysis of seaweed sample concentrations is shown in Figure 95. Aside from Diclo and Meclo, it is no immediately obvious of any increasing trend at the effluent site or other. However going back to standard deviation; although statistically high, in comparison to the actual concentration results, they are of little significance as it is relative to the initial result, and as shown on Figure 95, all acceptable.

Conc (ng/g)	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Upstream	5739.89±4268.70	366.97±266.84	1296.50±776.46	102.51±9.63	4938.97±1849.81	8180.56±2259.88	698.39±535.62	12361.19±3077.32	14206.81±2540.61
Effluent	2255.01±1006.06	450.47±630.74	239.53±338.75	109.41±17.34	5241.70±4120.04	1183.13±458.55	716.10±424.13	17704.05±1082.40	15578.34±3294.52
Downstream	1440.12±52.85	405.31±391.80	272.73±57.30	108.36±8.60	4260.02±5428.97	1155.20±4.53	3283.66±3738.48	43866.78±1934.03	43987.78±3774.47

Table 34 Concentration (ng/g) and standard deviation of target pharmaceuticals from River Medway seaweed samples from June 2010

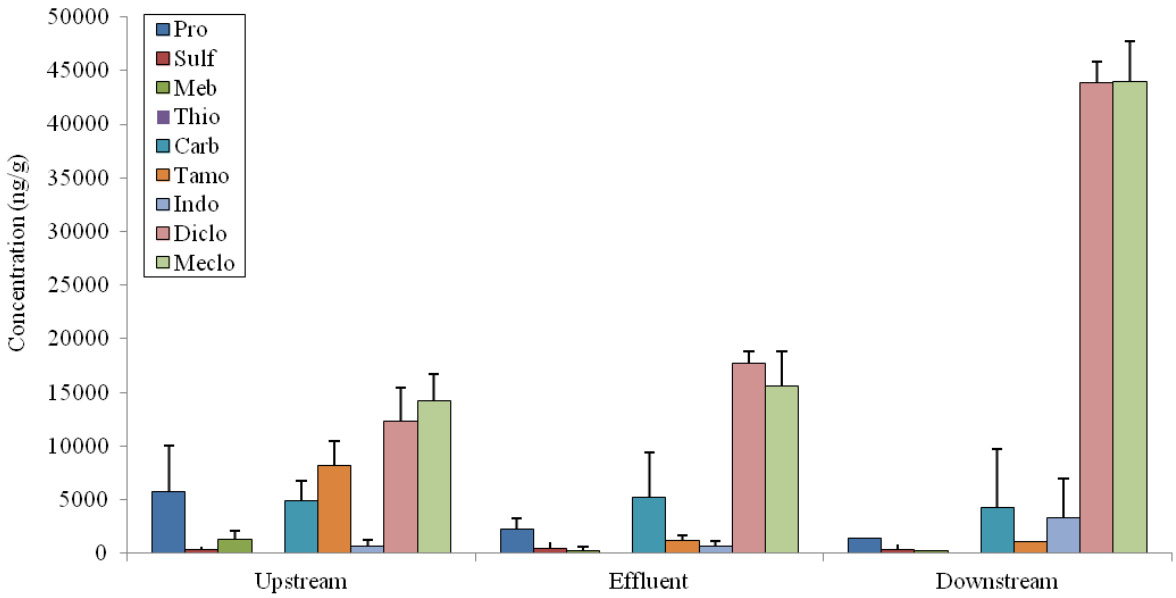

Figure 95 Total concentration of pharmaceuticals at River Medway sample locations for seaweed samples in June 2010

Chapter 5 - China Sample Analysis

5.1 Project background

A large study in China used a vast amount of samples to determine antibiotics in waters and surrounding environments. The samples were then sent for analysis of pharmaceuticals using the aforementioned developed method. As antibiotics are chemicals with the ability to inhibit the growth of microbes, a variety of antibiotics are currently been used to prevent and treat different diseases and infections in humans and animals. In addition, they are widely applied as growth promoter and feed additives in aquaculture and animal husbandry. Since antibiotics play a major role in medical treatment and modern livestock industries, they are used in large quantities worldwide. The global consumption of antibiotics is estimated to be 100,000-200,000 tons annually, of which approximately 13,000 tons were used in the EU countries and Switzerland, and 16,200 tons in the USA. Similarly more than 25,000 tons of antibiotics are used in China every year.

Due to the fact that most antibiotics are not completely metabolized by human and animal bodies, considerable fractions (about 25 ~ 75 %) of the administered doses are excreted directly with feces and ultimately enter the aquatic environment through various routes. Many antibiotics have been detected in different aquatic environments such as sewage effluents, surface water, groundwater and drinking water. Hence, aquatic organisms are subject to long-term exposure of antibiotics, with resulting chronic effects. For instance, the growth of Microcystis aeruginosa (freshwater cyanobacteria) was inhibited by exposure to chlortetracycline (CTC) or tetracycline (TC) at concentrations of less than 0.1 mg L^{-1}. On the other hand, tetracyclines can bind to the ribosome and impair protein synthesis. In addition, it was reported that horizontal transfer of some resistance genes on plasmids could occur between different bacteria in the environment. Evidently, more and more concern is being raised on the potential risks of antibiotics to aquatic organisms.

Till now, there have been limited studies investigating the environmental risks of antibiotics in China, e.g. in the Victoria Harbour and the Pearl River. In addition, studies of antibiotics tend to focus on rivers and harbors, with little attention to lakes. Ironically, lakes in China are generally abundant in biodiversity yet under intensive

pressure from human activities (e.g. aquaculture, agriculture). Thus, appropriate environmental risk assessment (ERA) on antibiotics in lakes plays a significant role in protecting aquatic organisms and ultimately human development.

Guidelines for ERA of pharmaceuticals were introduced by the European Agency for the Evaluation of Medicinal Products and the US Food and Drug Administration. Currently, the risk quotient (RQ) approach is recommended to assess the environmental risk of medicines, by predicting the potential of many compounds to cause adverse environmental effects in aquatic organisms. In order to evaluate the environmental risk of antibiotics, their toxicity to aquatic organisms should be tested through different trophic levels, such as algae, flea and fish. However, a major obstacle in such risk assessment of antibiotics is the lack of appropriate toxicity data, there is therefore an urgent need for a reliable tool to predict acute and chronic toxicity. Consequently, the EcoSAR models, which were considered to be robust in predicting toxicity, are widely adopted in ERA.

The aim of this study was to conduct the analysis pharmaceuticals in Baiyangdian (BYD) Lake, a major lake in North China, following analysis of antibiotics back in China. The seasonal variation of antibiotic concentrations were also investigated.

5.2 Preliminary sample preparation

5.2.1 Site description and sample collection

As the largest freshwater lake (maximum depth was 3 m in 2007) in North China, BYD Lake is located about 200 km southwest of Beijing and covers more than 366 km^2. It consists of more than 100 small and shallow lakes linked by thousands of ditches and large areas of reeds. Currently, there are more than 243,000 people living in 39 villages scattering in and around it. BYD Lake plays a key role in sustaining local aquaculture as well as poultry farming, which provides aquatic products and eggs for nearly seven million people in the cities of Baoding and Cangzhou. In the last two decades, the lake has become a semi-closed water body without any outflows, whilst receiving runoff from agricultural irrigation, and untreated wastewater and sewage effluents from villages and Baoding City. It is likely that various antibiotics commonly used by human and animals are discharged into the lake.

Ten sampling sites covering potential sources of antibiotic inputs, e.g. urban sewage, agricultural fields, were selected in BYD Lake. At each sampling site, surface water samples (0.5 m depth) were collected in three different time periods in July and November 2007 and April 2008, corresponding to summer, autumn and spring seasons, respectively. Samples could not be collected in winter due to ice coverage. Then, samples were transported in ice boxes to the laboratory and stored at -20 °C before treatment and analysis.

5.2.2 Reagents and materials

Reagents and materials followed the trialed and tested new methodology developed in chapter 3.2.

The solvents used, including methanol and acetonitrile, were of distilled-in-glass grade, purchased from Rathrburn, Scotland. Formic acid was of HPLC grade. Internal standard (diuron-d6) was acquired from Cambridge Isotope Laboratories, USA.

Unless otherwise indicated, the chemicals used in the study were of analytical grade without further purification. Separate stock solutions (1000 mg/L) of individual compounds and internal standard were prepared by dissolving an appropriate amount of each substance in methanol, which were then stored at -20°C, and further diluted before using. Solid-phase extraction (SPE) cartridges (Oasis HLB, 500 mg/mL) were obtained from Waters (Milford, MA, USA). Glass fiber filters (Millipore, 0.45 μm) were obtained from Waters (MA, USA). Ultrapure water was supplied by Maxima Unit from USF Elga, UK.

5.2.3 LC-MS/MS analysis

The LC-MS/MS analysis of samples followed that used in the existing pharmaceutical experimentation in chapter 3.2.5.

5.3 Beijing sample analysis

5.3.1 Biological Samples from Beijing

Average Concentration (ng/g)	Pro	Sulf	Meb	Thio	Carb
Narrow leaves waterweed	39.69±33.86	2.49±4.32	60.58±8.73	32.87±5.41	222.96±68.62
Wide leaves waterweed	3.58±1.06	2.01±3.49	10.90±7.47	7.62±1.45	65.71±18.98
Reed root	4.09±0.33	0.81±1.28	4.72±7.92	5.86±1.12	24.72±7.72
Carp	5.09±0.70	2.01±2.37	8.55±3.83	4.52±2.06	18.05±3.26
Caucian	4.53±1.10	1.01±1.11	21.71±19.80	2.53±1.07	17.22±2.72
Silvercarp	6.67±0.84	4.32±1.62	1.79±2.19	4.30±0.40	16.49±3.09
Shrimp	7.77±7.05	1.43±3.28	0.25±0.24	5.90±1.30	29.21±22.57
Catfish	31.83±2.22	10.85±1.75	8.32±1.058	6.20±0.20	49.84±3.57
Screw	26.50±1.25	4.70±5.64	12.75±22.09	9.37±0.55	56.97±2.35
Average Concentration (ng/g)	Tamo	Indo	Diclo	Meclo	
Narrow leaves waterweed	567.00±58.09	125.41±54.08	170.83±35.81	228.39±60.02	
Wide leaves waterweed	523.93±25.75	81.52±9.37	103.10±15.90	111.41±15.26	
Reed root	427.32±25.50	60.46±4.75	56.83±8.67	56.03±11.88	
Carp	455.74±87.94	66.05±6.77	72.95±17.39	62.48±6.44	
Caucian	476.25±36.63	68.33±5.02	71.02±5.05	58.41±2.64	
Silvercarp	467.13±36.63	61.80±4.48	59.00±4.65	46.38±6.42	
Shrimp	195.04±49.31	23.20±13.14	13.95±8.44	40.00±24.63	
Catfish	145.52±11.90	27.65±5.64	36.85±12.04	43.69±5.26	
Screw	136.89±2.28	16.32±2.37	35.75±5.47	30.25±25.73	

Table 35 Average concentration and standard deviation of target pharmaceuticals from biological samples in Beijing

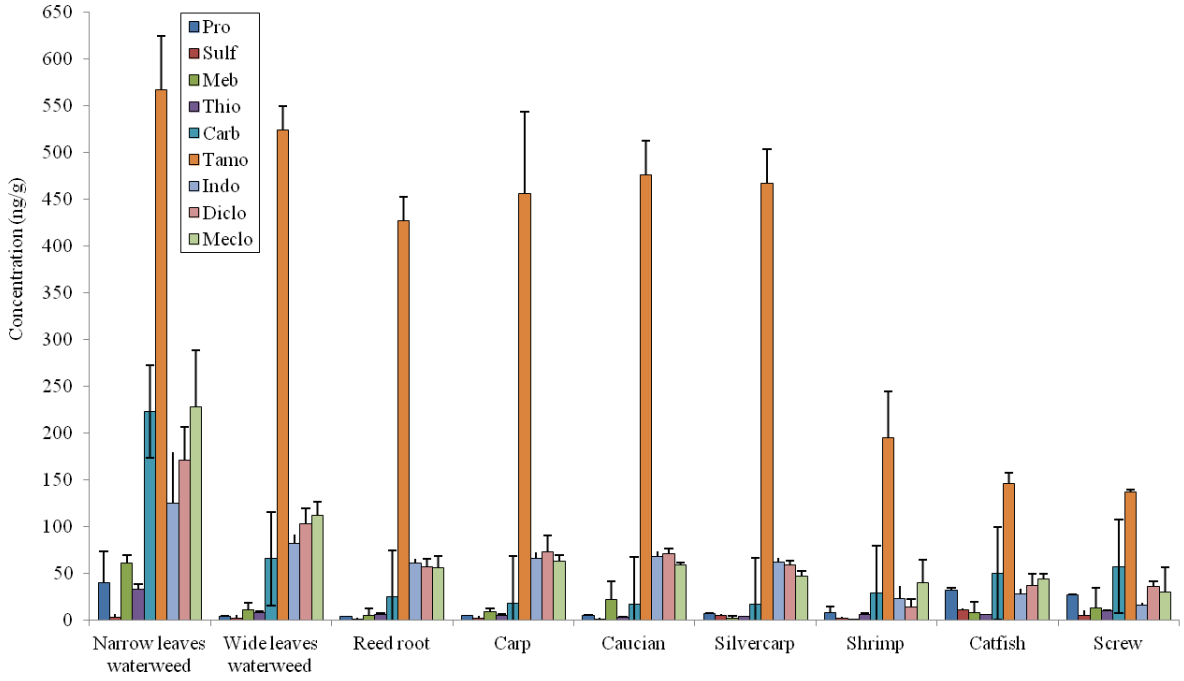

Figure 96 Total concentration of pharmaceuticals at Beijing sampling locations (all)

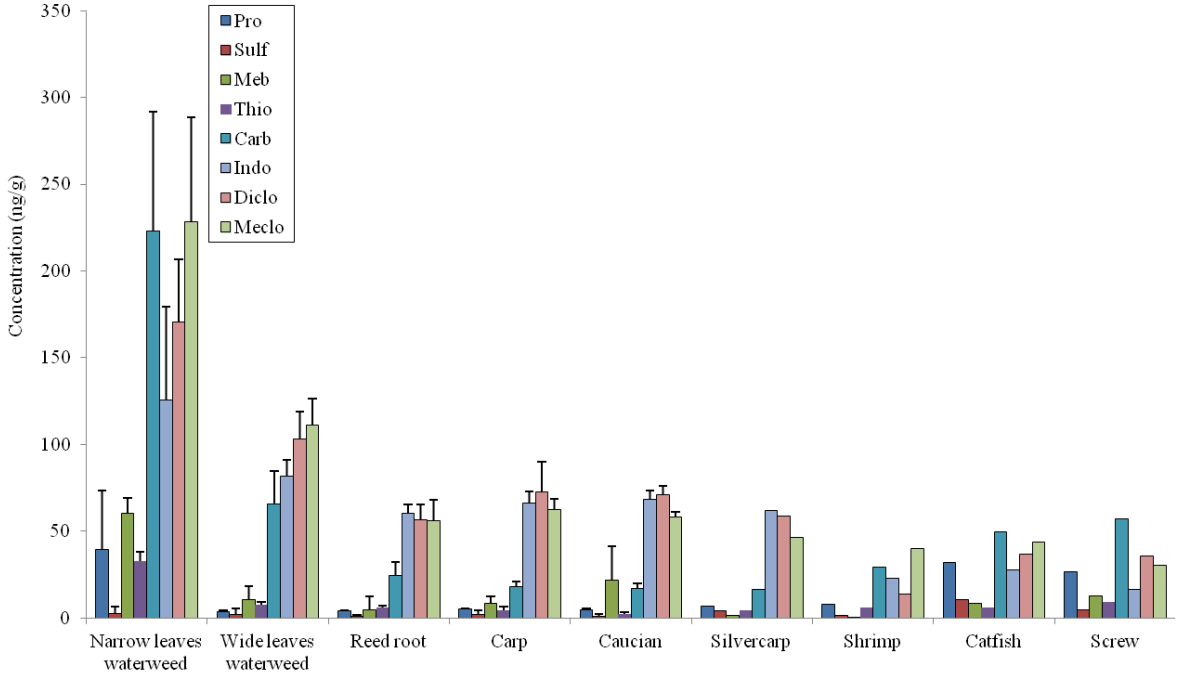

Figure 97 Total concentration of pharmaceuticals at Beijing sampling locations (normalized results)

Figure 95 clearly illustrates that Tamo is the contaminant with the highest concentrations observed in Beijing biological samples at between 136.89 ng/g and 567.00 ng/g (Screw and Narrow Leaves Waterweed, respectively) (Table 35). Samples were prepared in the same way as chapter 3 and 4. The samples of shrimp and catfish have low concentrations of Tamo of 195.04 ng/g and 145.52 ng/g . The lowest recorded concentration was 0.25 ng/g of Meb in the Shrip sample. Narrow waterweed leaves gave the highest recovery of compounds of all samples, indicating highest pharmaceutical contamination concentration up to 567.00 ng/g for Tamo. The lowest observed overall contaminant was Sulf with 0.81 ng/g at its lowest and only 10.85 ng/g as its highest concentration observed. The remaining compounds had concentration ranges as follows: Pro 3.58 – 39.69 ng/g, Meb 0.25 – 60.58 ng/g, Thio 2.53– 32.87 ng/g, Carb 16.49 – 222.96 ng/g, Indo 16.32 – 125.41 ng/g, Diclo 13.95 – 170.83 ng/g and finally Meclo 30.25 – 228.39 ng/g.

Figure 97 shows normalized results for biological samples from Beijing, ie with Tamo removed. It is sometimes not considered good practice to normalize results but however removing one to three compounds from the group on graphical analysis simply enhances those with lower concentrations and makes them visibly easier to analyze. Absolute concentrations are shown in the data tables and first graphical representations. Data for all samples except ‘narrow leaves waterweed’ are still difficult to decipher visually from this. The narrow waterweed (*Elodea*) leaves samples had concentrations between 2.49 ng/g (Sulf) and 567.00 ng/g (Tamo). All compounds had significantly high recoveries in comparison to other sample matrices tested.

Wide waterweed (*Elodea*) leaves also had good high concentrations of target compounds which range from 2.01 ng/g to 523.93 ng/g for Sulf and Tamo. Pro is similar in concentration to Sulf with only 3.58 ng/g. Two other compounds are low in comparison to the remaining four unmentioned compounds, which are Thio (7.62 ng/g) and Meb (10.90 ng/g). The remainder are 65.71 ng/g (Carb), 81.52 ng/g (Indo), 103.10 ng/g (Diclo) and 111.41 ng/g (Meclo).

In ascending order, reed root (*Phragmites australis,* or common reed) samples produced the following results for concentration of target compounds; 0.81 ng/g for Sulf, 4.09

ng/g for Pro, 4.72 ng/g for Meb, 5.86 ng/g for Thio, 24.72 ng/g for Carb, 56.03 ng/g for Meclo, 56.83 ng/g for Diclo, 60.46 ng/g for Indo and a large 427.32 ng/g for Tamo.

Carp (*Cyprinidae* family) was the first fish sample tested and had the highest concentrations for Tamo, Indo, Diclo and Meclo (455.75 ng/g, 66.05 ng/g, 72.95 ng/g and 62.48 ng/g correspondingly). The lowest observed concentration was Sulf with 2.01 ng/g quantified from sample analysis.

As shown in Table 35, the concentrations of the target compounds are low for Caucian. The lowest observed concentration was 1.01 ng/g for Sulf. Tamo appears to be anomalous, with a concentration far exceeding the trend for this sample of 476.25 ng/g) (Figure 95) Other observed concentrations were as follows, in ascending order; 2.53 ng/g (Thio), 4.53 ng/g (Prop), 17.22 ng/g (Carb), 21.71 ng/g (Meb), 58.41 ng/g (Meclo), 68.33 ng/g (Indo) and 72.95 ng/g (Diclo).

Silver carp (*Hypophthalmichthys molitrix*) had a highest observed concentration of 467.13 ng/g, followed by Indo with 61.80 ng/g. The lowest observed concentration was for Meb with 1.79 ng/g. Other compounds with low observed concentrations were, in ascending order, Thio, Sulf and Pro (4.30 ng/g, 4.32 ng/g and 6.67 ng/g). Remaining compounds had higher amounts of pharmaceuticals ranging from 16.49 – 59.00 ng/g.

Shrimp (*Malacostraca*) gave the lowest total concentrations observed in comparison to all other matrices analysed. Concentrations ranged from 0.25 ng/g for Meb to 195.04 ng/g for Tamo. Other compounds with low concentrations were Pro (7.77 ng/g), Sulf (1.43 ng/g) and Thio (5.90 ng/g).

Catfish (*Siluriformes*) gives the following observed concentrations for Pro at 31.83 ng/g, Sulf at 10.85 ng/g, Thio at 6.20 ng/g, Carb at 49.84 ng/g, Tamo at 145.52 ng/g, Indo at 27.65 ng/g, Diclo at 36.85 ng/g and Meclo at 43.69 ng/g. The highest and lowest concentrations observed were Tamo, and Thio, respectively.

Screw gave relatively low total concentrations compared to other matrices tested for the target pharmaceutical compounds. The highest concentration was recorded for Tamo (136.89 ng/g), which is the lowest recording for this compound of all sites. The same is true for Meclo and Indo.

5.3.2 Water samples from Beijing

5.3.2.1 Haihe River

Figure 98 Location of Haihe River in Beijing

Figure 98 displays the location of the Haihe River in Beijing. Figure 99 and Figure 99 highlight the 24 sampling locations (approximate) along the river.

Figure 99 Location of sampling sites from the Haihe river

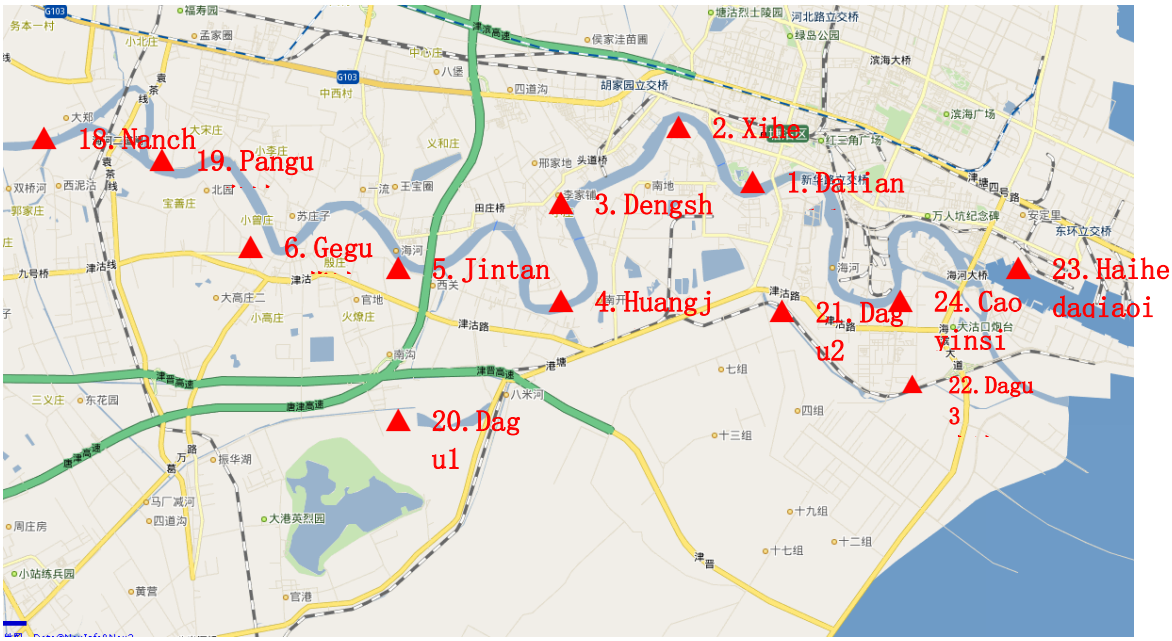

Figure 100 Location of sampling sites from the Haihe river

Concentrations from the Haihe River in Beijing range from a low of 0.00 ng/l for Meb at thirteen sites and Meclo at site Dagu3 to 3486.1 ng/l for Carb at site Nancha. Excluding non-detected samples, the minimum observed concentration was identified for Meb at Majiacaivuan with 14.0 ng/l (Table 36). Zero concentrations were identified for Meclo at Daliangdukou, Xihe, Dengshangu, Huangjiajuan, Tianjinzhan, Dongyangchang, Fengkoucun, Caizhuangzi, Nancha, Dagu1, 2, and 3 and Caoyinsi. A concentration of below LOD was also identified at Dagu3 for Meclo.

Across all sites, Carb outperforms all other compounds as the highest concentration from a minimum of 984.8 ng/l at Haihe Bridge (Figure 102) to the maximum of 3486.1 ng/l at Nancha (Figure 103). Figure 101 also displays results graphically. As a result, a second set of graphs (Figureure. 9-11) were drawn excluding Carb, as a measure of normalising results for analysis purposes which is spread over three separate graphs for ease.

Two compounds regularly give high concentrations above all others (excluding Carb), Diclo and Tamo with ranges between 156.8 – 1598.5 ng/l and 140.2 – 1089.8 ng/l respectively; minimum and maximum values for these compounds was observed at Tianjinzhan & Beicha and Dagu2 & Dagu2.

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Daliangdukou	99.2	170.0	0.0	206.4	1513.4	522.9	140.9	476.5	382.0
Xihe	131.0	219.0	0.0	241.7	1625.0	551.7	213.6	526.2	496.8
Dengshangu	155.8	297.5	0.0	231.6	3057.0	439.6	166.6	572.5	449.5
Huangjiajuan	148.0	212.3	0.0	287.5	2480.1	932.5	152.7	469.2	748.0
Jintanggaosu	116.2	390.6	14.6	301.7	2213.6	253.2	326.5	900.5	485.8
Gegu	114.2	62.5	323.8	218.7	1545.5	810.9	195.6	507.0	445.7
Tianjinzhan	110.3	176.2	14.7	208.2	2080.3	205.6	77.9	156.8	90.3
Tianjinzhan	120.9	153.5	0.0	200.6	2643.8	219.0	146.2	322.9	120.4
Haijin Brigge	194.7	155.7	223.0	283.2	2691.2	467.8	196.0	442.9	347.2
Zhangguizhuang	123.8	141.9	145.3	114.8	1828.5	895.3	159.8	310.1	410.0
Dongyangchang	128.8	173.6	0.0	312.7	2968.6	433.7	201.4	518.3	427.3
Fengkoucun	113.2	228.6	0.0	287.9	1249.5	969.0	175.3	388.1	355.0
Niwocun	97.6	139.3	309.7	212.6	1721.1	792.2	154.9	266.9	516.4
Majiacaiyuan	164.2	224.7	14.0	195.5	2631.4	307.2	161.9	417.1	500.3
Caizhuangzi	150.9	266.8	0.0	197.6	2100.1	729.9	262.2	553.2	504.5
Chaqian	164.6	493.9	95.6	207.1	3431.1	311.8	177.3	895.9	381.0
Beicha	113.1	332.8	1312.4	191.6	2102.4	264.0	202.4	1598.5	349.6
Nancha	145.8	397.6	0.0	199.3	3486.1	241.9	230.5	649.4	370.6
Pangu	201.2	486.0	9.5	224.4	2870.3	382.0	160.9	572.7	434.1
Dagu1	87.4	518.9	0.0	264.6	2445.5	1089.8	693.1	791.3	765.5
Dagu2	97.1	173.7	0.0	196.9	1157.6	140.2	133.8	537.3	108.2
Dagu3	148.4	83.4	0.0	273.0	1045.3	263.6	142.8	288.4	0.0
Haihe Bridge	148.8	88.8	22.6	212.0	984.8	943.1	246.7	381.2	343.7
Caoyinsi	168.5	260.4	0.0	197.0	3475.8	429.4	243.5	624.3	424.5

Table 36 Concentration (ng/l) of pharmaceutical compounds found in Haihe River surface water samples in June 2009

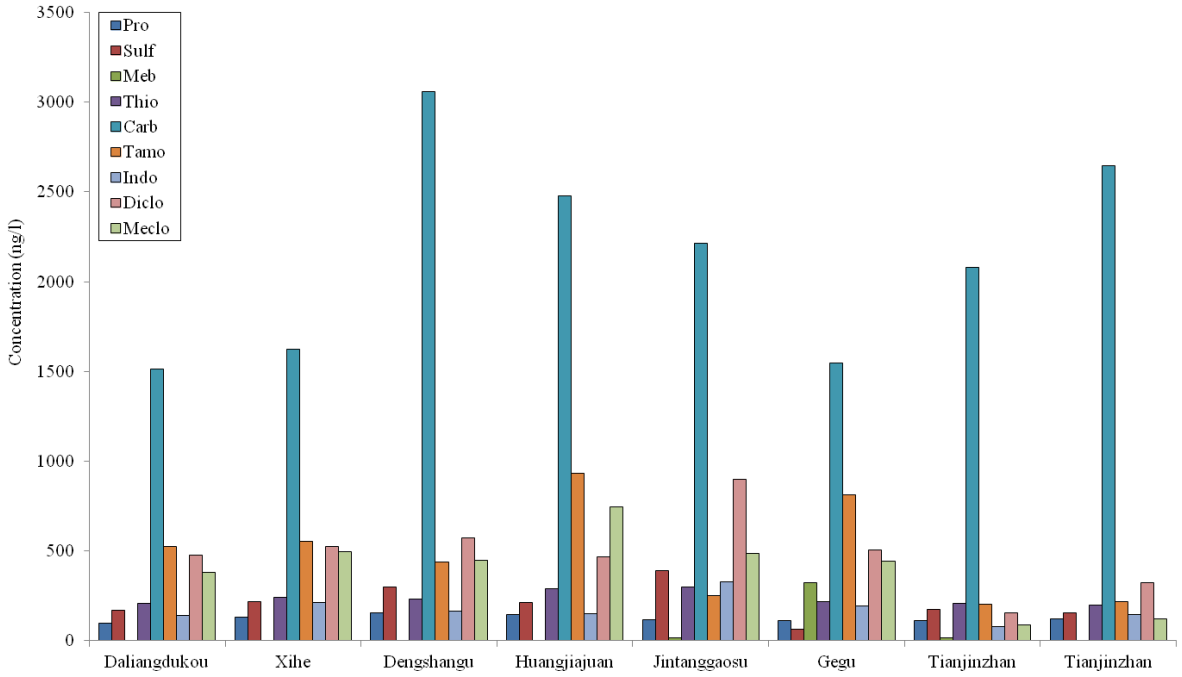

Figure 101 Target pharmaceutical compounds in surface water from Haihe River (ng/l) in June 2009 at the first 8 sampling locations

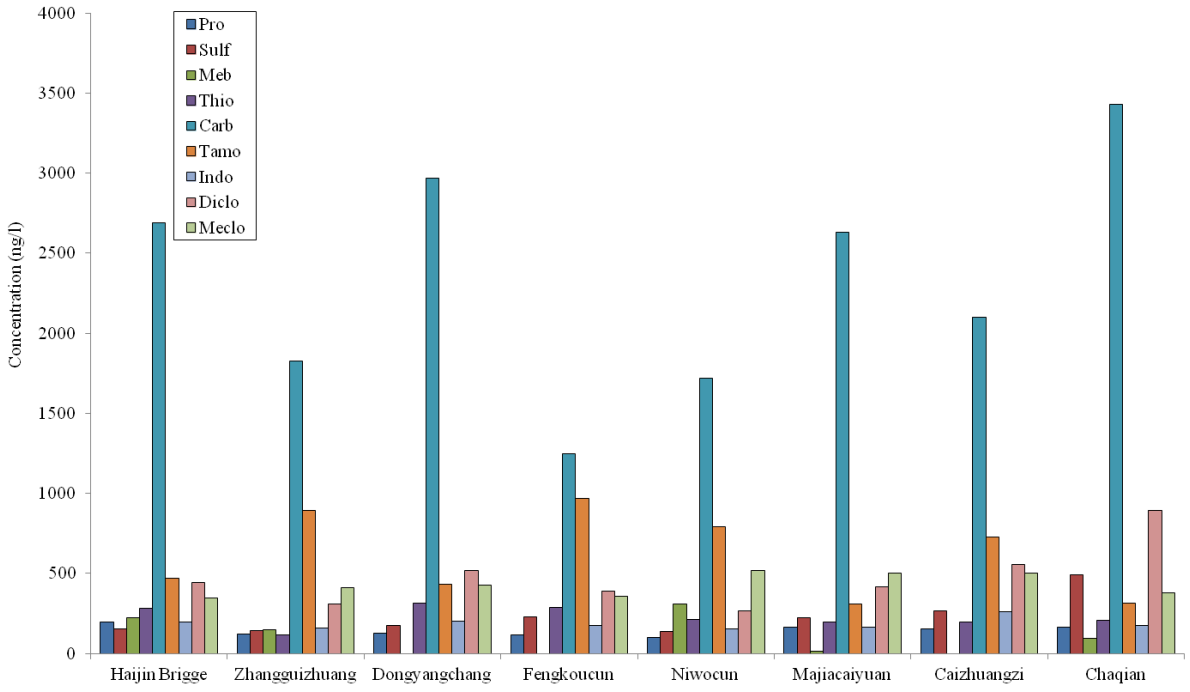

Figure 102 Target pharmaceutical compounds in surface water from Haihe River (ng/l) in June 2009 at the next 8 sampling locations

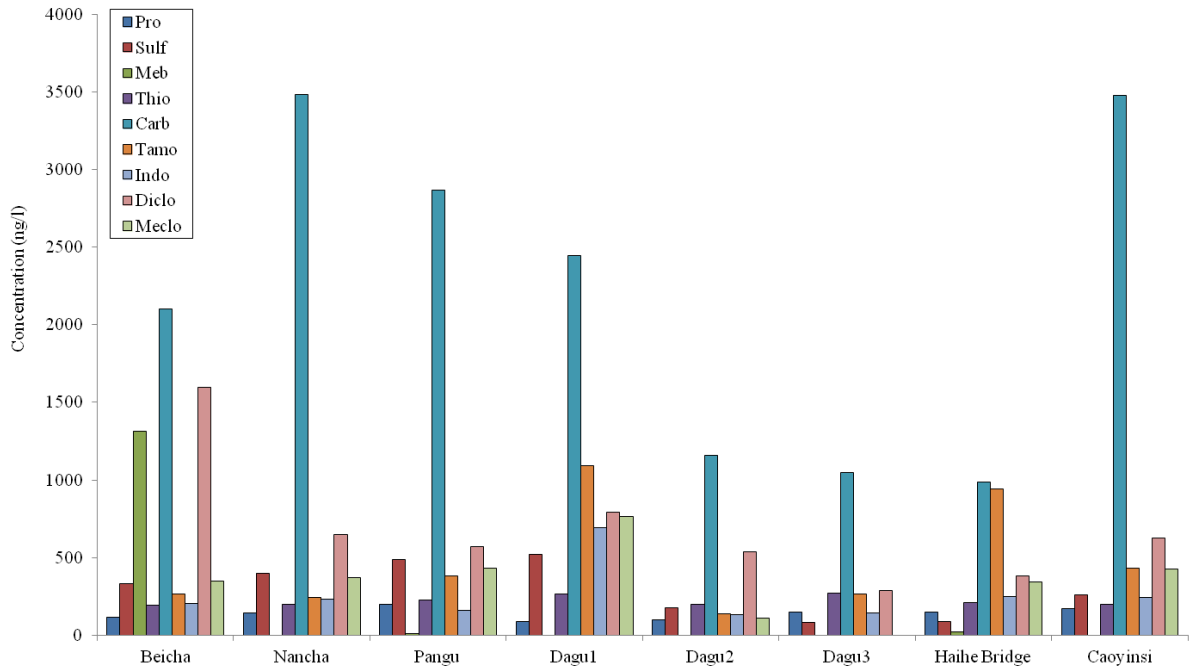

Figure 103 Target pharmaceutical compounds in surface water from Haihe River (ng/l) in June 2009 at the last 8 sampling locations

Site Dagu1 harbours the maximum value for four out of nine compounds across all sites, which is the highest number and suggests a high level of contamination. These are for Sulf (518.9 ng/g), Tamo (1089.8 ng/g), Indo (693.1 ng/g) and finally Meclo (765.5 ng/g). The maximum value for Pro is identified at Pangu (201.2 ng/g), maximum for Meb at Beicha (1312.4 ng/g), Thio at Dongyangchang (312.7 ng/g), Carbemazepine at Nancha (3486.1 ng/g), and Diclo at Beicha (1598.5 ng/g) (Figure 100 – 102).

For every site, the maximum identified concentration was for Carb with a range from 984.8 ng/g to 3486.1 ng/g (Table 36). Normalised results are displayed in Figures 103 – 105.

Excluding non-detected samples, minimum concentrations identified were as follows; 87.4 ng/g for Pro at Dagu1, 62.5 ng/g for Sulf at Gegu, 14.0 ng/g for Meb at Majiacaiyuan, 114.8 ng/g for Thio at Zhangguizhuang, 984.8 ng/g for Carb at the Haihe Bridge, 140.2 ng/g for Tamo at Dagu2, and finally 77.9 ng.g for Indo, 156.8 ng/g for Diclo and 90.3 ng/g for Meclo at Tianjinzhan1. This finding concludes that Tianjinzhan1 has the highest frequency of lowest concentrations identified suggesting it to be the least contaminated site sampled on the Haihe River in Beijing.

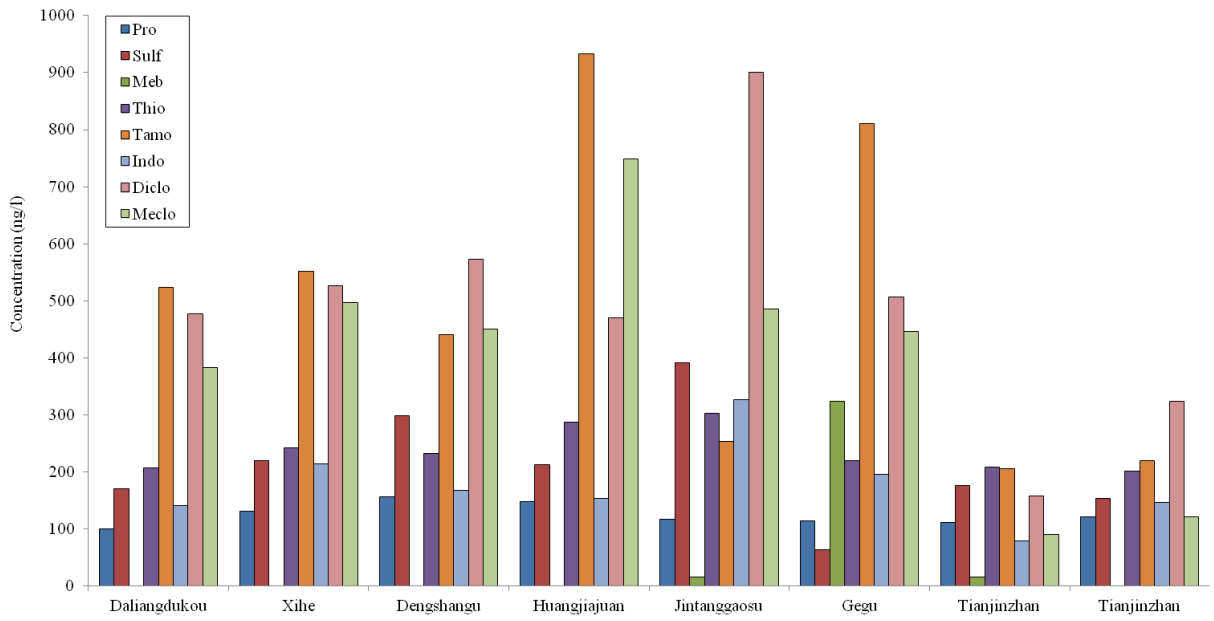

Figure 104 Target pharmaceutical compounds in surface water from Haihe River (ng/l) (Normalized results) in June 2009 at the first 8 sampling locations

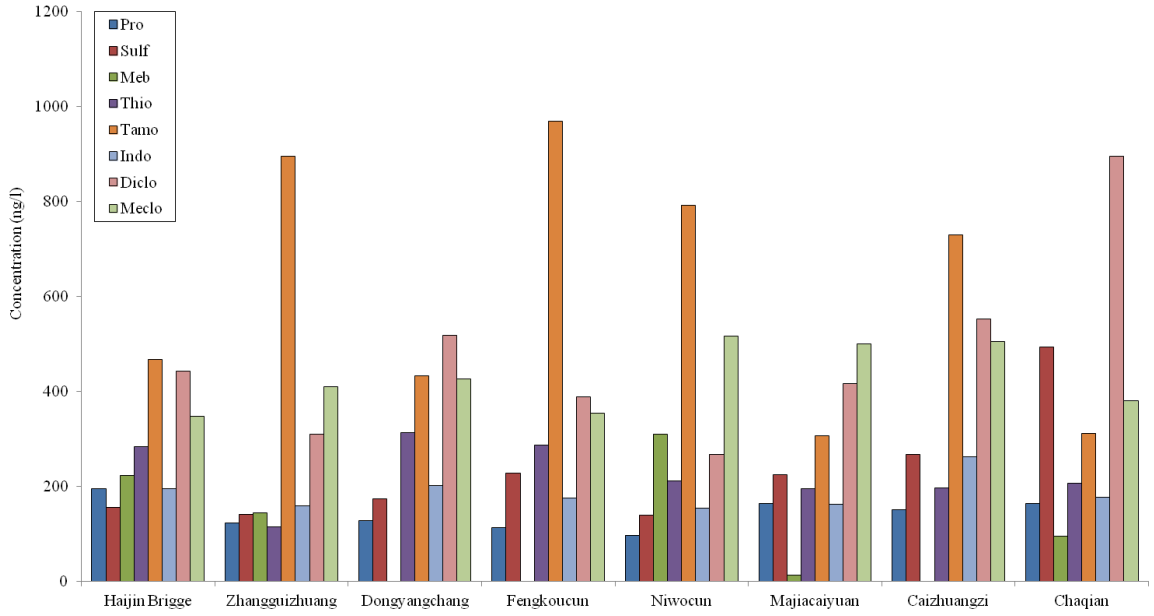

Figure 105 Target pharmaceutical compounds in surface water from Haihe River (ng/l) (Normalized results) in June 2009 at the next 8 sampling locations

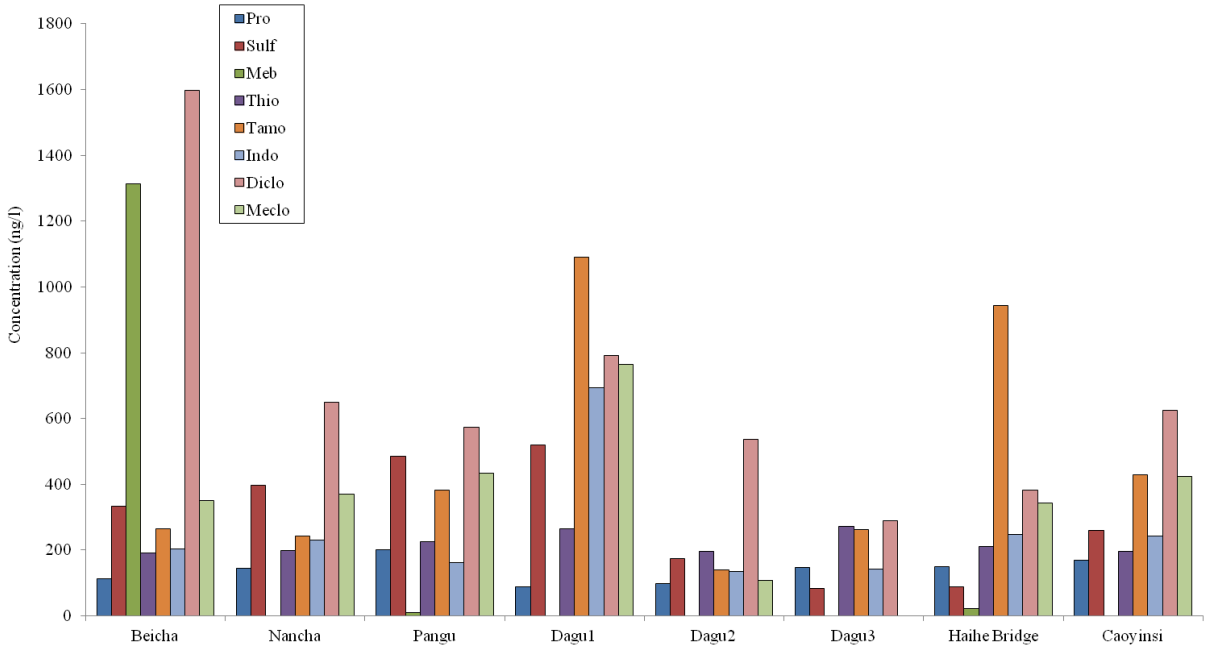

Figure 106 Target pharmaceutical compounds in surface water from Haihe River (Normalized results) in June 2009 at the last 8 sampling locations

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Daliangdukou	103.8	221.5	376.6	16.8	737.4	338.5	677.5	4149.4	3130.4
Xihe	153.9	133.2	0.0	14.6	1090.8	351.9	1110.0	8476.1	5238.9
Dengshangu	88.9	60.2	728.3	75.6	212.8	371.5	539.5	2988.3	3521.6
Huangjiajuan	65.7	367.5	816.8	13.3	845.9	476.1	821.4	6146.1	4553.7
Jintanggaosu	56.6	15.6	282.4	5.2	627.7	279.9	836.5	4055.2	3479.9
Gegu	201.5	409.8	774.5	8.3	1620.1	451.4	981.0	3992.2	7006.8
Tianjinzhan	74.6	68.7	117.1	10.5	272.9	259.6	475.6	2455.0	2983.4
Tianjin	107.2	1092.5	2486.8	54.2	789.6	625.1	753.1	5871.3	6506.9
Haijin Bridge	54.2	347.0	0.0	15.0	434.8	339.8	674.6	3356.1	4022.8
Zhangguizhuang	173.3	281.3	0.0	10.3	775.3	528.6	745.7	5020.9	6472.7
Dongyangchang	111.1	110.4	1058.9	13.6	795.8	465.6	875.5	3509.5	4583.4
Fengkoucun	103.5	521.9	169.6	5.0	280.4	448.3	777.2	2687.4	2183.7
Niwocun	160.8	442.3	38.8	9.7	1034.3	384.3	619.1	4285.3	4836.6
Majiacaiyuan	71.9	7.2	0.0	11.5	498.7	279.0	611.7	3093.7	3214.7
Caizhuangzi	93.1	445.8	121.6	15.8	774.7	492.8	750.9	3618.2	4820.1
Chaqian	163.0	610.0	0.0	2.6	1735.8	505.7	988.2	5948.3	8106.9
Beicha	84.7	3.8	195.1	9.5	164.0	311.9	405.8	2680.4	3765.9
Nancha	211.3	3762.8	599.1	10.2	3102.1	629.6	2500.0	6503.0	4654.6
Pangu	93.8	5.1	0.0	8.2	497.9	419.5	679.0	4985.3	3302.5
Dagu1	686.2	4780.7	932.8	16.3	4962.7	302.7	5693.1	6508.4	7372.7
Dagu2	392.2	3469.3	1142.2	7.4	3787.1	251.7	3967.0	3609.0	4071.9
Dagu3	315.8	1872.2	293.3	1.5	3935.3	386.3	2472.7	3512.1	4350.6
Haihe Bridge	147.5	197.4	344.4	11.3	427.7	389.7	545.4	3298.8	4386.5
Caoyinsi	98.8	40.6	317.4	46.3	834.9	414.8	1165.8	5162.2	5768.3

Table 37 Concentration (ng/l) of pharmaceutical compounds found in Haihe River surface water samples in November 2009

During November 2009, the concentrations of pharmaceutical compounds in the Haihe River surface water samples from the same locations as in Figure 106 - 108, are detailed in Table 37. The highest observed concentrations were for Meclo (8476.07 ng/l) and Diclo (8106.88 ng/l) at Xihe and Chaqian respectively. Meclo had the highest overall concentration of all compounds detected, with a range between 2454.98 ng/l at Tianjinzhan to the previously mentioned 8476.07 ng/l. The lowest observed concentrations were 0.00 ng/l, only observed for the compound Meb at a total of 6 locations (Table 37). However the highest observed value for Meb was 2486.76 ng/l at

Tianjin. Other than zero values, sampling location Dagu3 had a very low concentration of 1.47 ng/l for Thio. Thio also had the lowest concentration range, between 1.47 ng/l and 75.62 ng/l at Dengshangu.

Remaining target compound ranges were as follows; 54.17 ng/l to 686.18 ng/l for Pro, 3.85 ng/l to 4780.67 ng/l for Sulf, 164.05 ng/l to 4962.65 ng/l for Carb, 251.70 ng/l to 629.63 ng/l for Tamo, 405.78 ng/l to 5693.07 ng/l for Indo and 2183.73 ng/l to 8106.88 ng/l for Diclo.

For 17 of the total 24 sampling locations, Diclo is the highest observed compound concentration. The remaining 7 were identified for Meclo. Similarly, 15 out of a total 24 sampling locations recorded Thio as the lowest observed compound concentration, which only excludes 6 zero concentrations identified for Meb and three for Sulf. Excluding zero concentrations, Thio would hold 19 of the total 24 sampling locations as having the lowest concentration.

The sampling location with the highest observed concentration variation was Xihe, with a total range of 8476 ng/l. The lowest observed concentration variation was Fengkoucun, with a range of 2682 ng/l.

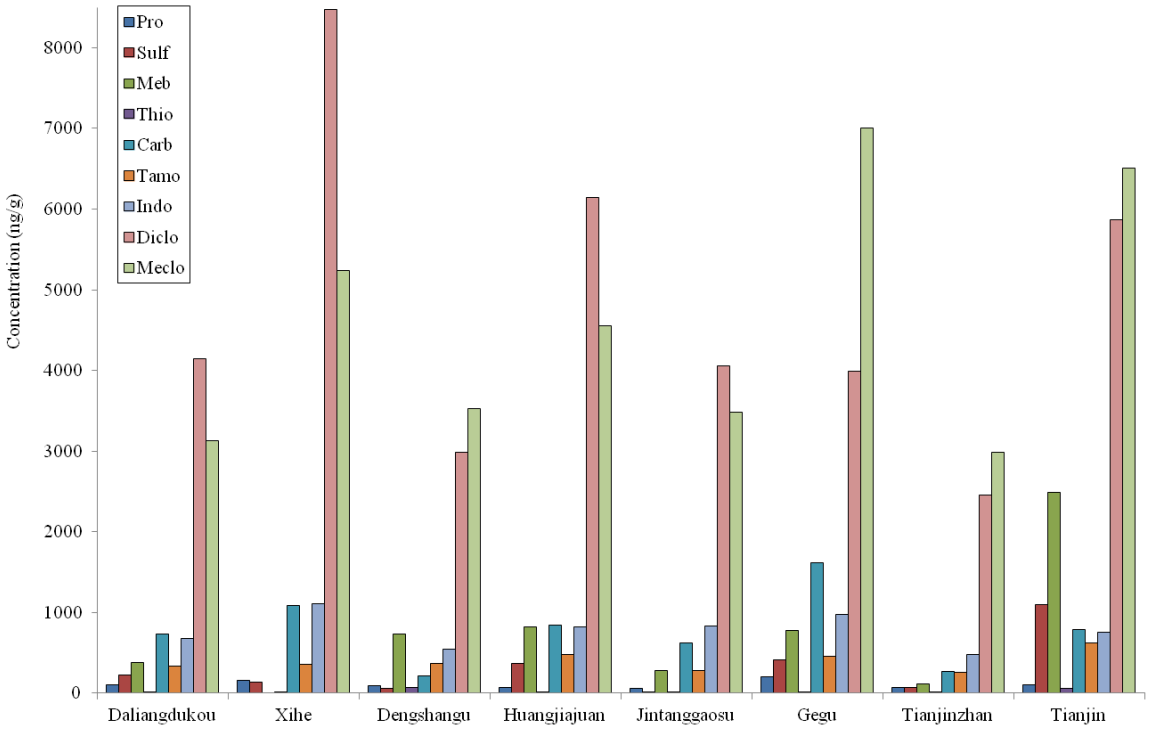

Figure 107 Target pharmaceutical compounds from the first 8 Haihe river surface water samples in November 2009

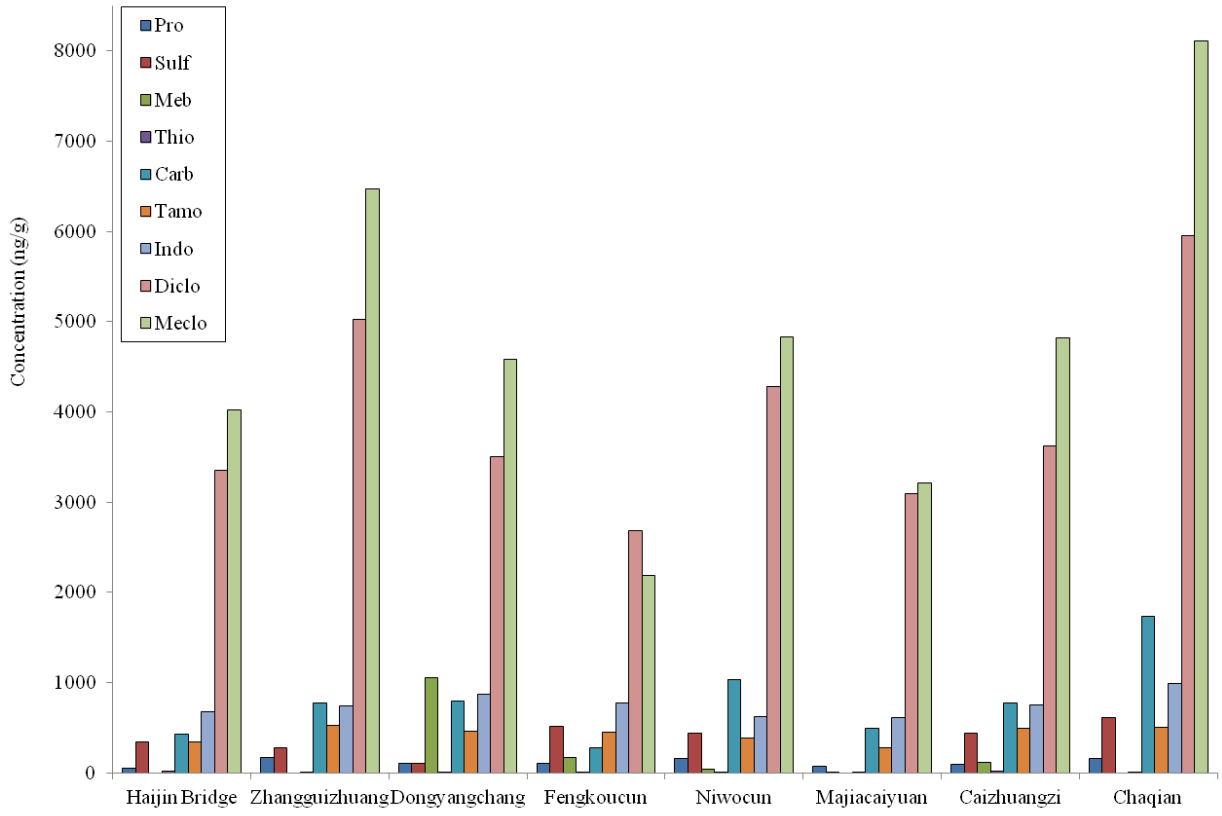

Figure 108 Target pharmaceutical compounds from the next 8 Haihe river surface water samples in November 2009

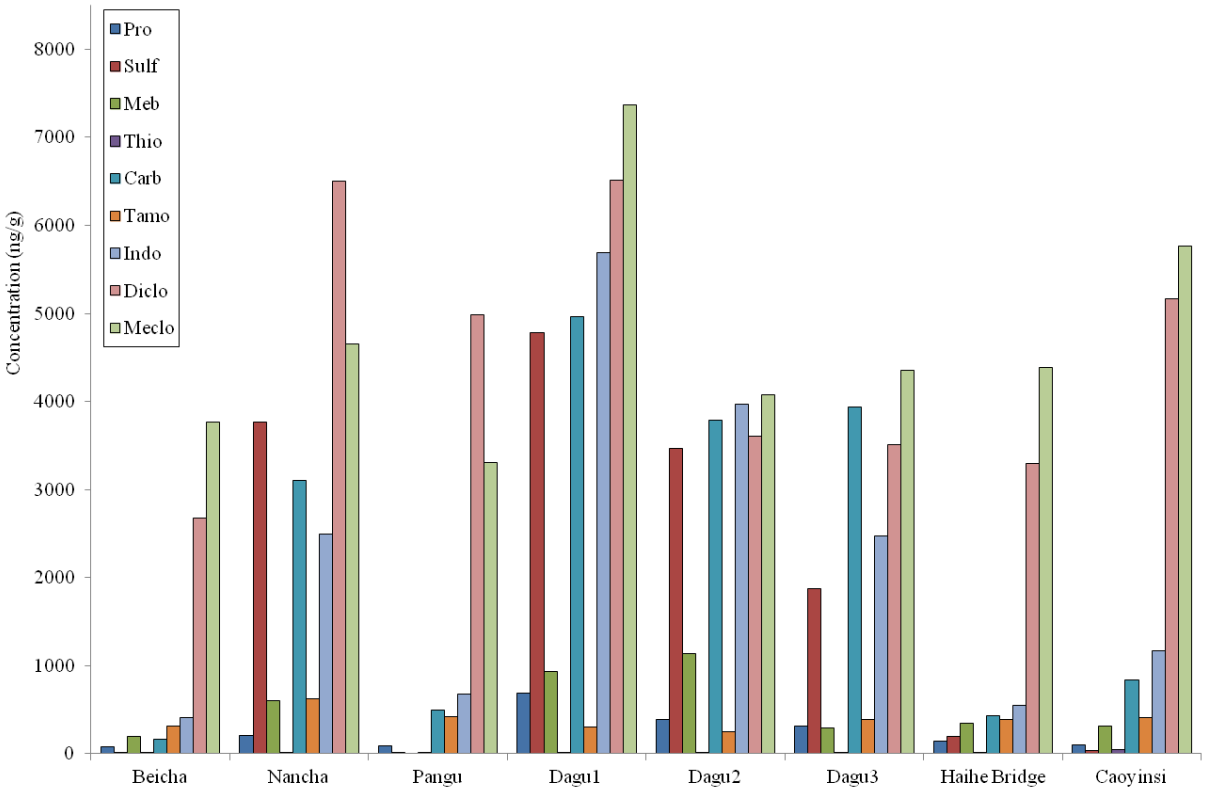

Figure 109 Target pharmaceutical compounds from the last 8 Haihe river surface water samples in November 2009

5.3.2.2 Bohai Bay

Below are two location maps for sampling in Bohai Bay, Beijing which was used on two occasions; June and November 2009. The first (Figure 109) is a general location map followed by Figure 110 which highlights the 18 sampling locations in more detail.

Figure 110 Location of Bohai Bay in Beijing

Figure 111 Location of sampling sites in Bohai Bay

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Shagouzi	150.5	57.7	50.2	117.6	565.0	943.4	184.3	206.9	246.0
Huangligang	237.1	115.3	34.0	154.9	851.8	659.9	305.9	462.8	258.8
Xujiabao	170.2	79.7	215.8	187.0	607.8	1071.9	180.6	583.0	301.3
Nanpaihe	175.3	69.7	44.5	45.2	541.6	1124.2	171.1	311.4	247.2
Ziyaxinhe	146.6	73.8	171.9	65.8	764.0	1152.4	138.8	199.0	273.1
Duliujianhe	135.8	97.0	92.3	200.1	1085.1	762.4	230.3	396.5	307.5
Lvjugou	120.2	15.2	6.3	156.4	486.5	966.8	119.7	293.6	262.5
Tianjinlingang	215.1	40.5	0.0	138.7	725.2	986.3	213.9	355.3	354.5
Tianjinlgang	193.5	86.2	214.2	87.6	747.0	1225.8	215.8	291.0	306.4
Tianjin Harbor	261.4	92.2	26.9	30.9	867.2	1052.5	233.4	373.4	401.8
Dongjianggang	153.4	71.5	102.4	110.5	505.3	865.3	142.3	177.5	224.9
Guanlanlu	201.9	2.4	0.0	100.8	1976.2	339.0	118.5	426.2	220.5
Luanhekou	54.6	30.5	5.4	97.6	208.7	364.2	77.6	73.9	180.7
Letinggongyeyuan	120.9	75.7	0.0	196.5	505.7	322.4	192.1	161.8	117.1
Xihekou	56.4	106.9	0.0	59.6	1567.2	292.2	79.8	595.0	161.7
Caofeidian	159.3	0.7	0.0	50.8	273.4	247.6	56.6	61.6	109.6
Heiyanzi	55.6	45.1	138.4	88.4	469.8	459.9	69.0	98.4	285.4
Beitangkou	61.6	136.4	0.0	102.6	1919.2	587.4	142.0	141.5	157.7

Table 38 Concentration (ng/l) of pharmaceutical compounds found in Bohai Bay surface water samples in June 2009

Table 38 details the surface water samples taken from 18 different sampling locations in Bohai Bay and the concentration (ng/l) observed at each. Minimum concentration values were identified for the compound Mebeverine at six sites; Tianjinlingang, Guanlanlu, Letinggongyeyuan, Xihekou, Caofeidian and Beitangkou. Excluding zero values, concentrations ranged from 0.7 ng/l to 1976.2 ng/l for Sulf at Caofeidian and Carb for Guanlanlu respectively. Tamo and Carb are by far the two compounds with the highest concentration identified across all sites.

Graphs to represent sampling data were produced (Figure 111-113), where it is immediately obvious that Tamo and Carb far outperform other compounds in concentration identified. For ease of data analysis, a normalized set of graphs were produced (Figure 114 - 116) excluding these two compounds.

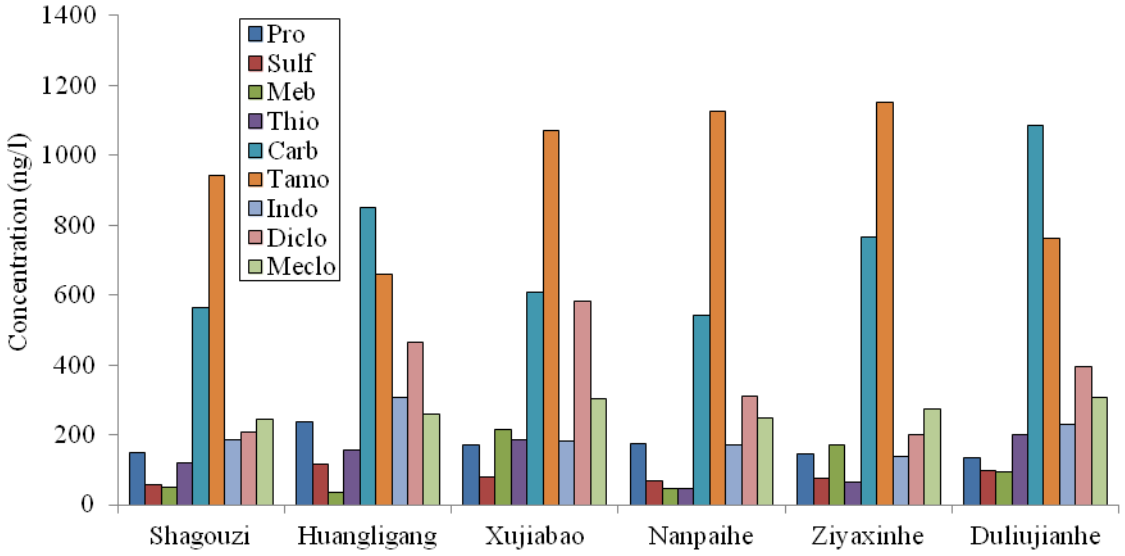

Figure 112 Target pharmaceutical compounds in surface water from Bohai Bay in June 2009 at the first 6 sampling locations

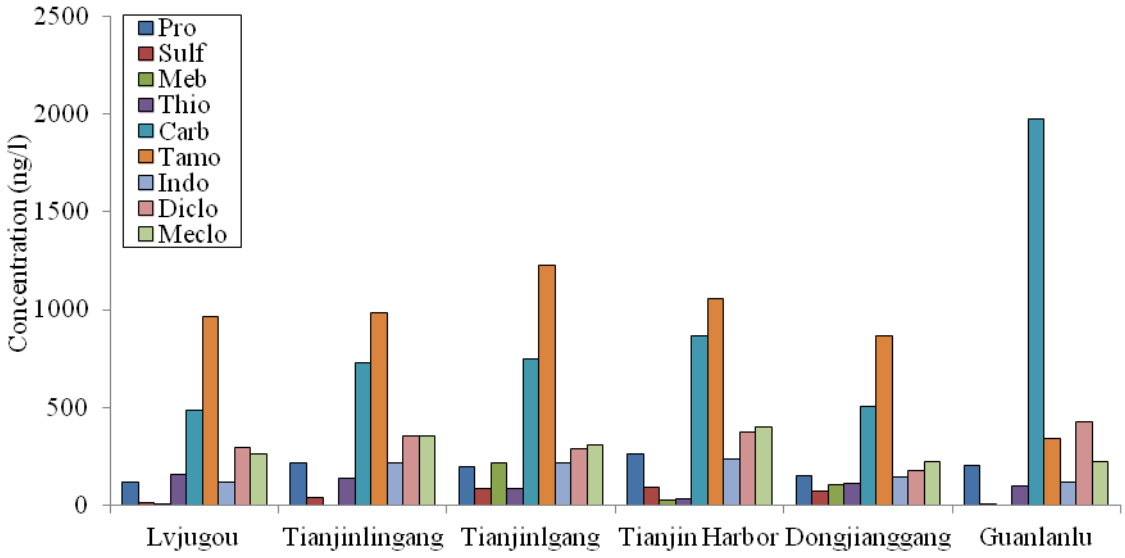

Figure 113 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in June 2009 at the next 6 sampling locations

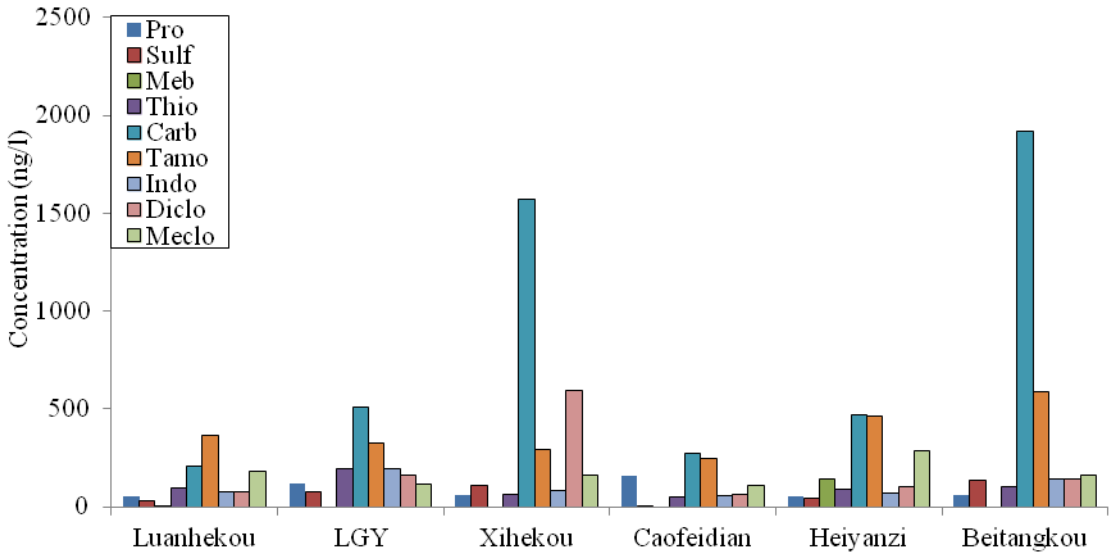

Figure 114 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in June 2009 at the last 8 sampling locations

In ascending order, maximum concentrations for each compound were as follows; 136.4 ng/l for Sulf (Beitangkou), 200.1 ng/l for Thio (Duliujianhe), 215.8 ng/l for Meb (Xujiabao), 261.4 ng/l for Pro (Tianjin Harbour), 305.9 ng/l for Indo (Huangligang), 401.9 ng/l for Meclo (Tianjin Harbor), 595.0 ng/l for Diclo (Xihekou), 1225.8 ng/l for Tamo (Tianjinlgang) and 1976.2 ng/l for Carb (Guanlanlu).

In ascending order, minimum concentrations for each compound were as follows (excluding zero values); 0.7 ng/l for Sulf (Caofeidian), 5.4 ng/l for Meb (Luanhekou), 30.9 ng/l for Thio (Tianjin Harbor), 54.6 ng/l for Pro (Luanhekou), 56.6 ng/l for Indo (Caofeidian), 61.6 ng/l for Diclo (Caofeidian), 109.6 ng/l for Meclo (Caofeidian), 208.8 ng/l for Carb (Luanhekou) and 247.6 ng/l for Tamo (Caofeidian).

No one site appears to be more highly contaminated than another, with only Tianjin Harbour having two of the nine maximum concentrations of target pharmaceutical compounds identified. Least contaminated site on the other hand is easier to identify, with Caofeidian having five out of nine (>50 %) of the observed minimum concentrations.

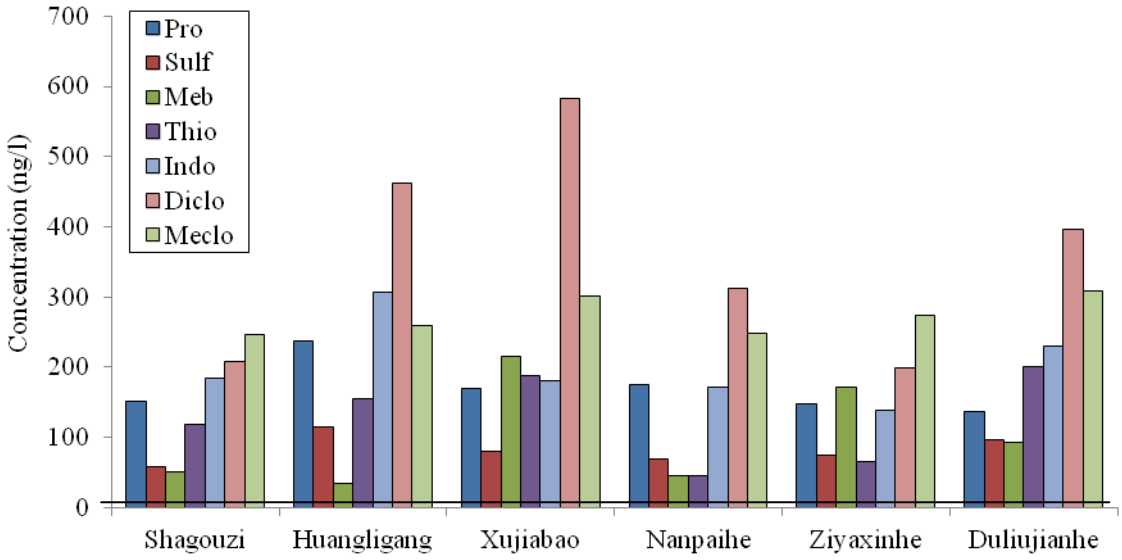

Figure 115 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in June 2009 at the first 6 sampling locations (Normalized results)

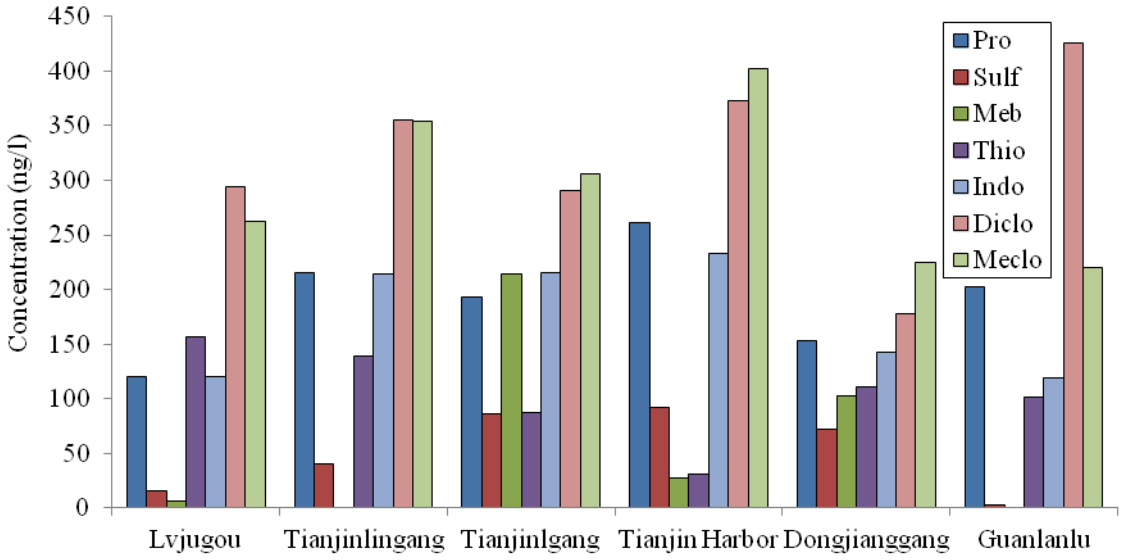

Figure 116 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in June 2009 at the next 6 sampling locations (Normalized results)

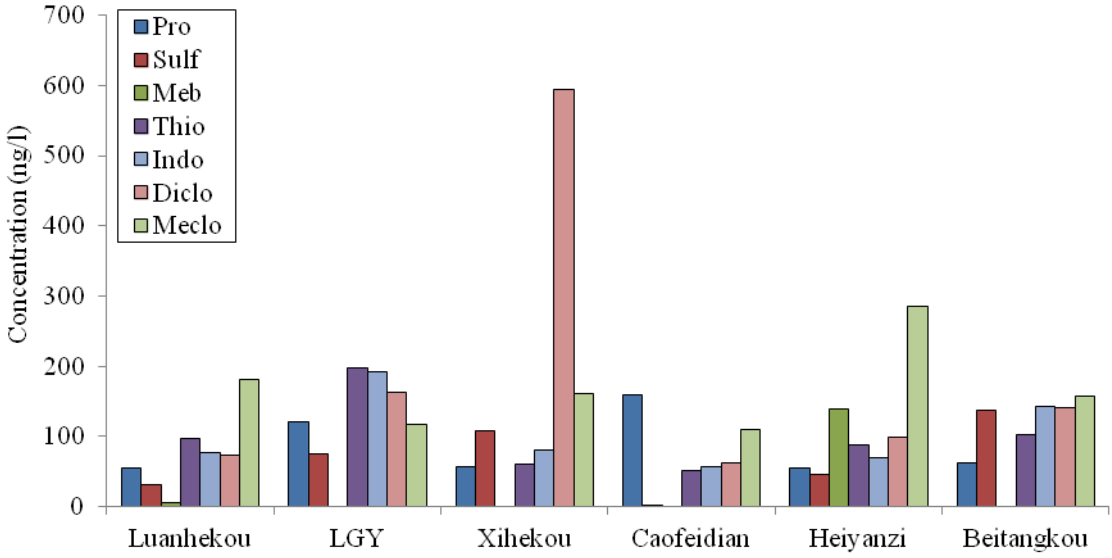

Figure 117 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in June 2009 at the last 6 sampling locations (Normalized results)

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Shagouzi	101.9	12.3	0.0	14.3	540.3	283.1	836.9	3064.4	2398.2
Huangligang	172.1	7.6	0.0	5.3	228.9	325.1	921.0	3747.7	2715.0
Xujiabao	121.9	4.4	39.5	8.7	202.7	304.9	719.5	2728.1	2636.8
Nanpaihe	100.2	151.5	0.0	9.3	811.9	346.6	882.2	7838.9	3876.4
Ziyaxinhe	125.1	1778.8	32.8	16.6	1143.4	319.1	1003.6	4776.3	4214.7
Duliujianhe	157.6	208.2	0.0	17.4	739.0	460.1	904.9	3505.5	5908.5
Lvjugou	103.6	54.8	46.3	22.4	251.6	346.4	648.5	2825.4	4184.8
Tianjinlingang	135.1	99.8	0.0	23.7	372.2	352.4	977.6	4213.0	2560.8
Tianjinlgang	110.3	54.1	26.3	10.3	210.4	375.6	701.6	3321.4	2942.7
Tianjin Harbor	103.8	192.7	0.0	8.3	310.1	283.4	531.0	2623.7	3777.7
Do ngjianggang	111.7	216.2	33.7	52.5	327.5	384.2	768.7	3399.9	3695.8
Guanlanlu	106.6	15.8	0.0	13.8	505.1	421.8	890.9	4331.5	4498.8
Luanhekou	138.0	398.6	0.0	46.2	198.5	419.9	568.4	2277.9	3053.3
Letinggongyeyuan	154.3	15.4	148.0	14.6	211.5	436.3	832.3	4163.3	3266.5
Xihekou	84.3	25.0	0.0	18.7	388.8	328.6	617.2	2916.0	4508.2
Caofeidian	181.5	0.0	152.9	43.2	208.6	505.7	949.6	4980.6	6011.0
Heiyanzi	76.2	186.1	103.8	16.9	615.1	307.1	460.9	2099.2	2803.4
Beitangkou	126.4	11.0	0.0	8.4	217.5	261.6	655.6	2528.3	3899.6

Table 39 Concentration (ng/l) of pharmaceutical compounds found in Bohai Bay surface water samples in November 2009

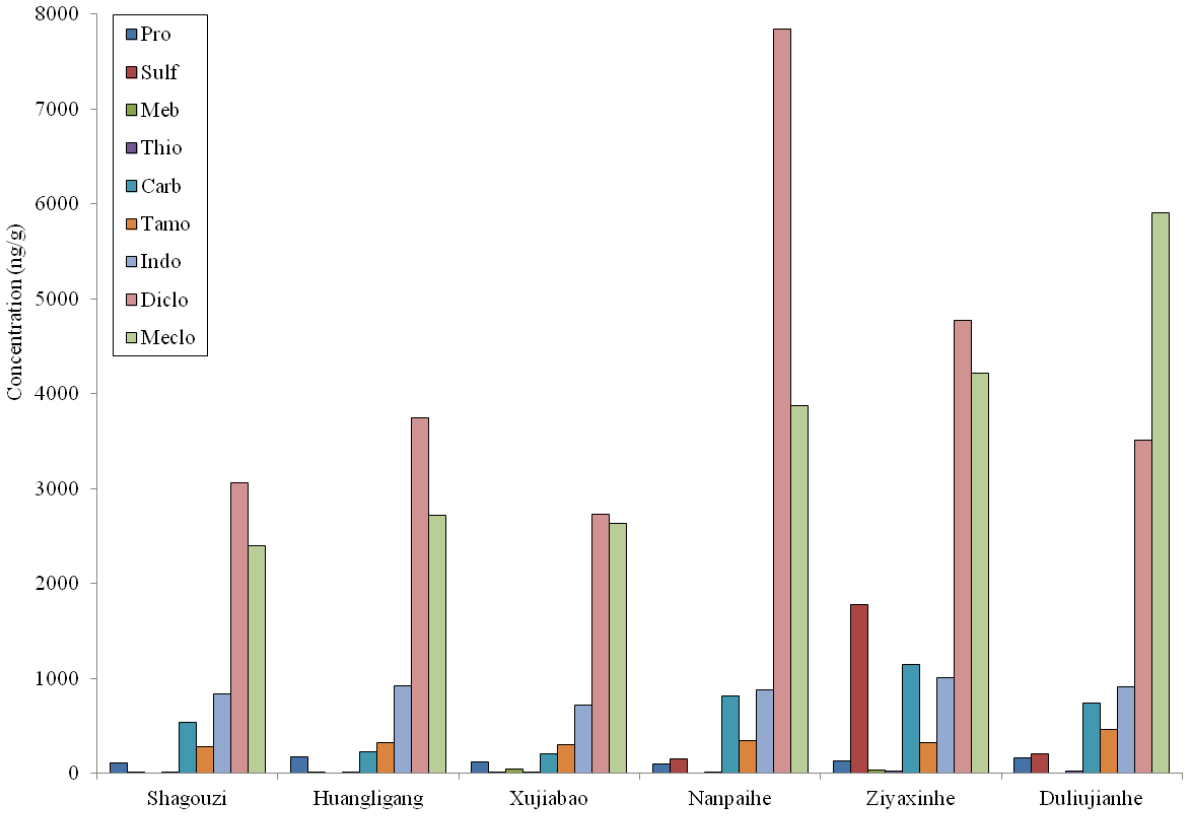

Figure 118 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in November 2009 at the first 6 sampling locations

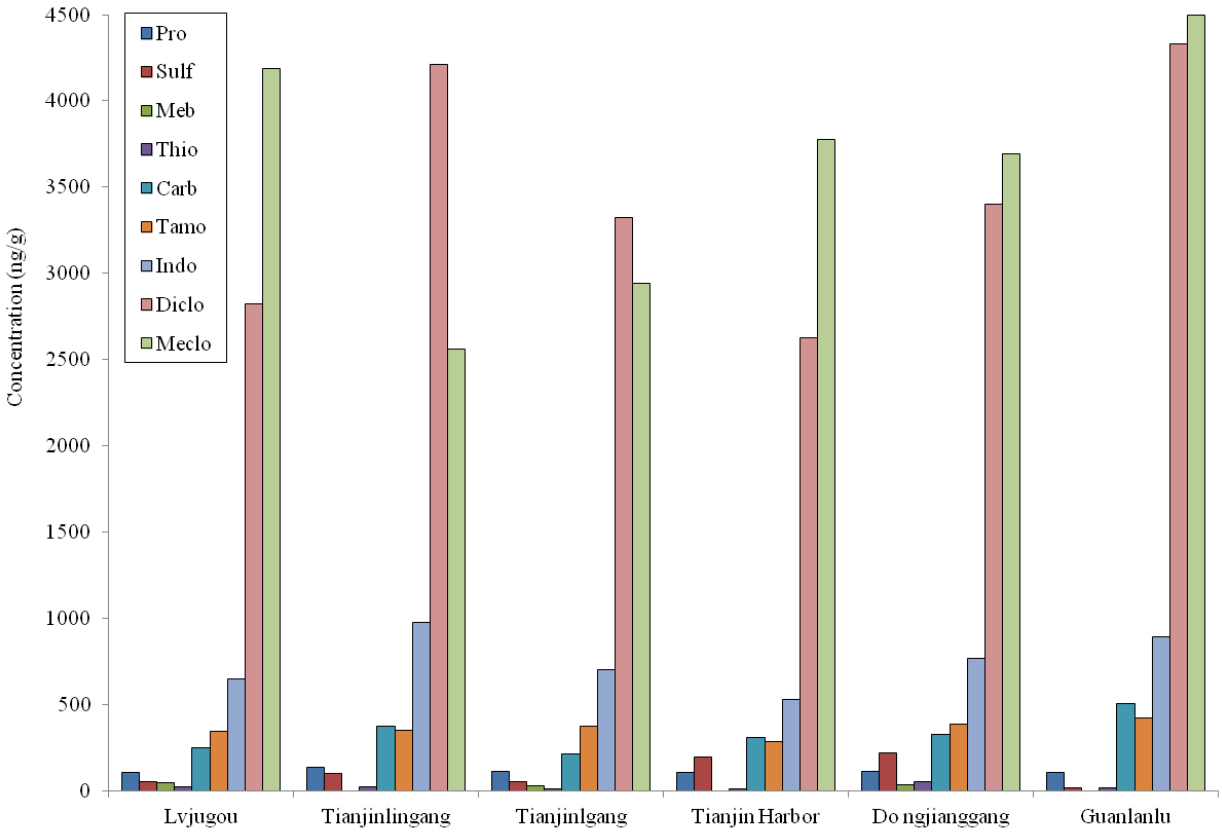

Figure 119 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in November 2009 at the next 6 sampling locations

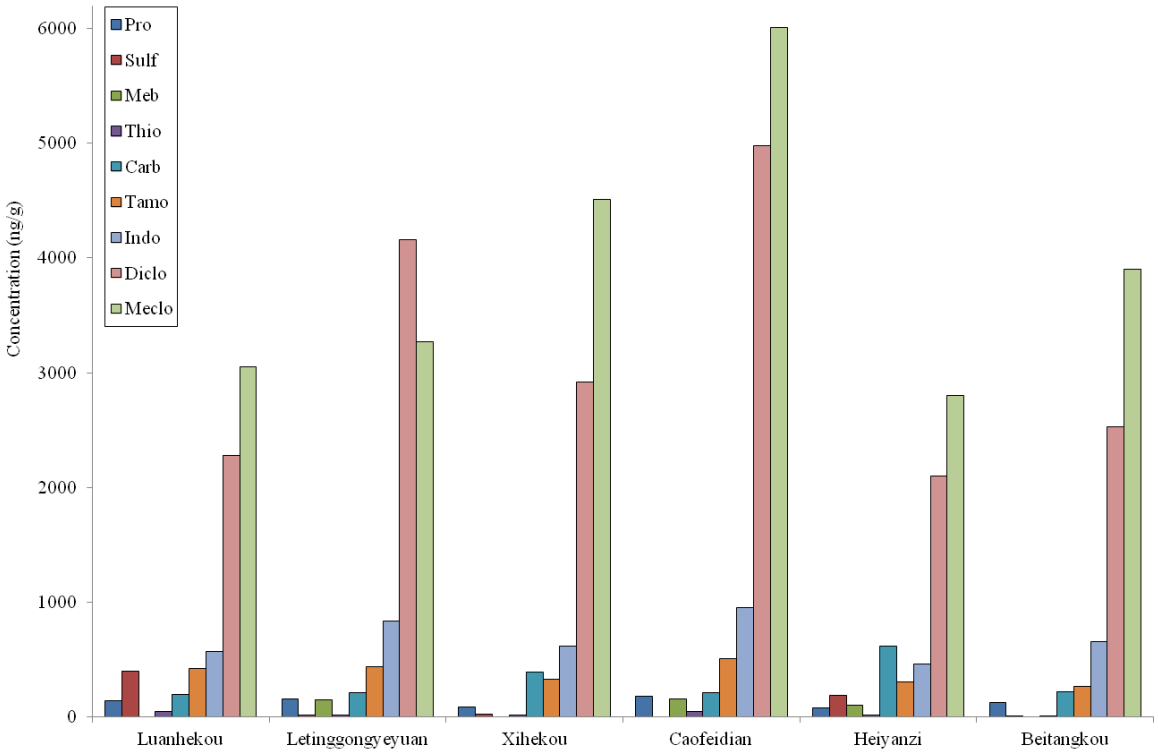

Figure 120 Target pharmaceutical compounds in surface water from Bohai Bay (ng/l) in November 2009 at the last 6 sampling locations

During November 2009, the highest observed concentration from Bohai Bay surface water samples was identified as 7838.94 ng/l for Diclo at the site Nanpaihe. The lowest observed concentration was below LOD for both Sulf (Caofeidian) and Meb (Shagouzi, Huangligang, Nanpaihe, Duliujianhe, Tianjinlingang, Tianjin Harbor, Guanlanlu, Luanhekou, Xihekou and Beitangkou; 10 of the total 18 sample locations). Excluding zero concentrations, the lowest observed was at Xujiabao for Sulf, with only 4.40 ng/l. All results are displayed in Figure 118 - 119.

The total range for each compound was as follows; 76.16 ng/l to 181.46 ng/l for Pro, 0.00 ng/l to 1778.80 ng/l for Sulf, 0.00 ng/l to 152.95 ng/l for Meb, 5.32 ng/l to 52.46 ng/l for Thio, 198.54 ng/l to 1143.37 ng/l for Carb, 261.64 ng/l to 505.69 ng/l for Tamo, 460.87 ng/l to 1003.65 ng/l for Indo, 2099.23 ng/l to 7838.94 ng/l for Diclo and 2398.23 ng/l to 6011.01 ng/l for Meclo (Table 39).

Ten out of the 18 sampling locations identified Meclo as the higest observed concentration. The remaining eight were identified for Diclo. Zero concentrations were

listed previously, but are seen in Table 39. Remaining lowest identified concentrations were 4.40 ng/l for Sulf at Xujiabao, and the rest for Thio; 16.64 ng/l at Ziyaxinhe, 22.35 ng/l at Lvjugou, 10.32 ng/l at Tianjinlgang, 14.64 ng/l at Letinggongyey and 16.85 ng/l at Heiyanzi.

6.3.2.3 Baiyangdian (BYD) Lake

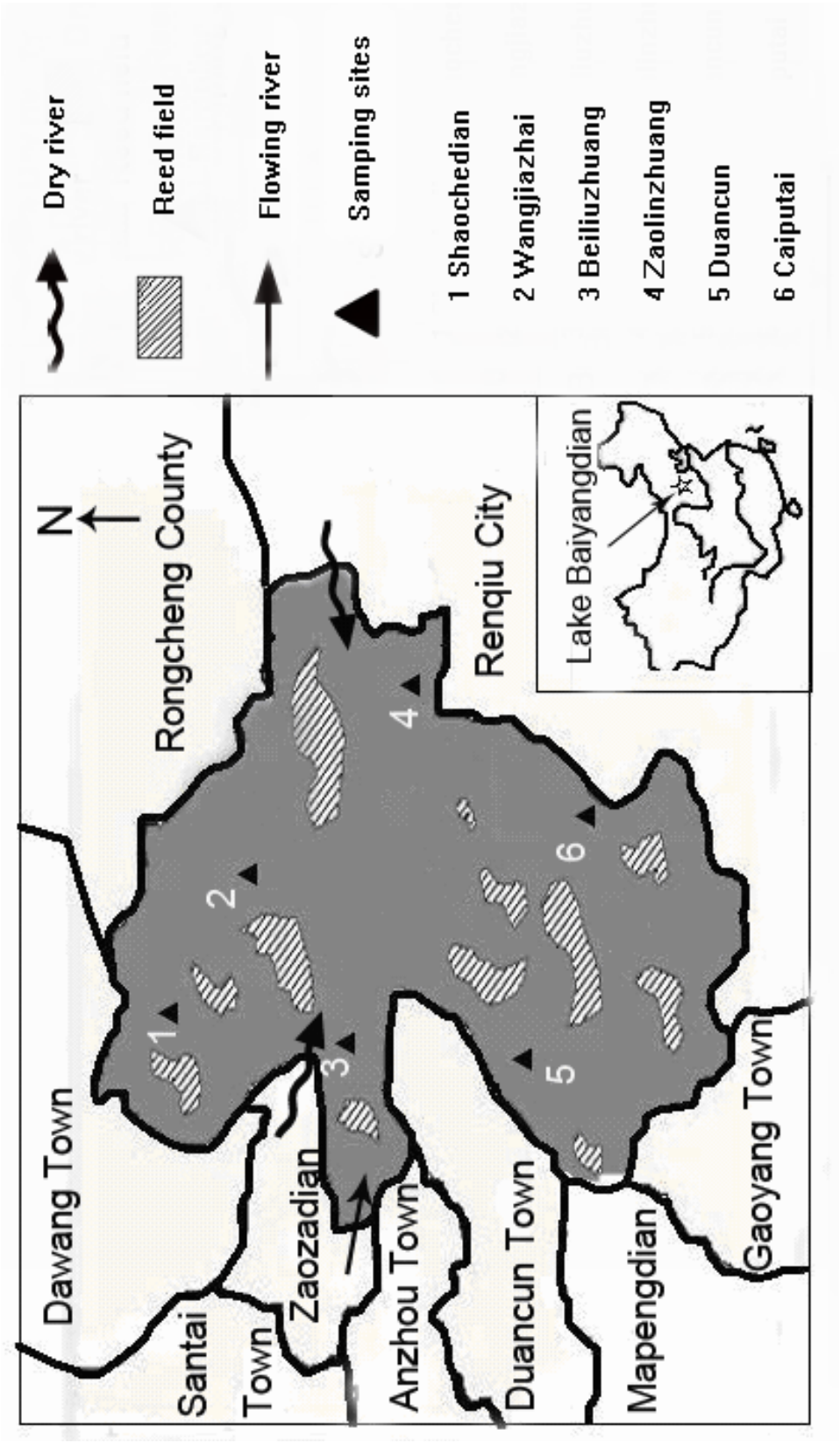

Figure 121 Location of sampling sites at BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	56.3	59.2	0.0	4.7	747.9	338.1	108.6	242.5	227.8
	Shaochedian	81.3	287.5	86.2	8.3	457.2	897.7	212.3	335.9	260.5
	Caiputai	35.8	12.1	14.0	3.6	354.4	359.2	63.8	97.9	169.8
	Duancun	45.9	27.4	0.0	7.0	701.6	325.8	62.9	179.7	94.5
	Beiliuzhuang	518.3	691.1	120.5	2.9	760.9	450.8	282.1	176.5	198.9
	Wangjiazhai	75.4	312.6	19.5	8.6	319.7	922.9	86.2	180.8	106.7
Overlying Water	Zaolinzhuang	117.9	18.7	22.1	4.7	291.4	455.7	24.6	68.8	64.8
	Shaochedian	43.6	272.2	0.0	6.7	378.8	169.3	98.0	207.3	80.9
	Caiputai	45.2	37.6	0.0	16.5	895.1	343.9	106.7	249.8	159.7
	Duancun	94.9	46.8	0.0	19.8	156.4	186.5	124.4	331.2	220.7
	Beiliuzhuang	284.2	316.1	0.0	20.3	240.6	127.4	163.2	392.6	80.0
	Wangjiazhai	82.7	454.3	0.0	18.7	579.3	237.0	191.9	216.8	123.6
Pore Water	Zaolinzhuang	46.5	0.0	0.0	4.6	486.1	183.0	106.9	93.6	71.5
	Shaochedian	81.5	57.2	0.0	3.9	840.9	578.7	119.7	157.2	261.7
	Caiputai	81.2	34.8	389.3	13.5	307.8	198.9	58.8	96.7	39.7
	Duancun	62.3	41.0	0.0	18.9	264.8	287.0	61.6	159.1	134.3
	Beiliuzhuang	81.1	71.5	0.0	20.0	300.5	970.8	123.7	381.2	102.9
	Wangjiazhai	81.1	67.2	0.0	14.6	1305.7	1006.3	123.7	1381.0	200.8
Water-release test	Zaolinzhuang	35.2	69.6	485.2	18.3	546.0	112.9	80.7	82.2	104.4
	Shaochedian	51.6	56.7	0.0	4.6	390.2	234.5	82.4	153.0	101.2
	Caiputai	51.2	187.0	180.2	15.3	768.4	311.6	75.3	110.6	125.7
	Duancun	51.4	40.7	60.8	17.2	196.9	405.8	93.6	158.1	54.5
	Beiliuzhuang	57.4	98.6	0.0	8.5	397.6	361.9	92.0	120.1	159.7
	Wangjiazhai	125.2	90.3	0.0	9.6	245.8	609.9	882.2	203.2	267.6

Table 40 Concentration (ng/l) of pharmaceutical compounds found in BYD Lake surface water samples in March 2009

Sampling locations are displayed in Figure 120. All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from March are listed in Table 40 and displayed in Figure 122 - 124.

The range of target compounds for surface water samples was <LOD for Meb at Zaolinzhuang and Duancun to 923 for Tamo at Wangjiazhai. For overlying water samples the range was between <LOD for Meb at 5 of 6 sampling locations (all but

Zaolinzhuang) to 895 ng/l for Carb at Caiputai. For pore water samples, the lowest identified concentration was for Meb at 5 out of 6 sampling locations excluding Caiputai of <LOD to 1381 ng/l for Diclo at Wangjiazhai. For the last sample group of water-release test samples, the lowest observed concentration was once again <LOD for Meb at Shaochedian, Beiliuzhuang and Wangjiazhai to 882 ng/l for Indo at Wangjiazhai.

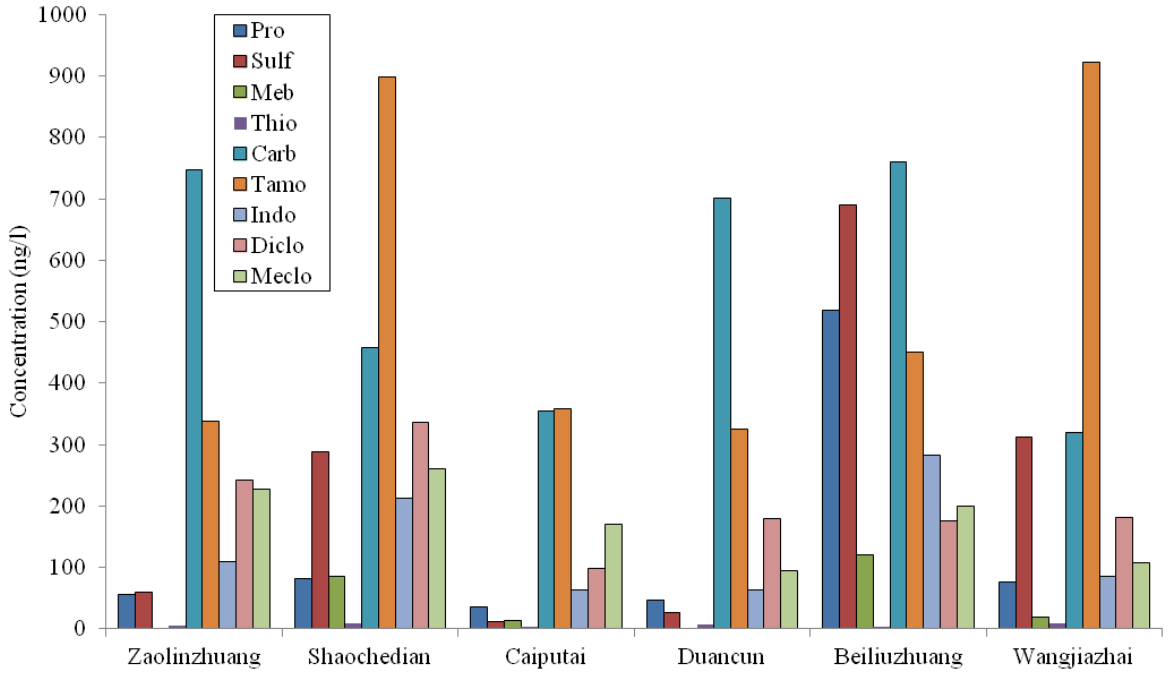

Figure 122 Concentration of target pharmaceutical compounds for March 2009 in Surface water samples for BYD Lake

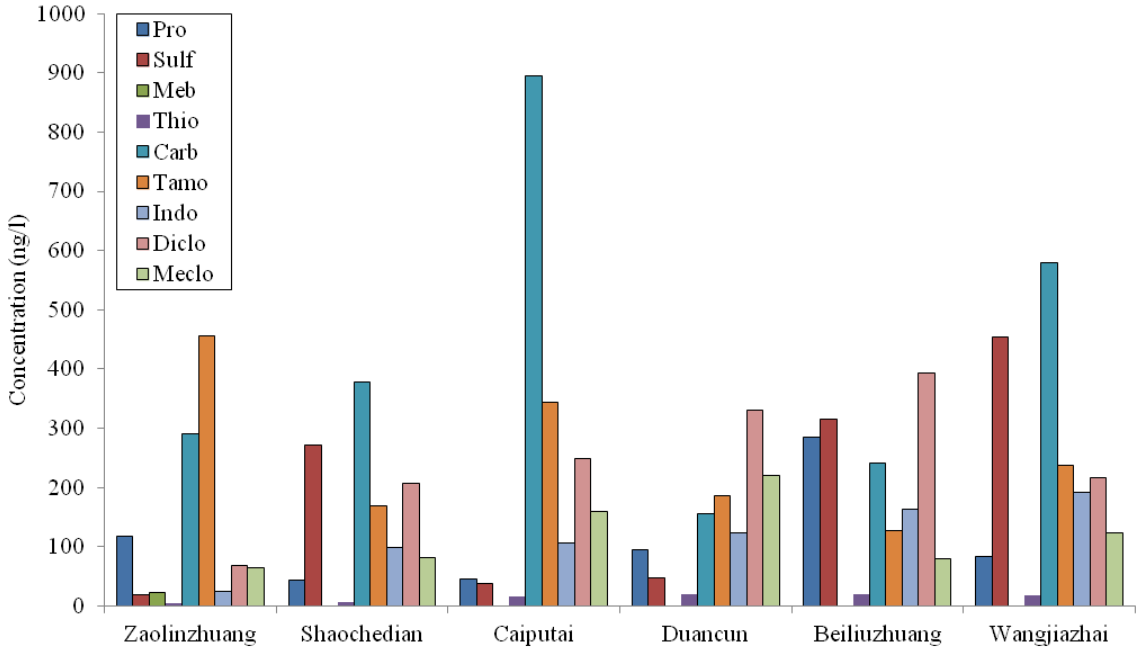

Figure 123 Concentration of target pharmaceutical compounds for March 2009 in Overlying water samples for BYD Lake

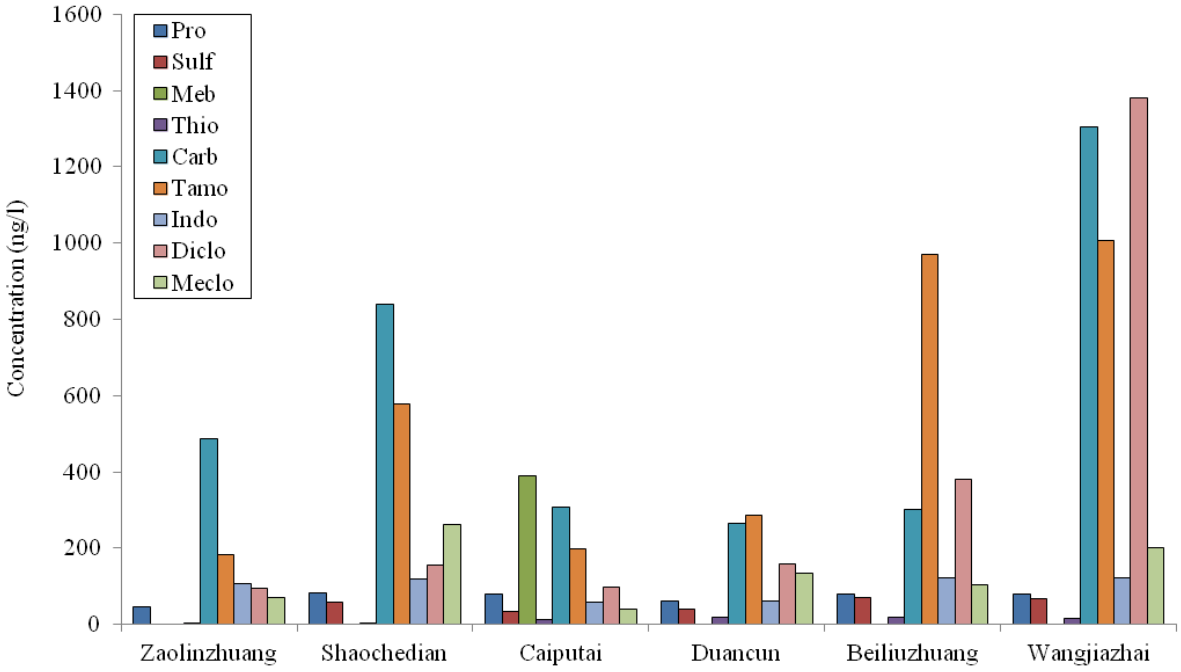

Figure 124 Concentration of target pharmaceutical compounds for March 2009 in Pore water samples for BYD Lake

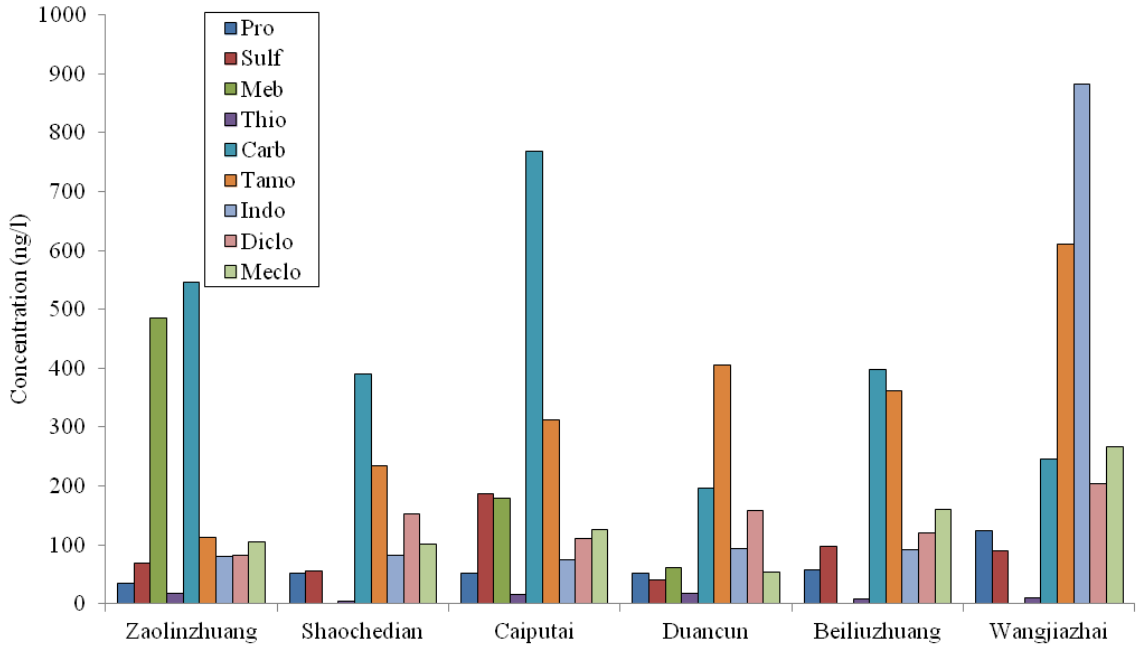

Figure 125 Concentration of target pharmaceutical compounds for March 2009 in Water-release test samples for BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	90.1	192.6	46.6	8.2	1091.9	410.0	94.8	154.2	152.6
	Shaochedian	1092.5	98.2	935.6	13.2	271.3	204.0	57.2	191.0	203.7
	Caiputai	27.3	206.0	107.5	14.2	239.8	287.7	65.7	116.7	168.7
	Duancun	12.1	45.6	0.0	16.2	641.6	157.4	71.2	101.2	90.9
	Beiliuzhuang	33.4	133.9	71.6	21.2	568.6	283.9	79.8	78.6	45.3
	Wangjiazhai	56.5	191.8	0.0	10.2	566.9	220.6	342.3	915.2	82.9
Overlying Water	Zaolinzhuang	23.4	194.4	418.2	4.1	486.2	255.5	32.1	159.3	64.4
	Shaochedian	20.5	6.3	0.0	9.2	618.4	117.6	0.0	171.7	68.1
	Caiputai	13.6	116.4	0.0	18.2	422.5	149.4	66.5	218.7	57.2
	Duancun	23.4	52.7	25.0	6.2	838.6	533.7	215.7	216.5	72.1
	Beiliuzhuang	10.8	59.9	791.9	23.2	578.6	147.3	94.6	152.3	266.5
	Wangjiazhai	75.9	212.7	139.4	19.5	105.7	384.8	313.4	1009.1	191.7
Pore Water	Zaolinzhuang	21.6	154.3	7.5	13.2	308.4	296.5	23.8	60.1	68.9
	Shaochedian	21.6	154.3	7.5	13.2	381.4	300.1	23.8	60.1	68.9
	Caiputai	34.8	62.2	95.1	18.0	200.0	151.1	61.6	51.8	58.9
	Duancun	26.8	56.1	22.7	21.2	390.5	217.7	49.3	59.2	92.7
	Beiliuzhuang	23.7	0.0	16.3	19.2	271.9	235.9	51.4	63.0	52.8
	Wangjiazhai	21.0	0.0	56.2	16.2	209.7	545.0	37.7	32.3	81.5
Water-release test	Zaolinzhuang	33.2	558.9	0.0	1.1	232.3	578.2	102.8	984.0	60.3
	Shaochedian	15.7	1.2	725.8	15.2	500.6	293.1	41.3	170.3	41.8
	Caiputai	29.1	38.8	0.0	12.1	229.4	190.5	58.2	51.4	102.4
	Duancun	30.0	199.2	0.0	2.3	841.8	540.6	55.7	45.5	8.2
	Beiliuzhuang	38.5	16.1	0.0	16.2	308.8	649.1	52.9	79.0	68.8
	Wangjiazhai	28.3	20.0	0.0	12.2	220.5	1051.5	49.8	58.7	0.0

Table 41 Concentration (ng/l) of pharmaceutical compounds found in BYD Lake surface water samples in April 2009

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from April are listed in Table 41 and displayed in Figure 126 - 128.

The highest observed concentration for surface water samples was 1092 ng/l at Shaochedian for Pro, closely followed by Carb at Zaolinzhuang with 1092 ng/l. The lowest observed concentration for this group of samples was <LOD for Meb at Duancun and Wangjiazhai. For overlying water samples, the lowest observed concentration was

once again <LOD for Meb (Shaochedian and Caiputai) and Indo (Shaochedian). The highest observed concentration for this group of samples was of 1009 ng/l for Diclo at Wangjiazhai. For pore water samples, the minimum concentration identified was once again <LOD for Meb at Beiliuzhuang and Wangjiazhai and the highest was for Tamo at 545 ng/l at Wangjiazhai. The concentration range for the water-release test sample group was between <LOD for Meb at all locations except Shaochedian and Meclo at Wangjiazhai to 1052 ng/l for Tamo at Wangjiazhai.

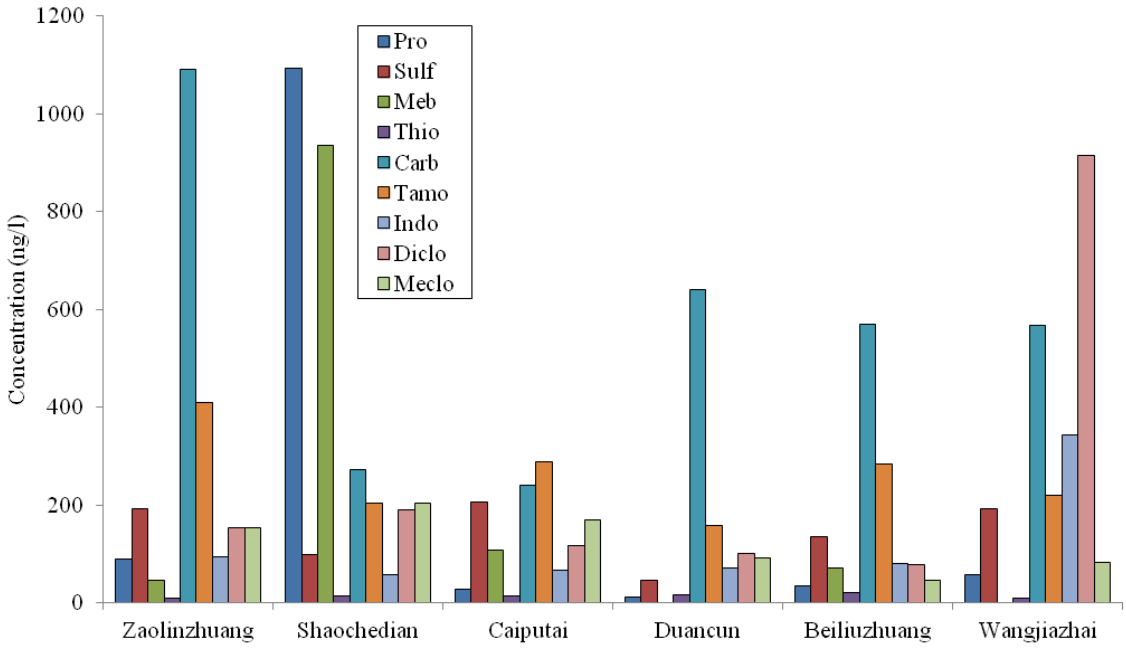

Figure 126 Concentration of target pharmaceutical compounds for April 2009 in Surface water samples for BYD Lake

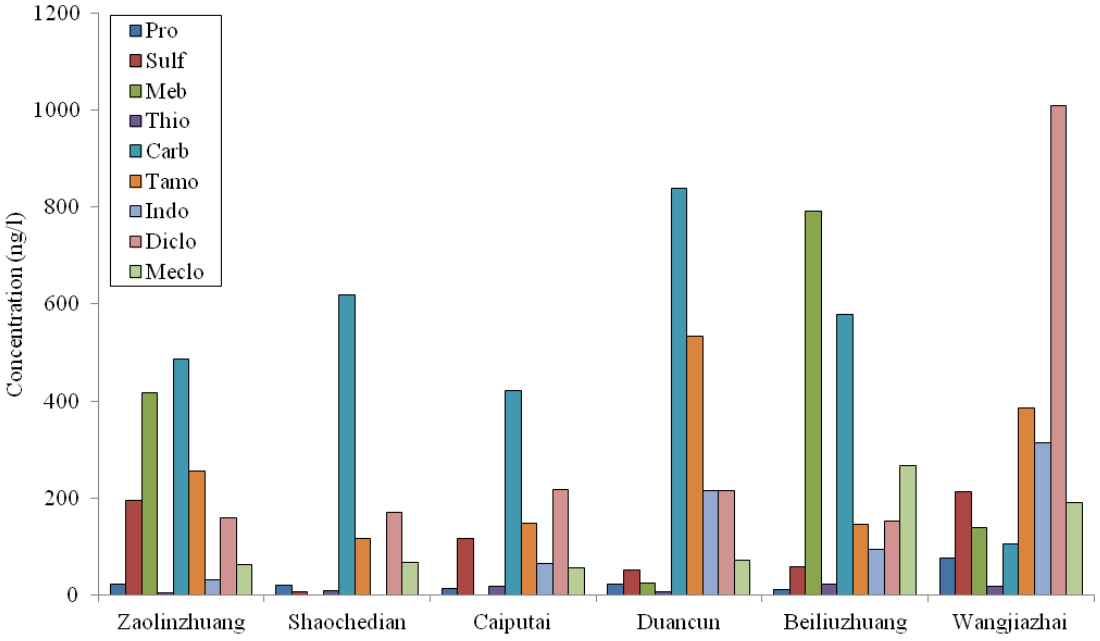

Figure 127 Concentration of target pharmaceutical compounds for April 2009 in Overlying water samples for BYD Lake

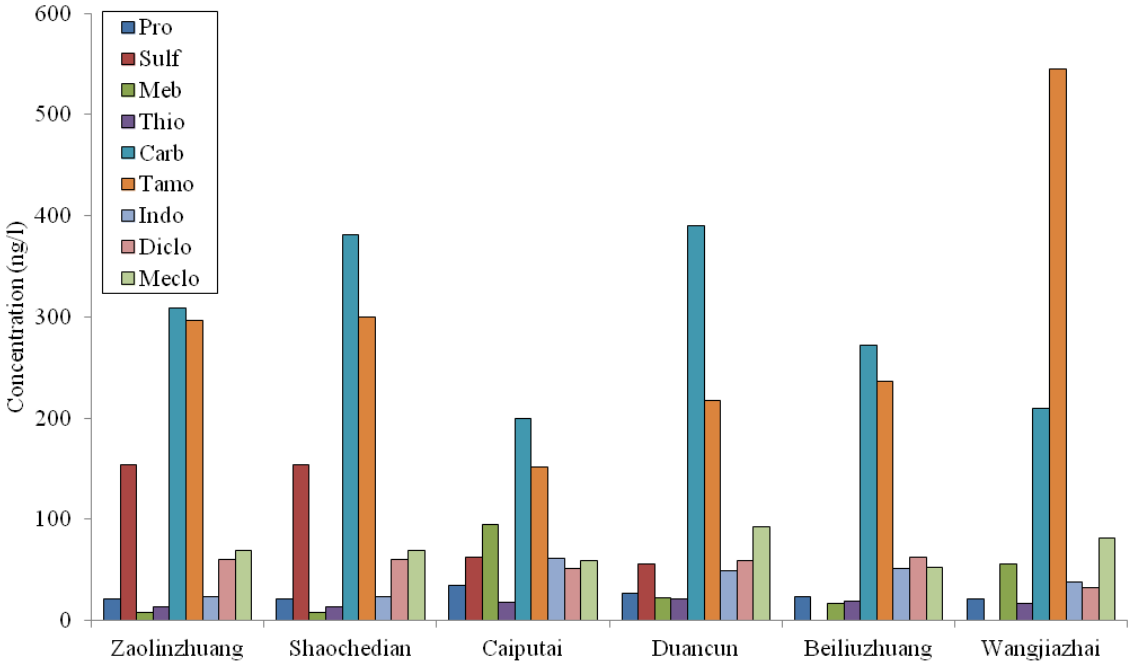

Figure 128 Concentration of target pharmaceutical compounds for April 2009 in Pore water samples for BYD Lake

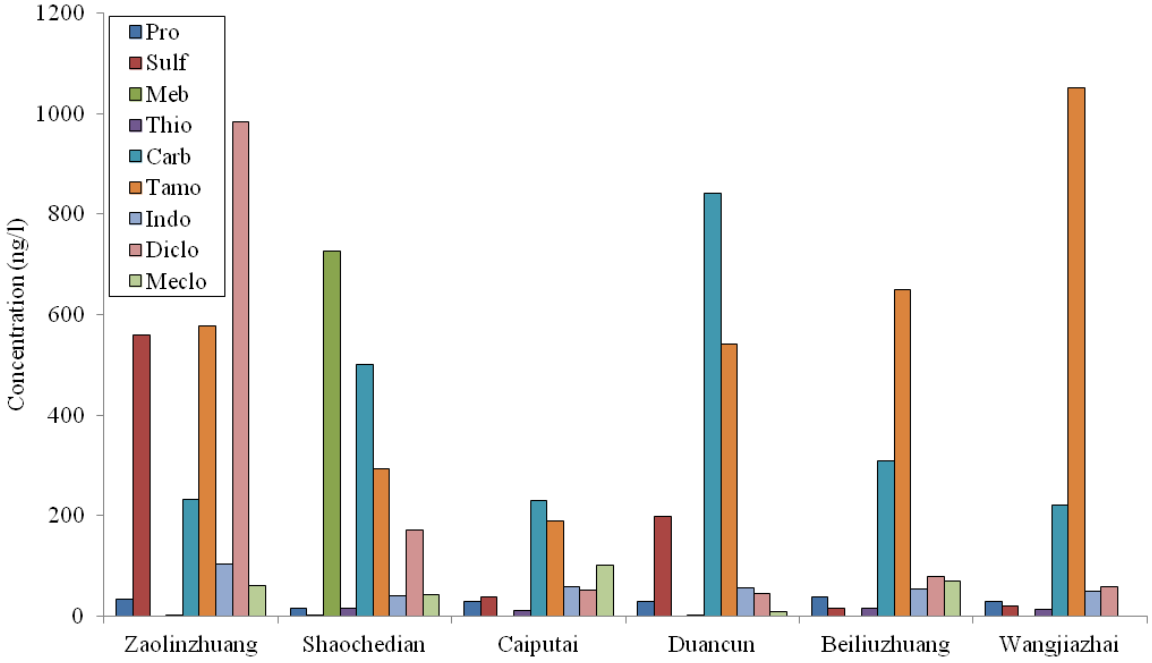

Figure 129 Concentration of target pharmaceutical compounds for April 2009 in Water-release test samples for BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	51.8	825.3	0.0	4.7	433.4	504.5	85.8	774.9	12.6
	Shaochedian	28.3	113.1	0.0	15.2	794.4	456.2	385.7	0.0	85.3
	Caiputai	11.8	6.2	215.2	14.2	336.8	255.0	182.2	37.8	8.0
	Duancun	35.0	16.9	41.2	9.1	357.8	325.5	233.9	149.3	365.0
	Beiliuzhuang	9.5	24.3	0.0	7.2	662.6	201.6	41.8	193.4	10.3
	Wangjiazhai	24.4	428.9	118.0	4.3	106.2	388.7	0.0	0.0	159.5
Overlying Water	Zaolinzhuang	6.6	215.5	776.4	6.2	333.0	448.8	94.2	0.0	131.7
	Shaochedian	19.4	45.9	119.6	15.2	361.8	139.5	101.9	0.0	13.1
	Caiputai	34.3	151.6	0.0	4.2	367.1	412.6	25.3	79.2	190.9
	Duancun	11.7	12.7	61.9	16.2	42.9	106.6	23.4	0.0	16.9
	Beiliuzhuang	18.9	24.4	258.5	13.0	567.3	508.3	133.9	175.5	110.6
	Wangjiazhai	112.1	1106.8	168.7	11.2	1056.8	98.2	207.9	1330.2	100.0
Pore Water	Zaolinzhuang	3.7	215.8	1276.2	10.2	475.8	120.6	83.7	31.2	101.5
	Shaochedian	6.4	23.9	704.4	5.2	180.1	314.0	31.7	37.3	108.5
	Caiputai	27.4	6.8	0.0	6.1	179.0	346.5	35.5	221.7	8.3
	Duancun	13.8	0.0	9.4	8.1	88.3	693.6	31.8	36.3	61.7
	Beiliuzhuang	8.1	2.0	0.0	14.2	227.2	350.9	41.5	27.0	80.7
	Wangjiazhai	9.7	129.7	0.0	5.1	1160.5	987.0	31.6	411.9	23.7
Water-release test	Zaolinzhuang	14.1	7.8	114.6	6.2	522.2	187.8	88.2	220.0	145.0
	Shaochedian	19.9	8.3	41.3	5.2	263.0	333.9	86.6	0.0	125.6
	Caiputai	12.2	1.6	339.2	1.2	1217.4	977.4	86.8	94.4	127.3
	Duancun	19.0	73.4	0.0	13.2	229.8	339.6	66.2	164.6	8.8
	Beiliuzhuang	10.4	4.5	88.4	9.1	254.9	249.1	23.6	89.8	4.6
	Wangjiazhai	15.3	315.5	0.0	5.1	413.6	225.9	34.7	695.6	122.1

Table 42 Concentration (ng/l) of pharmaceutical compounds found in BYD Lake surface water samples in May 2009

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from May are listen in Table 42 and displayed graphically in Figure 130 - 132.

In the sample group of surface water samples, the highest observed concentration was for Sulf of 825 ng/l at Zaolinzhuang, and the lowest observed concentration was <LOD for Meb at Zaolinzhuang, Shaochedian and Beiliuzhuang, Indo at Wangjiazhai and Diclo at Shaochedian. For overlying water samples, the lowest observed concentration was once again <LOD at Caiputai for Meb and at Zaolinzhuang, Shaochedian and Duancun for Diclo. The highest observed concentration was of 1330 ng/l for Diclo at Wangjiazhai, closely followed by Sulf and Carb for the same location with 1107 ng/l and 1057 ng/l respectively. For pore water samples, concentrations identified ranged from <LOD to 1276 ng/l. The lowest was observes at 4 locations; Duancun for Sulf and at Caiputai, Beilizhuang and Wangjiazhai for Meb. The highest was observed at Zaolinzhuang for Meb. Lastly, the highest and lowest observed concentrations for water-release test samples were <LOD and 1217 ng/l. The lowest was recorded for both Duancun and Wangjiazhai for Meb and Shaochedian for Diclo and the highest was observed at Caiputai for Carb.

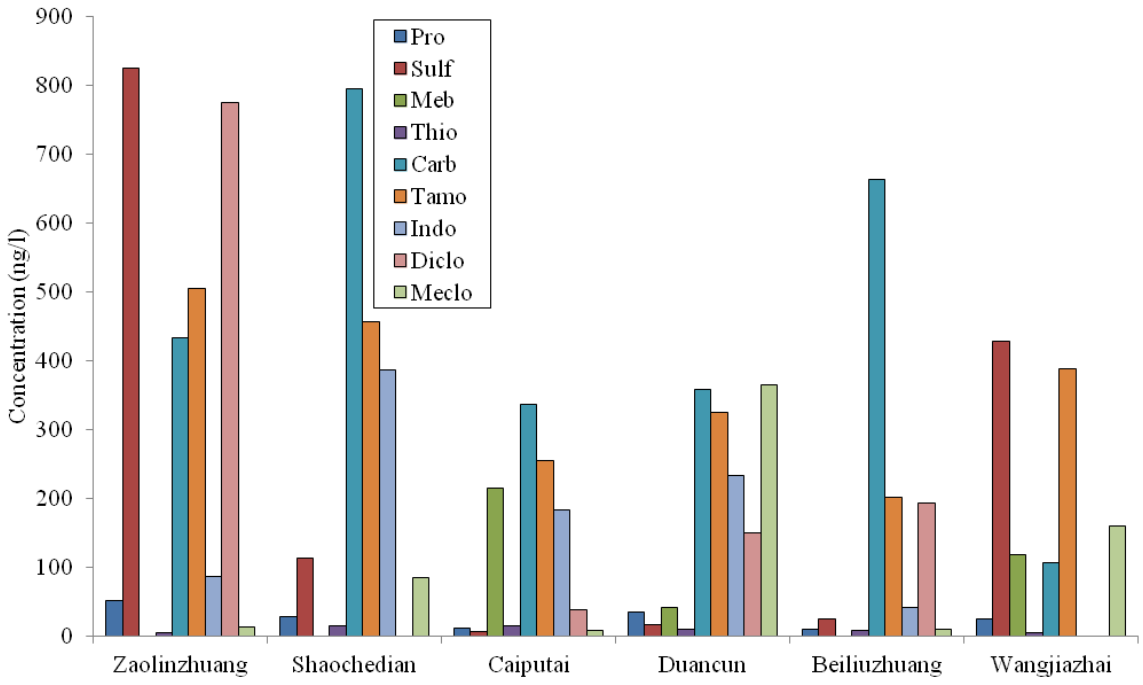

Figure 130 Concentration (ng/l) of target pharmaceutical compounds for May in Water-release test samples for BYD Lake

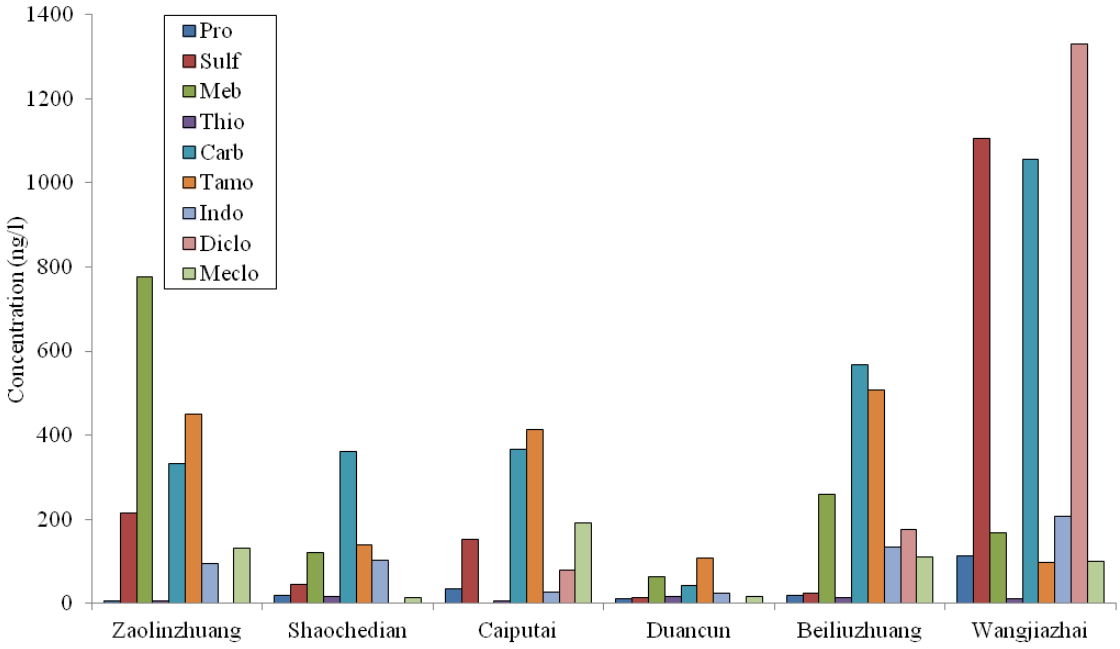

Figure 131 Concentration of target pharmaceutical compounds for May 2009 in Pore water samples for BYD Lake

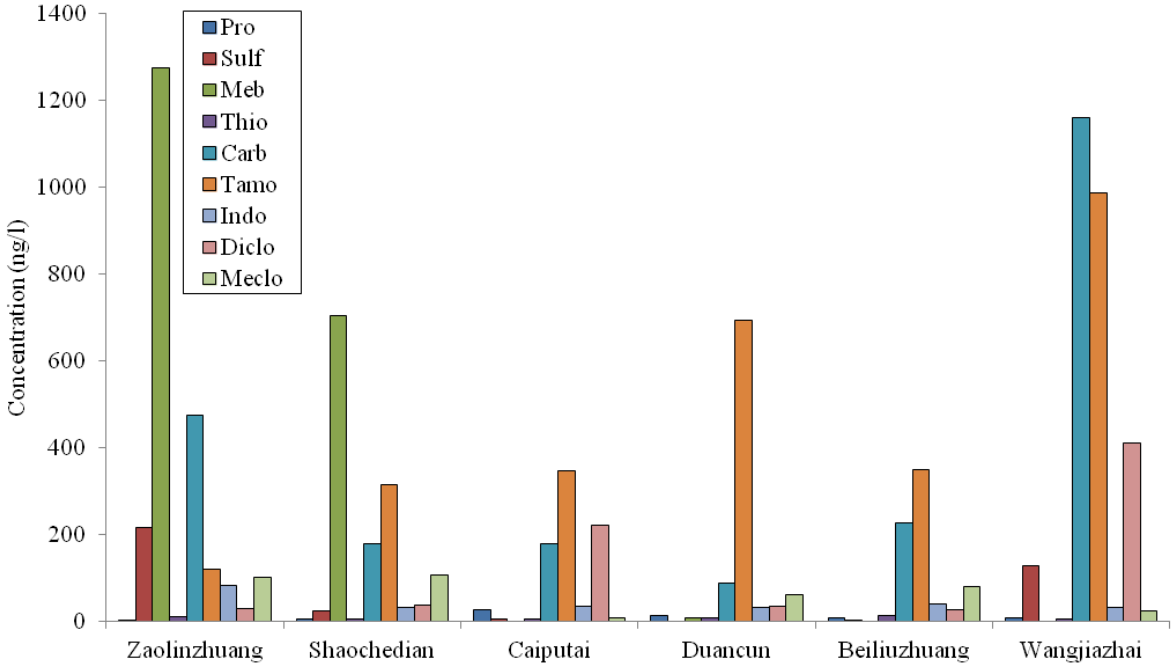

Figure 132 Concentration of target pharmaceutical compounds for May 2009 in Overlying water samples for BYD Lake

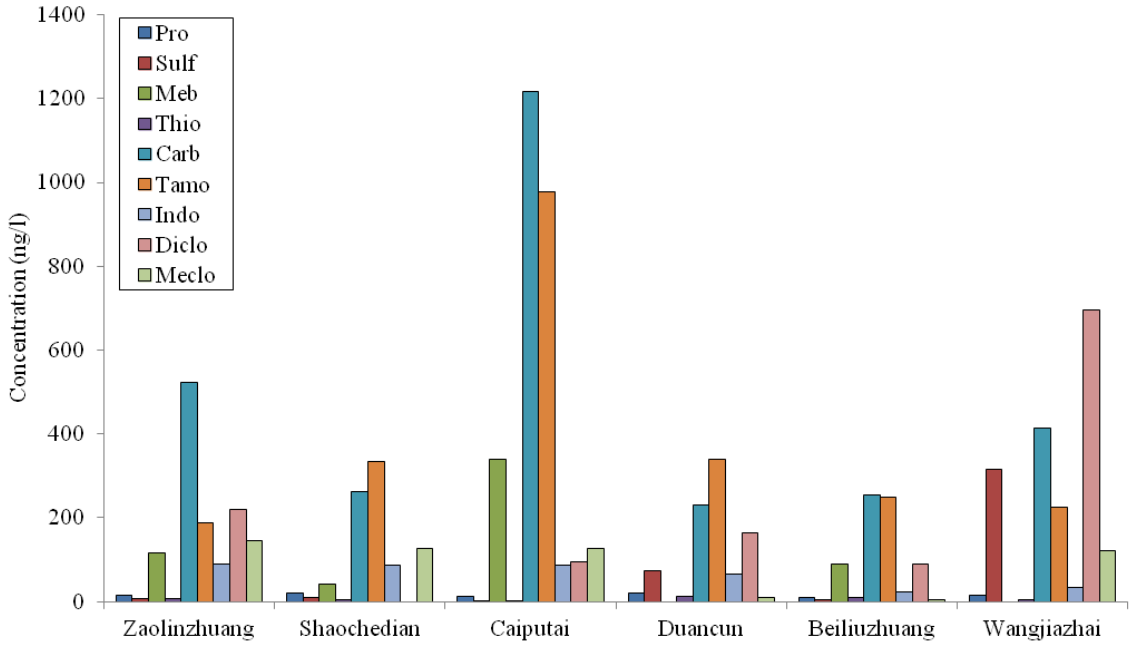

Figure 133 Concentration of target pharmaceutical compounds for May 2009 in Surface water samples for BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	23.4	84.8	66.2	14.2	1155.2	170.6	50.2	52.3	0.0
	Shaochedian	0.0	1.4	134.8	6.2	497.9	577.6	0.0	272.9	104.6
	Caiputai	0.0	9.0	71.6	14.2	506.8	117.9	16.9	339.9	96.4
	Duancun	12.7	297.8	37.8	4.2	1030.5	193.9	178.1	324.0	148.1
	Beiliuzhuang	27.2	678.3	211.1	6.1	123.7	137.5	0.0	207.8	7.2
	Wangjiazhai	21.6	577.3	0.0	13.2	441.8	273.7	77.2	285.5	166.4
Overlying Water	Zaolinzhuang	7.5	41.6	0.0	10.2	336.3	888.8	2.0	154.5	171.9
	Shaochedian	26.6	42.4	875.3	8.2	1712.6	215.2	74.1	102.2	148.3
	Caiputai	4.9	0.0	166.6	4.2	395.3	184.9	0.0	36.6	19.4
	Duancun	25.7	99.7	0.0	6.2	491.6	813.6	144.3	403.8	431.2
	Beiliuzhuang	83.9	1712.2	0.0	13.2	798.1	392.3	301.8	1904.0	0.0
	Wangjiazhai	103.7	685.5	85.0	10.2	687.3	737.6	69.7	940.7	255.3
Pore Water	Zaolinzhuang	42.3	77.0	273.1	11.0	730.2	1324.2	6.0	127.0	89.2
	Shaochedian	13.9	29.7	0.0	16.2	324.7	334.6	33.5	55.6	182.0
	Caiputai	57.3	4.6	0.0	3.1	73.5	338.6	46.8	343.1	307.5
	Duancun	65.3	9.3	54.8	13.2	28.0	1344.3	18.3	32.6	38.0
	Beiliuzhuang	112.0	853.9	269.7	17.2	369.2	899.9	157.4	179.2	309.0
	Wangjiazhai	21.8	21.2	26.6	11.2	656.6	191.8	0.0	174.8	58.6
Water-release test	Zaolinzhuang	13.4	40.5	52.7	3.1	298.2	189.1	51.9	73.4	29.6
	Shaochedian	5.9	84.9	0.0	5.2	488.4	180.4	82.7	16.6	115.4
	Caiputai	0.7	7.7	97.2	8.2	126.9	118.5	36.8	76.7	10.4
	Duancun	35.6	8.3	1144.5	5.2	160.5	167.9	63.5	0.0	121.1
	Beiliuzhuang	2.4	1200.0	0.0	5.2	315.3	156.6	6.8	91.7	146.1
	Wangjiazhai	907.5	197.8	815.9	1.2	204.1	453.2	429.6	798.7	1716.3

Table 43 Concentration (ng/l) of pharmaceutical compounds found in BYD Lake surface water samples in June 2009

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from June are listed in Table 43 and displayed graphically in Figure 134-136.

Starting with the group of 6 surface water samples, the highest observed concentration was 1155 ng/l for Carb at Zaolinzhuang and the lowest was for 60. ng/l values found at Zaolinzhuang for Meclo, Shaochedian for Pro and Indo, Caiputai for Pro, Beiliuzhuang

for Indo and Wangjiazhai for Meb. The highest identified concentration for the group of overlying water samples was 1904 ng/l at Beiliuzhuang for Diclo. Other high identified compounds were Sulf at Beiliuzhuang and Carb at Shaochedian with 1712 ng/l and 1713 ng/l respectively. For the pore water samples, non detection was once again identified three times as the lowest value for Meb at Shaochedian and Caiputai and for Indo at Wangjiazhai. For the water-release test samples the highest observed concentration was 1200 ng/g at Beiliuzhuang for Sulf and the lowest of <LOD at Shaochedian and Beiliuzhuang for Meb and Duancun for Diclo. A low concentration of 0.7 ng/l was also identified at Caiputai for Pro.

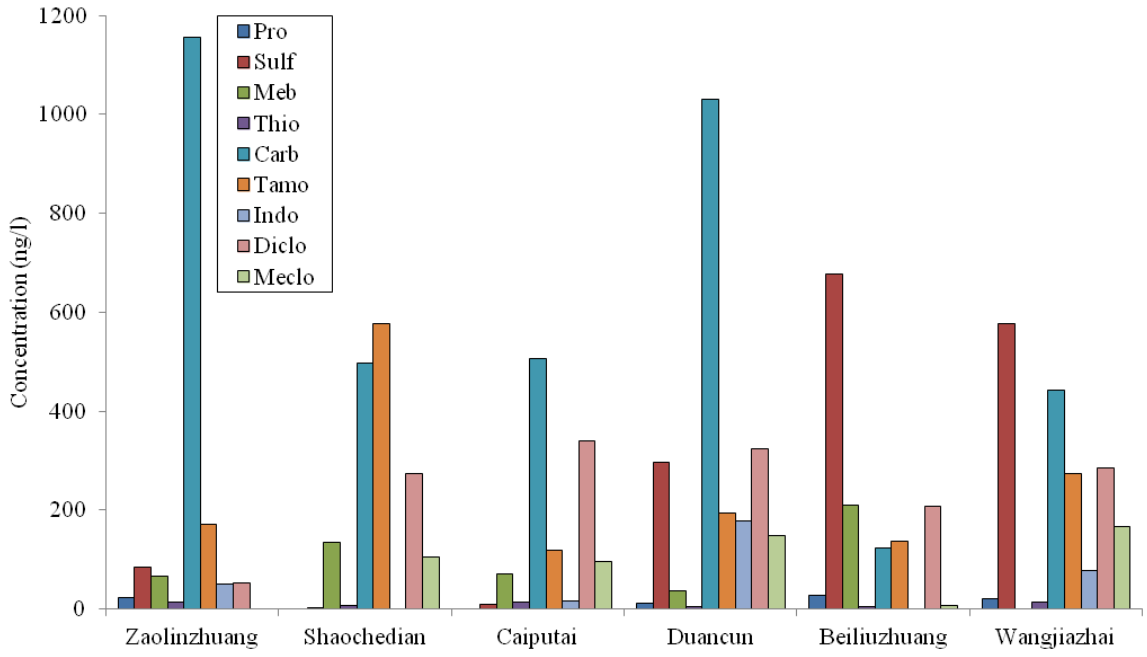

Figure 134 Concentration (ng/l) of target pharmaceutical compounds for June in Water-release test samples for BYD Lake

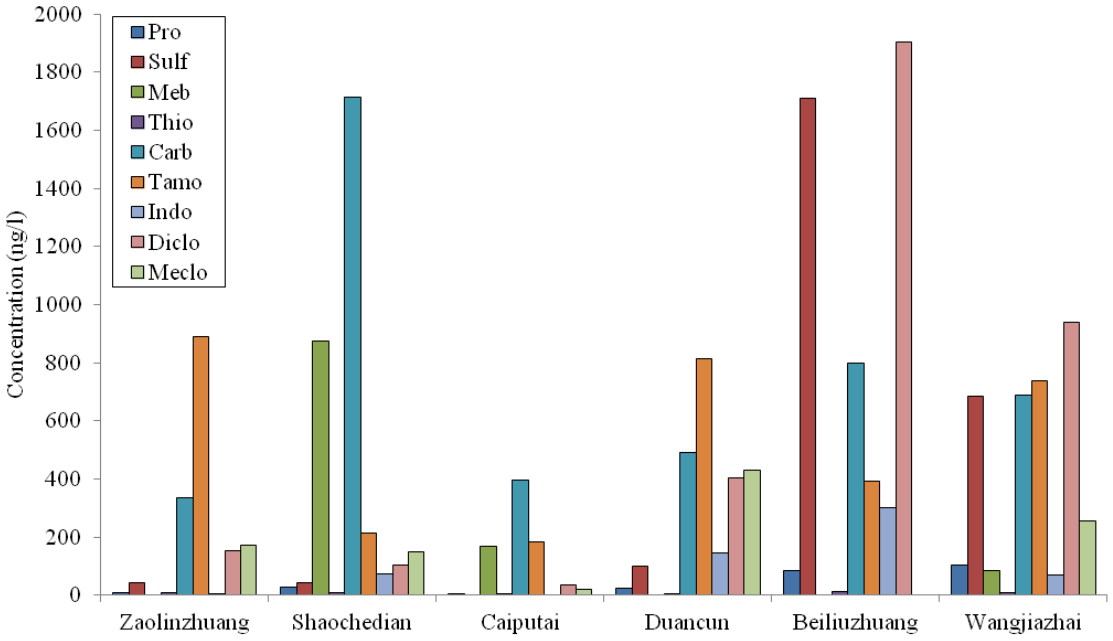

Figure 135 Concentration of target pharmaceutical compounds for June 2009 in Pore water samples for BYD Lake

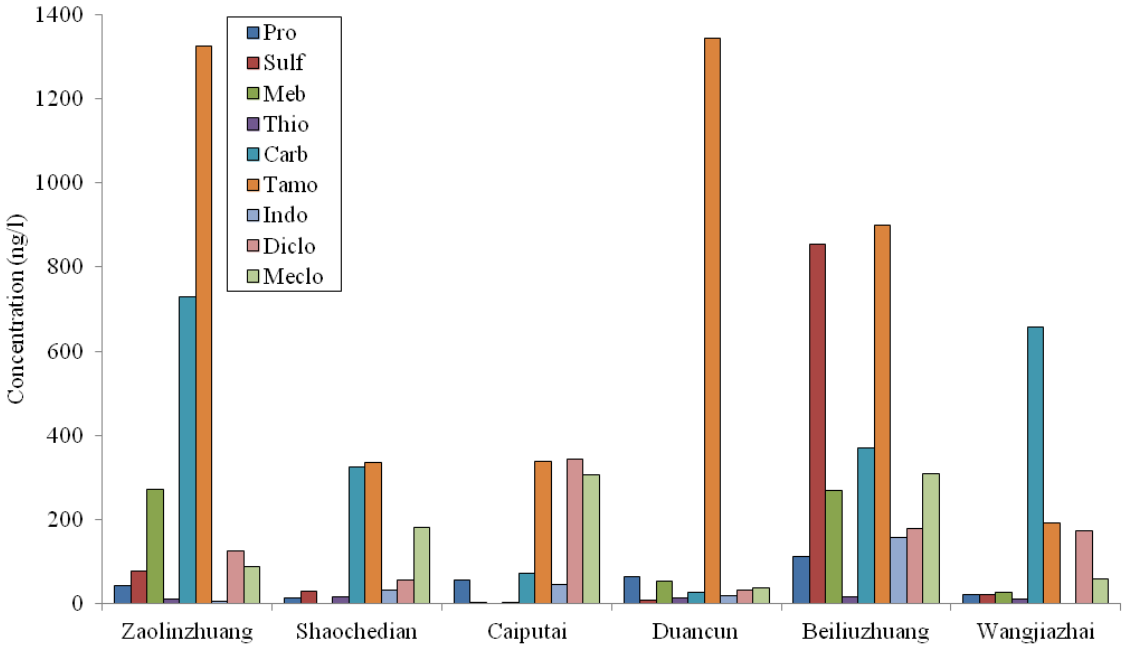

Figure 136 Concentration of target pharmaceutical compounds for June 2009 in Overlying water samples for BYD Lake

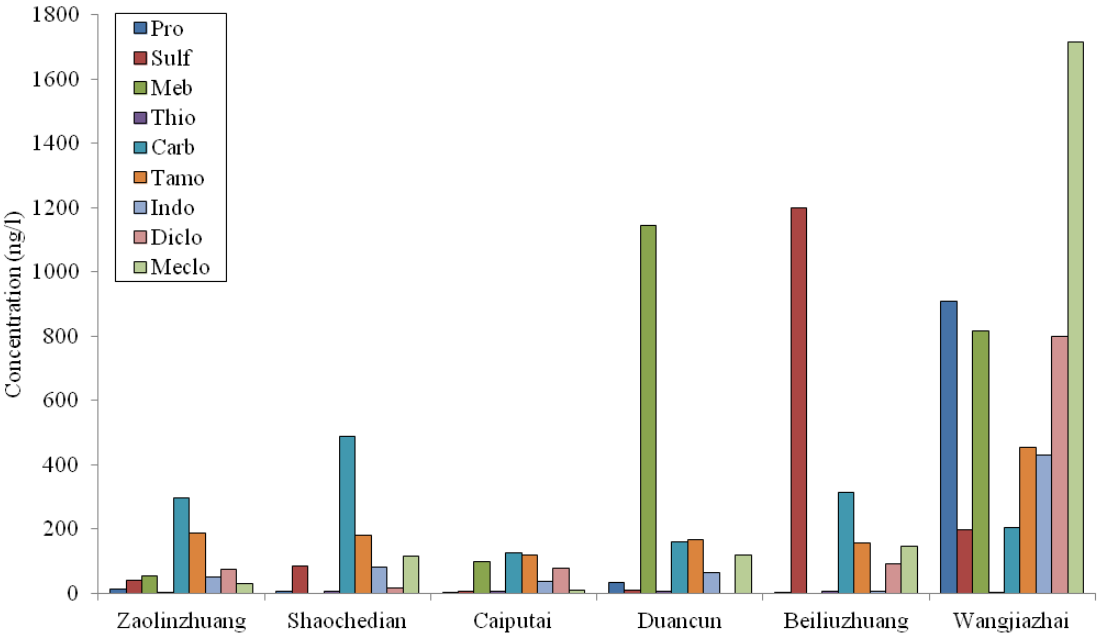

Figure 137 Concentration of target pharmaceutical compounds for June 2009 in Surface water samples for BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	1.7	636.0	1597.8	16.2	398.2	491.8	446.1	330.2	234.1
	Shaochedian	0.9	126.2	249.7	22.2	725.9	110.6	63.9	33.0	59.0
	Caiputai	0.0	28.6	0.0	15.2	1346.1	97.3	0.0	0.0	142.3
	Duancun	12.3	78.0	0.0	16.2	1218.4	461.2	59.2	511.5	373.9
	Beiliuzhuang	124.4	209.5	217.6	19.2	529.4	131.6	591.0	360.5	331.9
	Wangjiazhai	101.8	1194.7	27.7	8.2	136.9	230.0	628.1	1148.1	434.3
Overlying Water	Zaolinzhuang	5.9	157.7	516.9	3.2	988.3	177.3	111.9	191.8	474.1
	Shaochedian	26.7	80.2	1004.3	14.0	167.0	232.3	222.7	405.3	91.6
	Caiputai	264.5	0.0	1292.1	10.2	200.3	133.9	293.6	472.1	518.8
	Duancun	49.4	90.8	1089.0	14.2	1093.2	216.1	0.0	678.5	233.4
	Beiliuzhuang	164.7	263.7	0.0	5.2	466.1	78.0	667.1	632.8	134.1
	Wangjiazhai	278.6	1084.5	1491.5	11.2	540.6	380.2	355.2	1718.5	471.9
Pore Water	Zaolinzhuang	29.9	73.0	0.0	9.2	357.8	390.5	44.6	490.5	168.3
	Shaochedian	117.1	132.2	0.0	6.2	611.9	433.8	356.5	439.0	244.4
	Caiputai	1.4	0.0	154.3	12.3	258.7	67.7	31.9	107.3	73.2
	Duancun	0.0	0.0	0.0	13.2	503.3	226.8	52.6	277.8	224.2
	Beiliuzhuang	119.8	108.8	239.6	11.2	243.8	563.9	511.1	368.6	404.8
	Wangjiazhai	18.8	0.0	0.0	15.2	114.9	161.4	17.1	457.6	211.4
Water-release test	Zaolinzhuang	24.6	80.2	211.8	4.2	334.4	176.1	48.3	92.5	62.7
	Shaochedian	284.0	62.1	568.4	5.2	519.5	909.2	128.3	79.0	380.5
	Caiputai	157.7	15.6	0.0	8.2	272.7	1304.6	102.4	204.8	62.8
	Duancun	26.4	150.7	0.0	2.1	301.3	247.5	78.5	103.6	78.3
	Beiliuzhuang	49.8	969.3	0.0	3.3	258.2	110.2	235.9	1287.1	141.9
	Wangjiazhai	22.2	687.1	0.0	5.2	216.8	282.4	117.7	263.5	211.0

Table 44 Concentration (ng/l) of pharmaceutical compounds found in BYD Lake surface water samples in July 2009

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from July are listed in Table 44 and are displayed graphically in Figure 138 - 140.

For samples in July, the higest observed concentration of target compound identified in surface water samples was 1598 ng/l at Zaolinzhuang for Meb and the lowest was for <LOD for Pro at Caiputai, Meb for Caiputai and Duancun, Indo for Caiputai and Diclo for Caiputai. For overlying water samples, the highest observed concentration was 1718 ng/l for Diclo at Wangjiazhai. There were also other very high concentrations; 1084 ng/l for Sulf at Wangjiazhai, 1492 ng/l for Meb at Wangjiazhai and 1093 ng/l for Carb at Duancun. The highest observed concentration for pore water samples was 611.9 ng/l for Carb at Shaochedian. The lowest were <LOD identified at Duancun for Pro, Sulf and Meb, Wangjiazhai for Sulf and Meb, Caiputai for Sulf, and Shaochedian and Zaolinzhuang for Meb. Low concentrations were also identified of 1.4 ng/l for Pro at Caiputai and 6.2 ng/l for Thio. Thio generally has a low concentration through out the sampling in July 2009 , with a range from 2.1 ng/l to 22.2 ng/l. For water-release test samples, the highest observed concentration was of 1305 ng/l for Tamo at Caiputai, and the lowest of <LOD for all sites excluding Shaochedian and Zaolinzhuang for Meb.

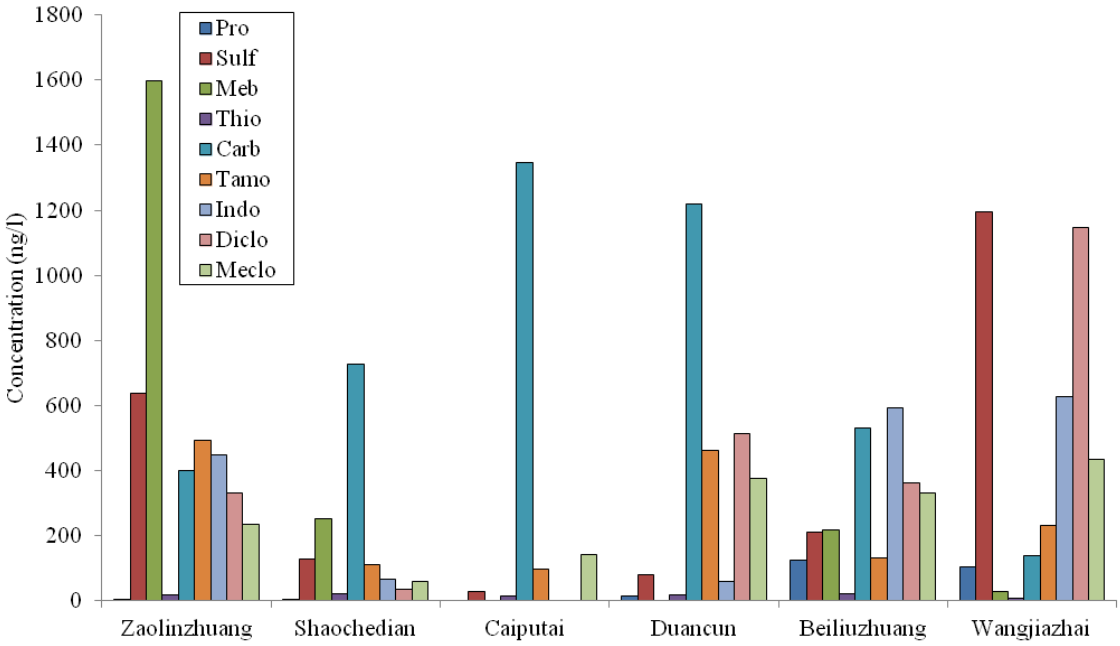

Figure 138 Concentration of target pharmaceutical compounds for July 2009 in Water-release test samples for BYD Lake

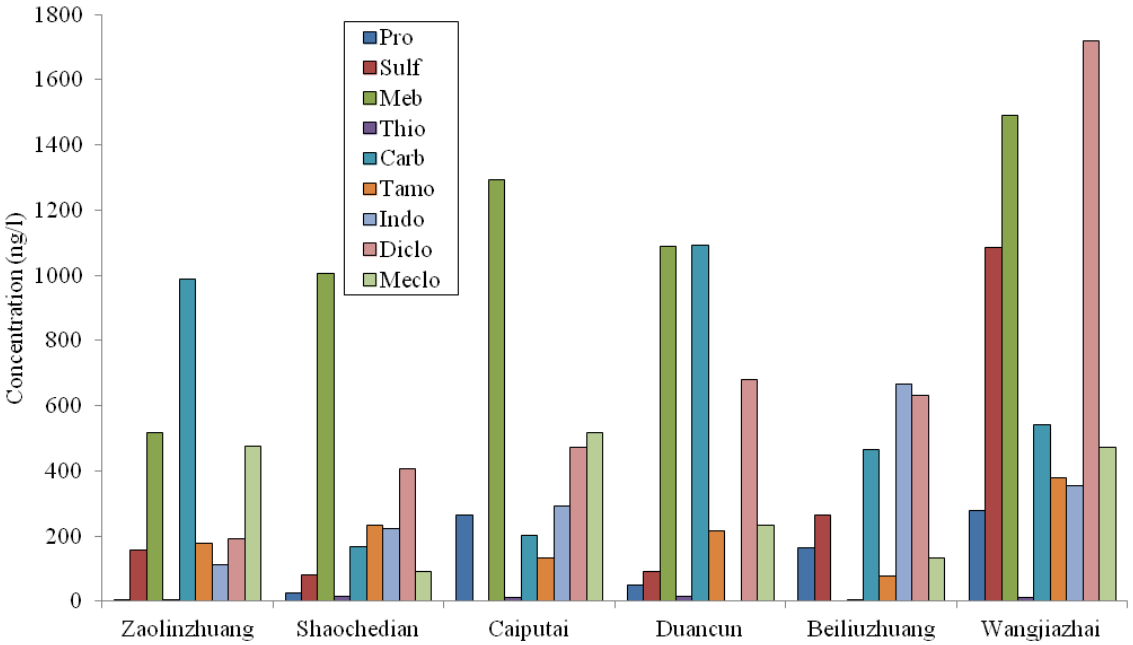

Figure 139 Concentration of target pharmaceutical compounds for July 2009 in Pore water samples for BYD Lake

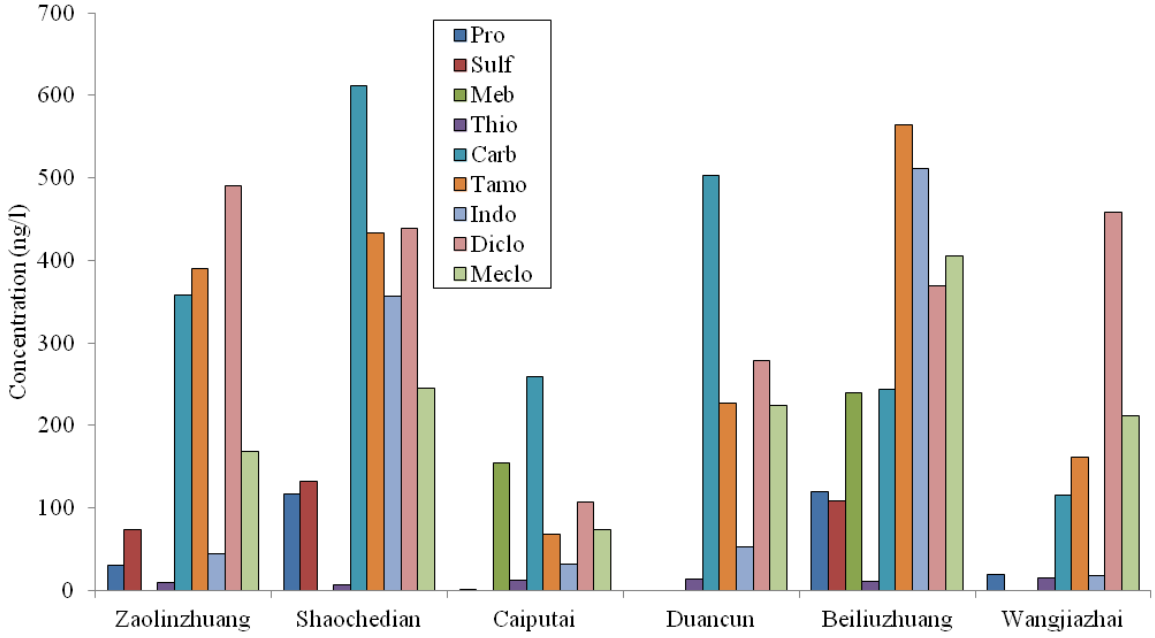

Figure 140 Concentration of target pharmaceutical compounds for July 2009 in Overlying water samples for BYD Lake

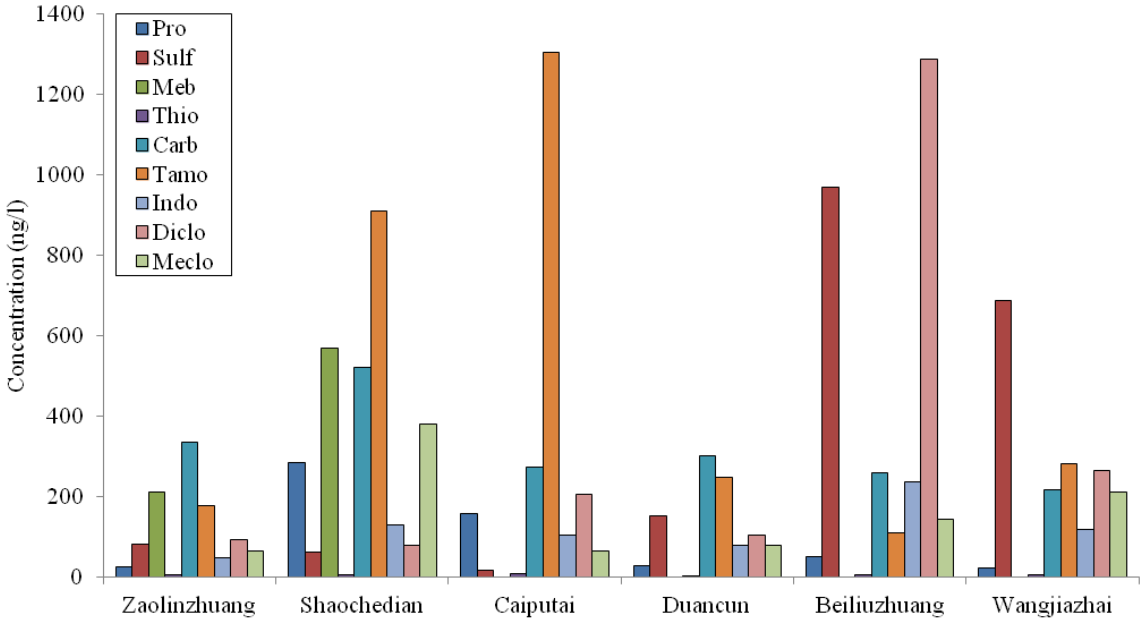

Figure 141 Concentration of target pharmaceutical compounds for July 2009 in water release test samples for BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	93.8	718.1	20961.5	4.7	660.6	4961.7	4679.0	1828.1	3577.9
	Shaochedian	8.6	195.5	8384.2	15.3	1113.0	737.5	2469.2	3928.9	234.1
	Caiputai	33588.1	97358.6	24385.9	12.4	24590.4	57124.6	107137.0	43706.6	84160.9
	Duancun	176.9	359.2	1638.9	7.2	641.9	2865.6	3869.8	1079.7	6566.1
	Beiliuzhuang	261.3	1219.2	2979.7	6.3	1709.6	4354.8	10753.3	1442.5	2159.3
	Wangjiazhai	140.1	291.1	2505.7	19.3	12681.3	5006.9	10119.7	11219.5	26962.6
Overlying Water	Zaolinzhuang	158.8	238.7	301.8	45.7	873.1	664.4	2363.6	1054.7	107.1
	Shaochedian	109.6	0.0	194.8	12.6	0.0	1015.8	2072.5	0.0	434.4
	Caiputai	39.5	63.8	0.0	10.2	960.0	1441.9	1665.3	3480.3	3061.6
	Duancun	159.3	203.7	2953.3	6.4	578.2	1371.0	7165.4	30524.0	0.0
	Beiliuzhuang	652.7	8121.1	68438.3	9.6	44078.2	24356.9	37485.0	59631.1	43439.4
	Wangjiazhai	0.0	10806.6	9467.3	45.2	5339.2	8490.1	24026.5	0.0	3384.2
Pore Water	Zaolinzhuang	0.0	0.0	8786.1	32.5	1444.5	14495.4	17871.5	4406.5	1521.2
	Shaochedian	0.0	134.9	2229.8	6.2	259.0	727.1	968.7	536.5	0.0
	Caiputai	1821.6	4285.9	18030.3	15.3	0.0	6238.8	49350.9	36499.4	12835.9
	Duancun	298.4	13.9	221.5	18.2	109.0	1225.4	1528.1	1242.6	60997.1
	Beiliuzhuang	670.7	1901.4	0.0	7.2	336.5	641.7	769.6	1958.0	1350.1
	Wangjiazhai	138.2	592.0	1550.6	11.3	67.0	1188.5	5202.8	6820.9	46470.5
Water-release test	Zaolinzhuang	5.1	9627.7	1383.9	3.2	149.5	591.5	1050.3	3611.1	1287.3
	Shaochedian	8.6	25.1	33.5	63.2	7.2	285.8	285.8	156.3	0.0
	Caiputai	335.1	27820.7	2235.6	12.4	161.5	1450.8	3183.3	0.0	2948.0
	Duancun	61.3	446.7	0.0	7.2	389.6	1294.0	2530.8	2652.1	4332.6
	Beiliuzhuang	63.5	0.0	678.9	14.3	342.9	181.2	1272.1	485.5	449.0
	Wangjiazhai	160.0	1119.2	14767.2	5.6	803.1	2302.8	1566.7	1753.1	0.0

Table 45 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples in September 2009

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from September are listed in Table 45 and are displayed graphically in Figure 142 - 144.

It is immediately obvious when looking at Figure 142 that the sampling location with the highest identified concentrations for surface water samples in September is Caiputai. This is also the case for Beiliuzuang for overlying water samples Figure 143.

The concentration of target pharmaceutical compounds in samples from September measured much higher concentrations than identified before. The maximum concentration identified in surface water samples was 107137 ng/l for Indo at Caiputai. The minimum was a very small 2.7 ng/l in comparison for Thio at Zaolinzhuang. Thio had overall the lowest identified concentration with a range between 4.7 ng/l and 19.3 ng/l (Wangjiazhai), which is also the case across all sample groups during this month with a range between 3.2 ng/l (at Zaolinzhuang during the water-release test) and 63.2 ng/l (at Shaochedian during the water-release test). The highest identified concentration for the overlying water sample group was 68438.3 ng/l at Beiliuzhang for Meb. The lowest was a value of <LOD on seven occasions for Pro, Sulf, Meb, Carb, Diclo and Meclo. For pore water samples, the highest identified concentration for target pharmaceutical compounds was 60997 ng/l for Meclo at Duancun. The lowest (excluding six 0.00 ng/l values) was 6.2 ng/l for Thio at Shaochedian. The maximum concentration identified during the water-release test sample group was 27821 ng/l for Sulf at Caiputai, and the lowest (once again excluding <LOD values) was 3.2 ng/l for Thio at Zaolinzhuang.

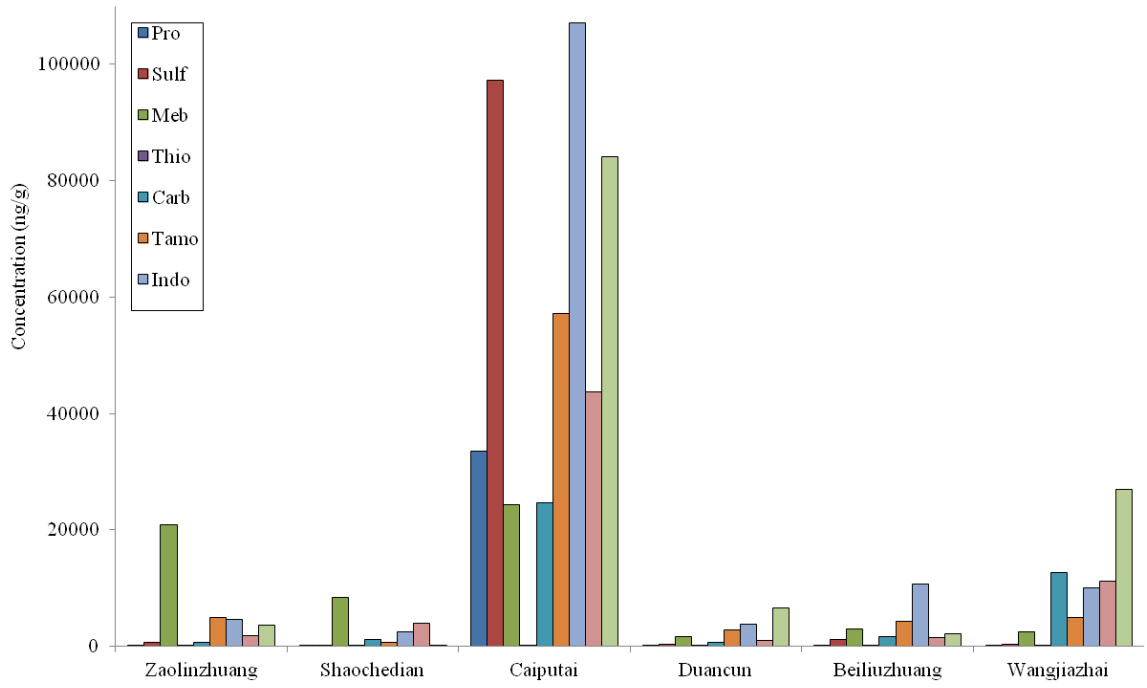

Figure 142 Concentration of target pharmaceutical compounds for September 2009 in Surface water samples for BYD Lake

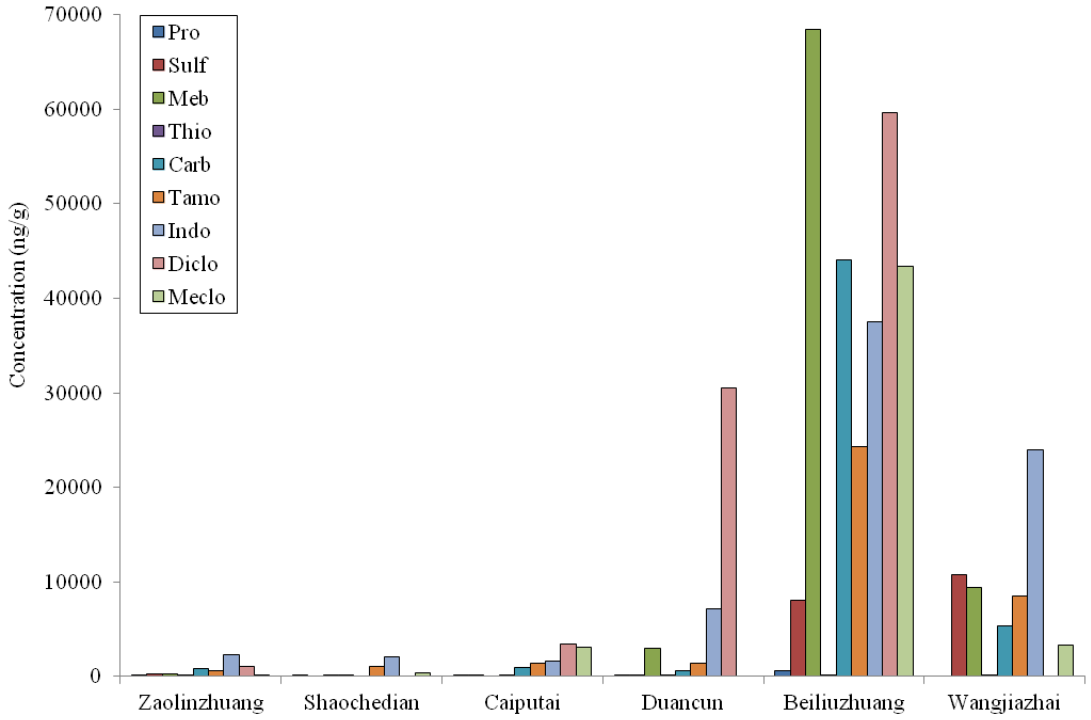

Figure 143 Concentration of target pharmaceutical compounds for September 2009 in Overlying water samples for BYD Lake

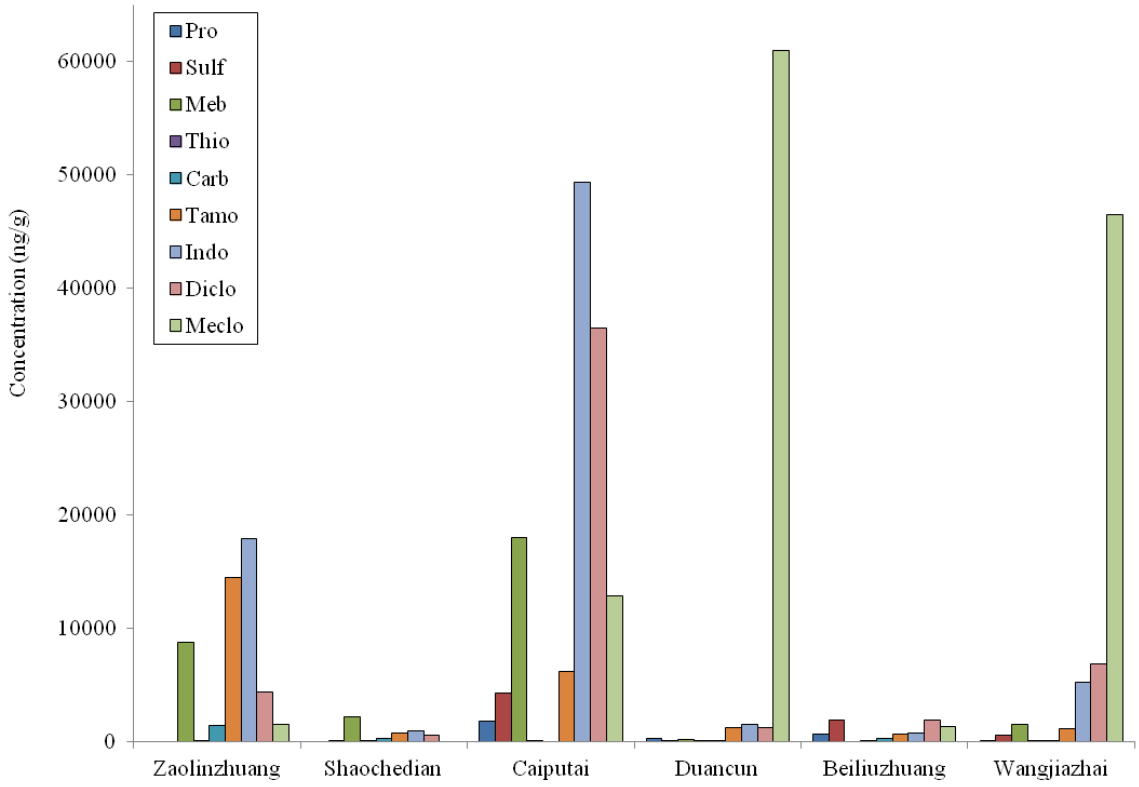

Figure 144 Concentration of target pharmaceutical compounds for September 2009 in Pore water samples for BYD Lake

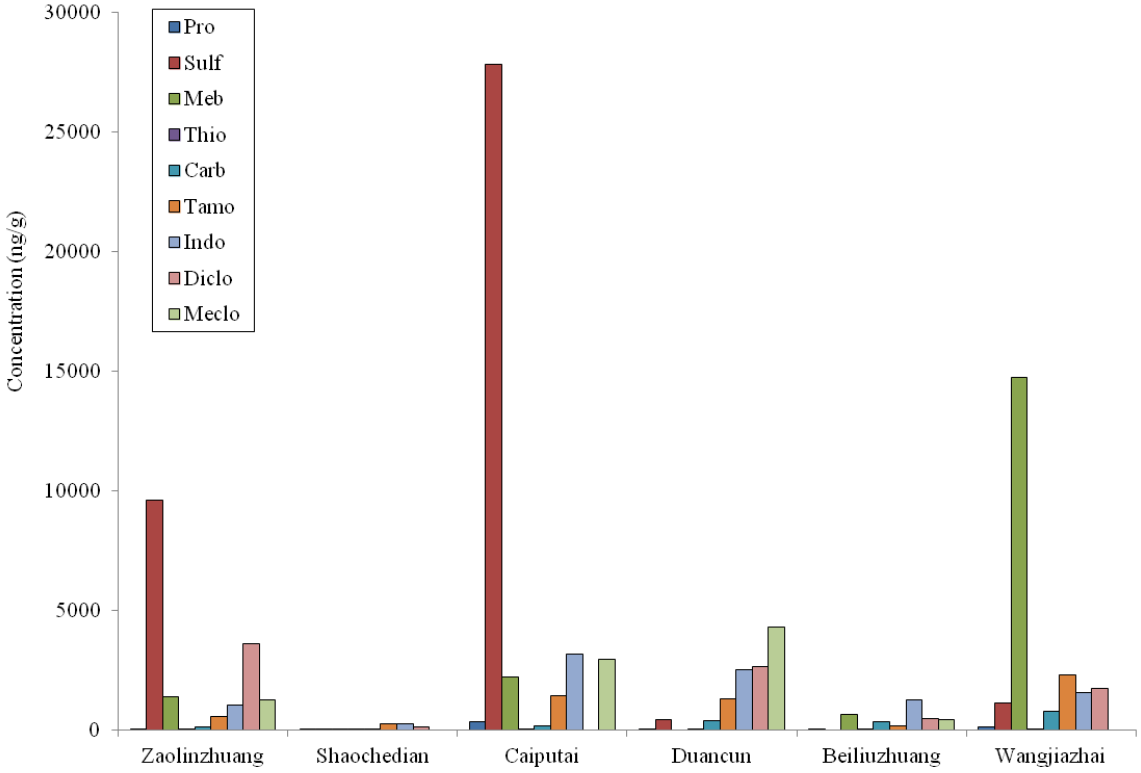

Figure 145 Concentration of target pharmaceutical compounds for September 2009 in water-release test samples for BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	878.4	633.9	0.0	16.3	4732.2	0.0	14311.9	6215.7	8665.2
	Shaochedian	8.2	0.0	10248.9	4.3	7734.5	1360.3	24901.0	0.0	2483.9
	Caiputai	2769.5	1899.1	0.0	11.4	234.0	67.7	817.2	3803.6	573.6
	Duancun	4.6	62.8	1409.9	9.3	1.4	37.2	143.5	453.4	2480.6
	Beiliuzhuang	101.8	259.4	2830.3	8.7	3161.6	467.4	2357.2	7204.2	0.0
	Wangjiazhai	4.8	4.1	0.0	12.3	539.3	34.3	74.5	274.1	142.9
Overlying Water	Zaolinzhuang	0.0	0.0	212.0	10.2	497.5	169.0	3344.4	1935.6	1917.6
	Shaochedian	23.2	114.6	212.2	6.3	2402.0	1943.8	2234.6	4723.6	0.0
	Caiputai	101.9	598.3	0.0	8.7	265.2	208.3	929.0	4310.5	7087.1
	Duancun	54.2	3543.6	6272.3	19.2	0.0	361.9	8072.0	359.3	2494.9
	Beiliuzhuang	2398.3	5249.0	0.0	11.7	6177.1	1913.5	8666.8	3895.8	4188.7
	Wangjiazhai	33.2	372.2	0.0	10.3	4499.5	7270.2	3729.7	0.0	0.0
Pore Water	Zaolinzhuang	5.5	0.0	7799.3	7.2	120.3	26.5	512.4	5269.5	6237.8
	Shaochedian	71.5	1215.2	201.6	86.2	14.9	112.4	695.9	7014.0	86.8
	Caiputai	288.1	2475.6	8041.7	86.3	129.6	2552.4	1563.2	0.0	5677.0
	Duancun	29.3	1090.8	3750.9	53.2	144.6	1013.2	4746.4	183.2	7669.7
	Beiliuzhuang	0.0	26.4	8654.9	26.6	0.0	875.0	2838.5	2219.3	0.0
	Wangjiazhai	4931.6	1140.3	4764.7	7.2	0.0	1560.7	5719.4	6757.5	0.0
Water-release test	Zaolinzhuang	723.4	0.0	6246.1	16.4	378.1	1039.3	2048.4	3594.4	157.1
	Shaochedian	518.5	6616.6	6247.3	2.7	278.4	629.5	0.0	0.0	1508.5
	Caiputai	33.7	0.0	0.0	10.6	0.0	1391.5	7727.9	4152.3	4130.3
	Duancun	902.4	2270.7	0.0	14.5	1223.8	7283.3	1382.0	0.0	4464.7
	Beiliuzhuang	63.2	3978.1	2733.5	60.0	4920.8	3767.4	7196.8	3502.9	7764.6
	Wangjiazhai	106.2	0.0	0.0	53.2	0.0	1803.5	7902.3	1427.7	0.0

Table 46 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples in October 2009

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from October are listed in Table 46, displayed graphically in Figure 146 - 148.

During October, the target pharmaceutical compound with the overall lowest observed concentrations was Thio, with a range between 2.7 ng/l (Shaochedian during water-release tests) and only 86.3 ng/l (Caiputai during pore water sampling). The highest overall observed compound concentration was 24901 ng/l for Indo at Shaochedian during surface water sampling. This compound along with Meclo gave very high concentration results. <LOD were identified across a variety of sites and sample groups

at all compounds excluding Thio on at least one occasion. The minimum observed concentration for surface water samples excluding <LOD values, was 1.4 ng/l for Carb at Duancan. Other minimum values for sample groups were as follows (excluding zero values); 6.3 ng/l for Thio at Caiputai (overlying water), 5.5 ng/l for Pro at Zaolinzhuang (pore water) and 2.7 ng/l for Thio at Shaochedian (water-release tests). Remaining maximum sample concentrations observed at all sites (excluding aforementioned maximum overall concentration) were as follows; 8667 ng/l for Indo at Beiliuzhuang (overlying water), 8655 ng/l ng/l for Meb at Caiputai (pore water) and 7902 ng/l for Tamo at Wangjiazhai (water-release test samples).

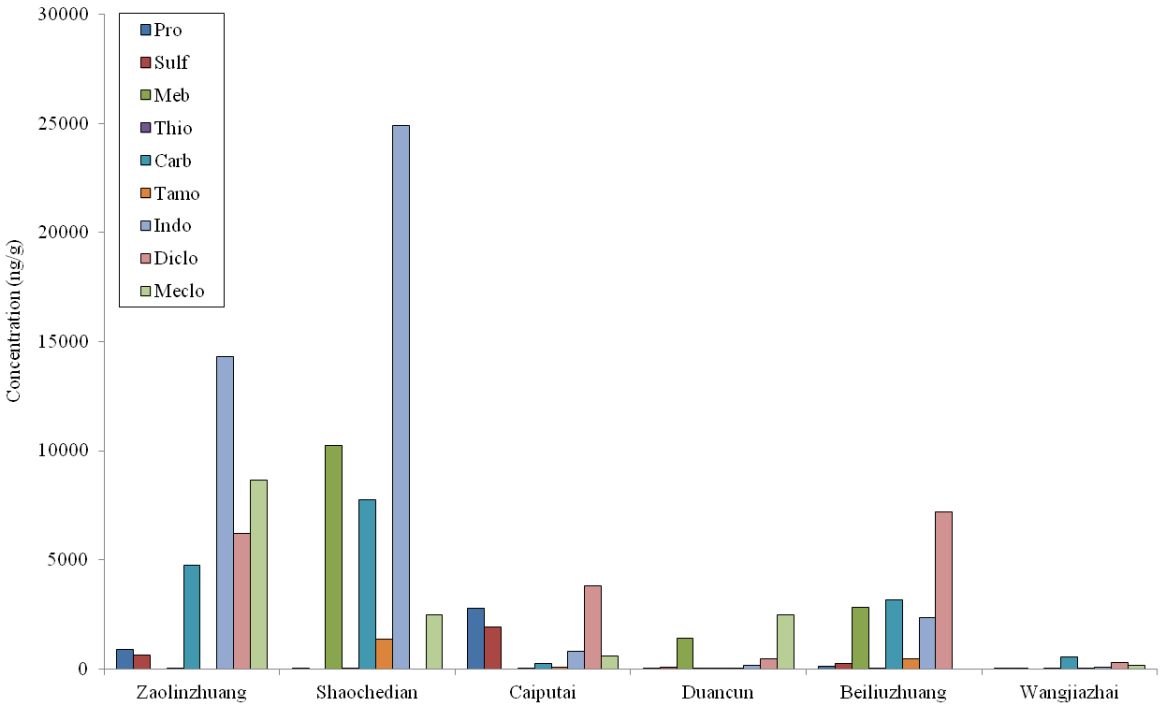

Figure 146 Concentration of target pharmaceutical compounds for October 2009 in Surface water samples for BYD Lake

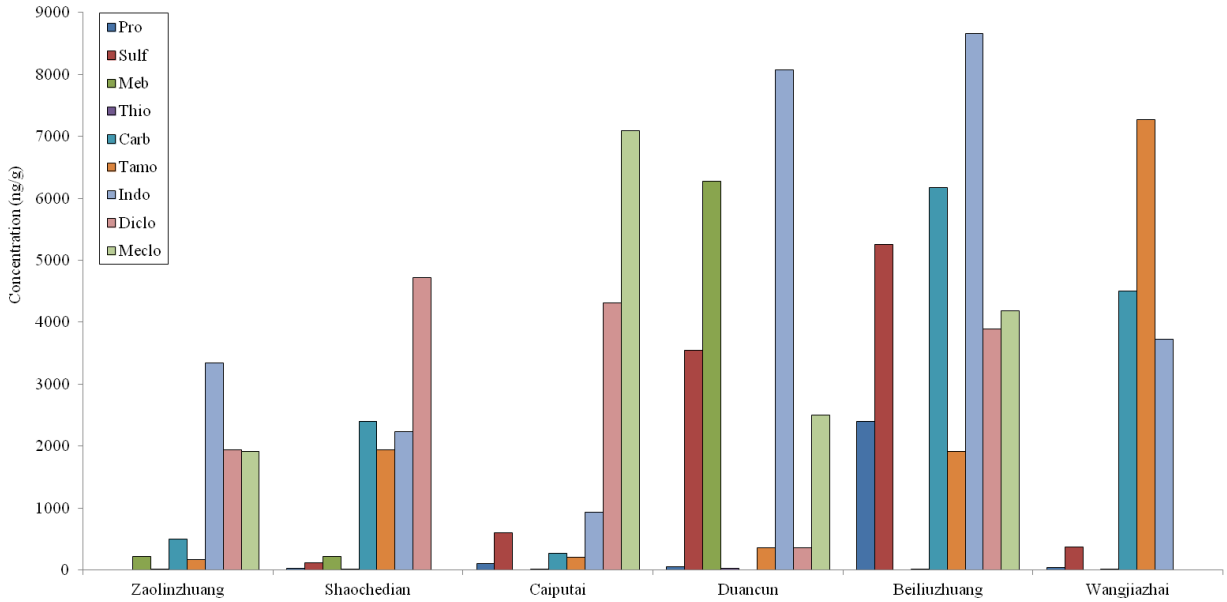

Figure 147 Concentration of target pharmaceutical compounds for October 2009 in Overlying water samples for BYD Lake

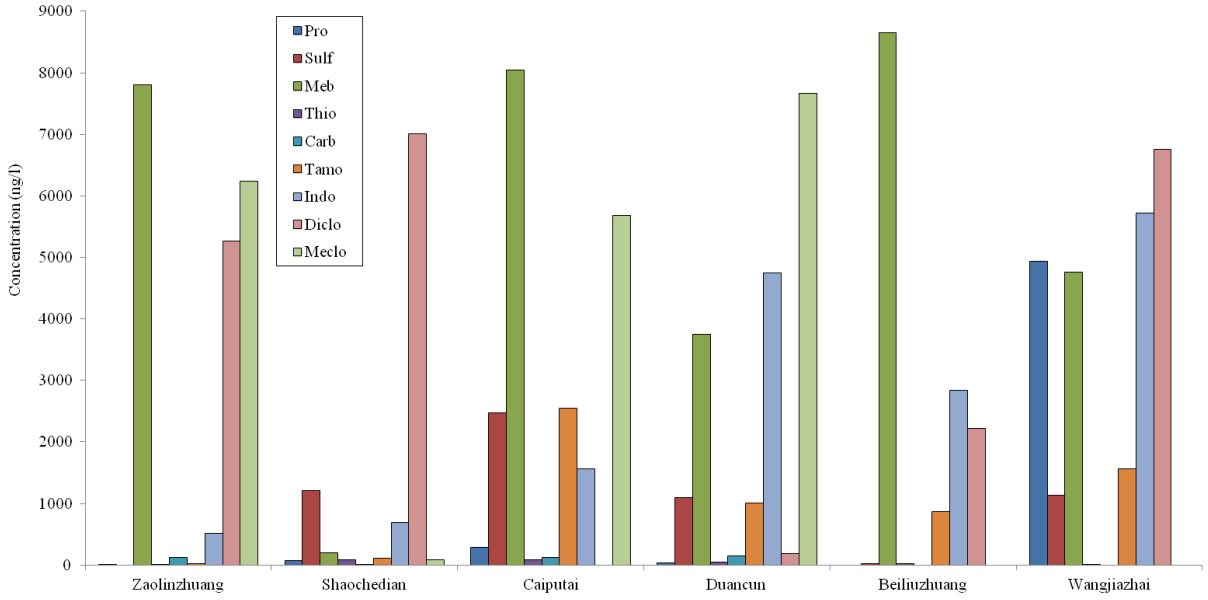

Figure 148 Concentration (ng/l) of target pharmaceutical compounds for October 2009 in Pore water samples for BYD Lake

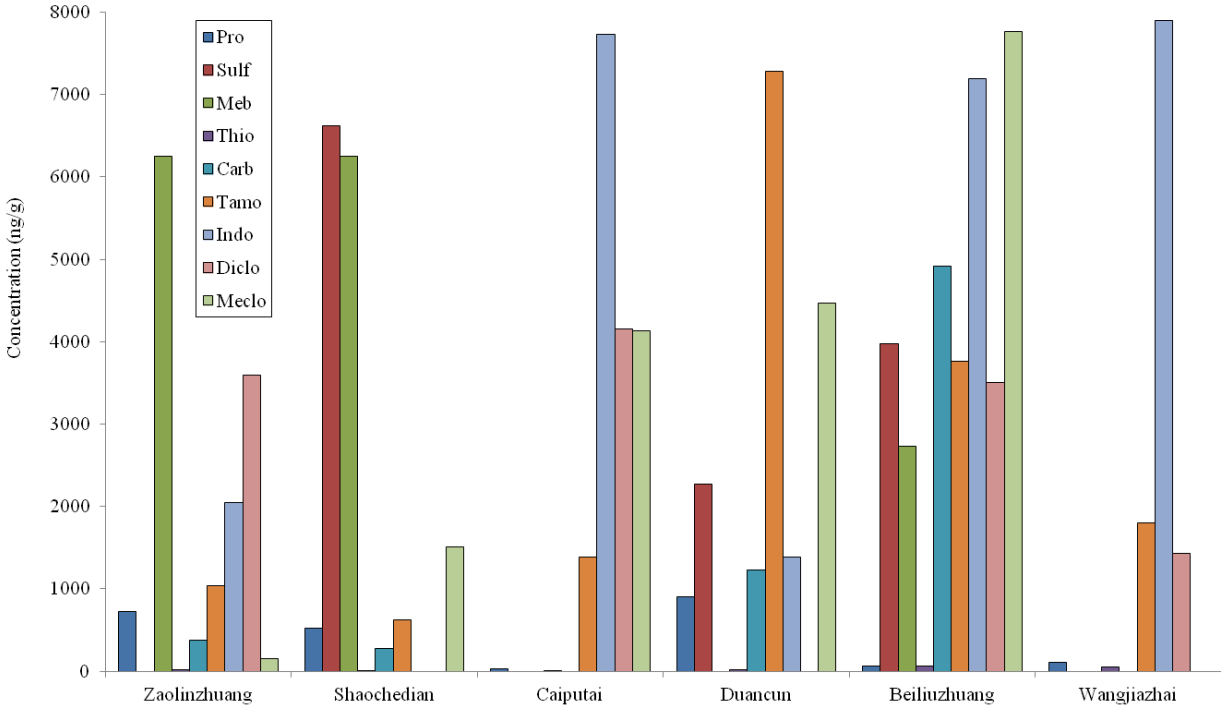

Figure 149 Concentration (ng/l) of target pharmaceutical compounds for October 2009 in water-release test samples for BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Surface Water	Zaolinzhuang	179.4	8314.5	0.0	4.7	435.6	4537.3	4273.0	0.0	2481.0
	Shaochedian	1806.2	12584.8	13151.2	23.4	1093.7	1698.3	3479.0	0.0	0.0
	Caiputai	23.2	1565.5	0.0	52.4	450.6	234.5	3066.9	477.2	0.0
	Duancun	2773.0	0.0	0.0	48.6	412.8	502.7	1167.1	6275.7	439.7
	Beiliuzhuang	0.0	1701.3	0.0	16.3	12015.3	3612.7	12675.3	12827.5	12351.1
	Wangjiazhai	0.0	769.8	6626.4	97.4	2427.4	1779.0	284.2	0.0	6878.5
Overlying Water	Zaolinzhuang	81.6	0.0	1698.6	46.3	0.0	2642.4	0.0	6988.6	3132.7
	Shaochedian	286.1	0.0	8996.5	31.2	5195.2	1080.2	1434.5	0.0	3129.4
	Caiputai	3788.6	3063.4	0.0	10.3	0.0	3793.4	5682.0	0.0	0.0
	Duancun	77.6	0.0	7027.9	52.2	389.2	61.4	9578.7	5261.4	11610.0
	Beiliuzhuang	201.0	4608.9	3807.0	86.4	10529.7	288.1	6669.5	6194.4	4871.7
	Wangjiazhai	296.6	5866.9	12628.4	94.3	1497.1	34.5	765.3	4591.2	744.2
Pore Water	Zaolinzhuang	311.6	2534.8	12588.8	43.3	619.8	2678.1	769.0	897.6	6176.5
	Shaochedian	0.0	2119.0	0.0	15.3	513.8	5729.0	2172.5	2962.2	0.0
	Wangjiazhai	70.7	2688.9	4239.1	16.3	1259.3	7057.5	8931.4	927.8	0.0
Water-release test	Zaolinzhuang	456.2	607.5	7562.3	72.3	847.2	1436.3	1882.5	0.0	847.4
	Shaochedian	238.7	77.2	0.0	38.0	716.0	2614.9	1787.9	490.9	3604.5
	Caiputai	0.0	0.0	0.8	46.3	15.9	165.6	116.0	406.6	484.5
	Duancun	44.1	2.4	0.7	95.2	92.1	55.2	201.1	774.3	580.8
	Beiliuzhuang	62.5	55.7	7.7	18.4	225.5	75.0	309.3	2698.1	671.5
	Wangjiazhai	46.8	6.8	0.0	45.2	115.9	81.8	232.9	782.4	533.4

Table 47 Concentration (ng/l) of pharmaceutical compounds found in BYD Lake sediment samples in November 2009

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from November are listed in Table 47 are displayed graphically in Figure 150 - 152.

The overall highest concentration observed during sample analysis in November from BYD lake water samples was 13151 ng/l for Meb at Shaochedian (surface water samples). Meb also had the overall highest concentrations observed. Seven out of nine compounds observed <LOD concentrations on at least one occasion exclusing Thio and Tamo. Surface water samples had a lowest concentration (excluding zero concentrations) of 4.7 ng/l for Thio at Zaolinzhuang. Thio was also the lowest observed

concentration (excluding <LOD) for overlying water samples and pore water samples for Caiputai (10.3 ng/l) and Shaochedian (13.3 ng/l) respectively. The lowest observed for water-release test samples excluding zero concentrations was for Meb with a concentration of 0.7 ng/l at Duancan, closely followed by 0.8 ng/l at Caiputai. Maximum concentrations for each sample group were as follows (excluding aforementioned highest overall observed); 12628 ng/l for Meb at Wangliazhai (overlying water samples), 12589 ng/l for Meb at Zaolinzhuang (pore water samples) and 7562 ng/l for Meb at Zaolinzhuang (water-release test samples). Only three sampling locations were collected for pore water samples as opposed to the regular six; Zaolinzhuang, Shaochedian and Wangjiazhai, for logistical and access reasons.

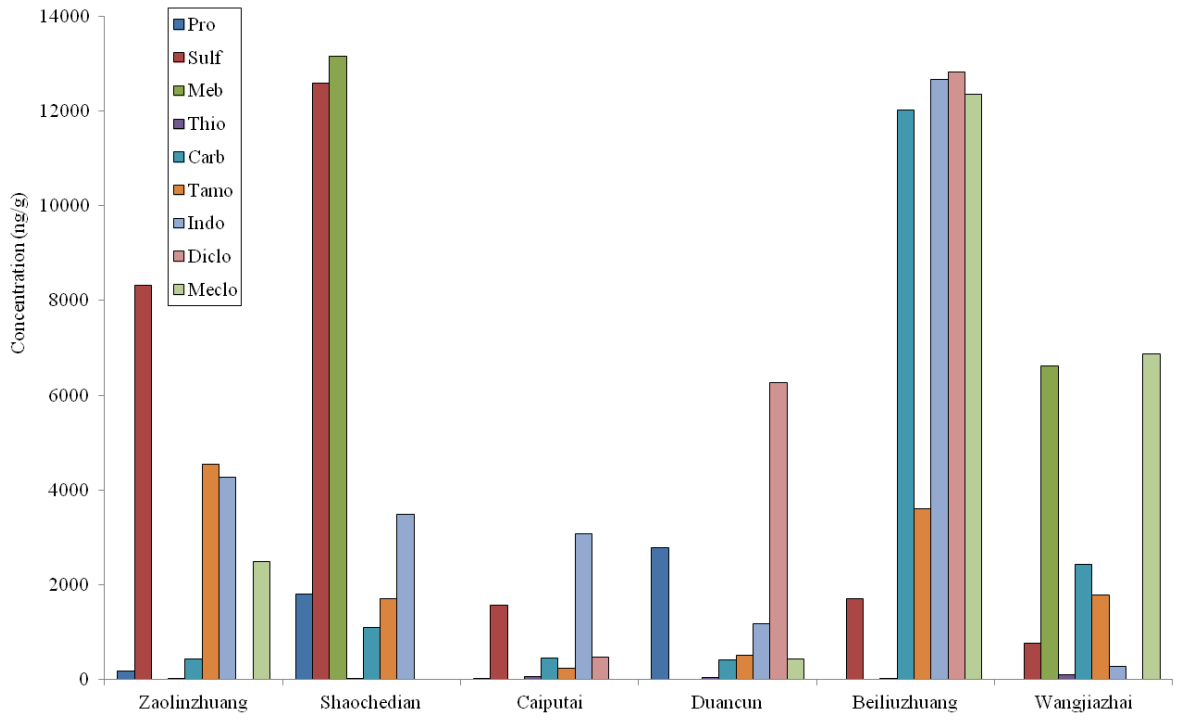

Figure 150 Concentration (ng/l) of target pharmaceutical compounds for November 2009 in Surface water samples for BYD Lake

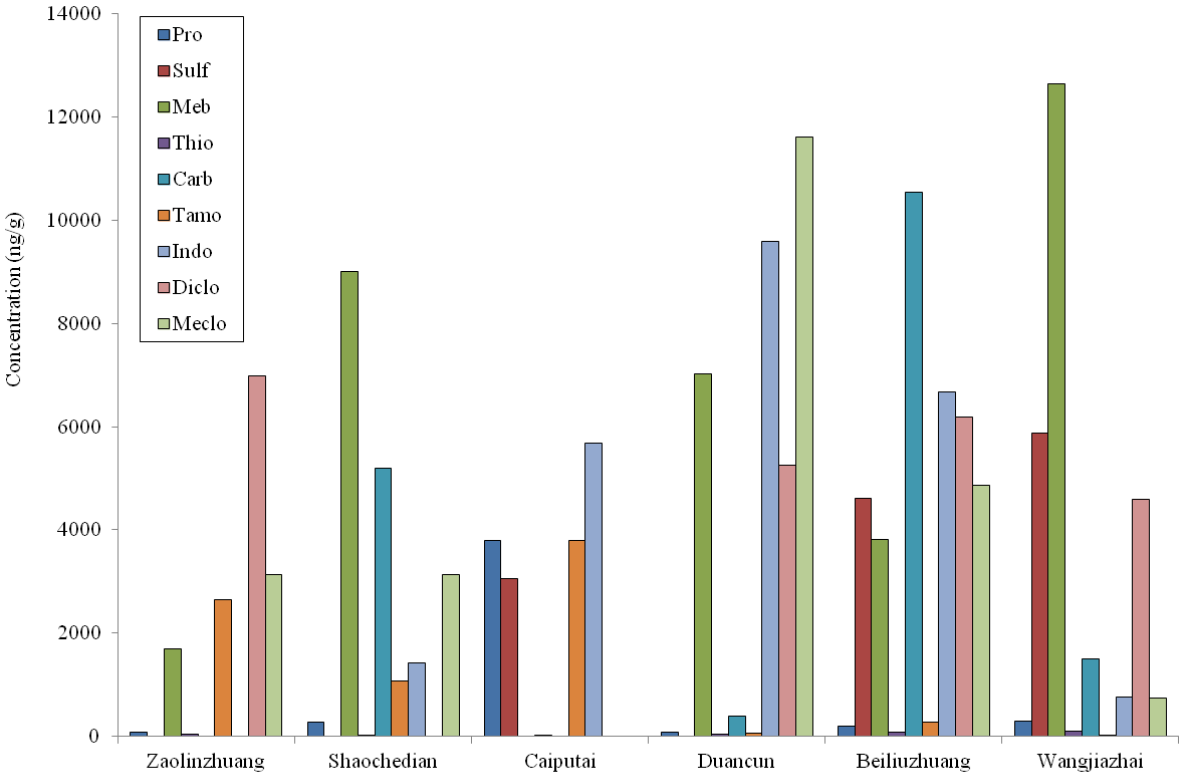

Figure 151 Concentration (ng/l) of target pharmaceutical compounds for November 2009 in Overlying water samples for BYD Lake

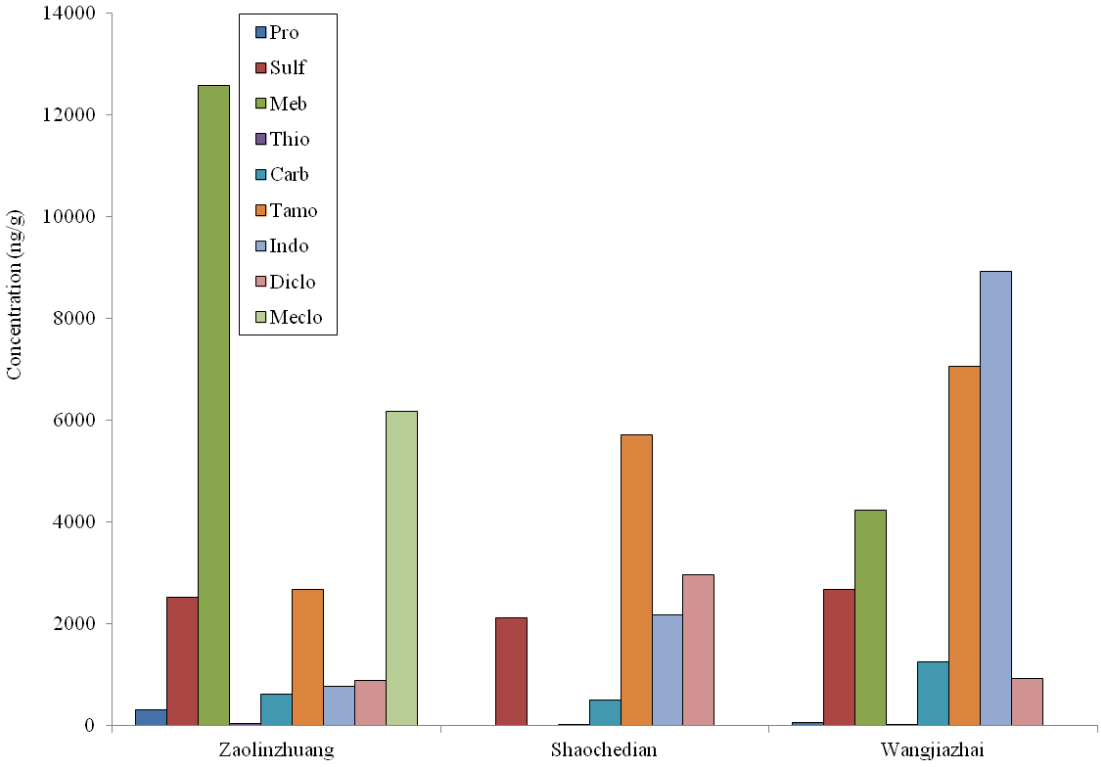

Figure 152 Concentration (ng/l) of target pharmaceutical compounds for November 2009 in Pore water samples for BYD Lake

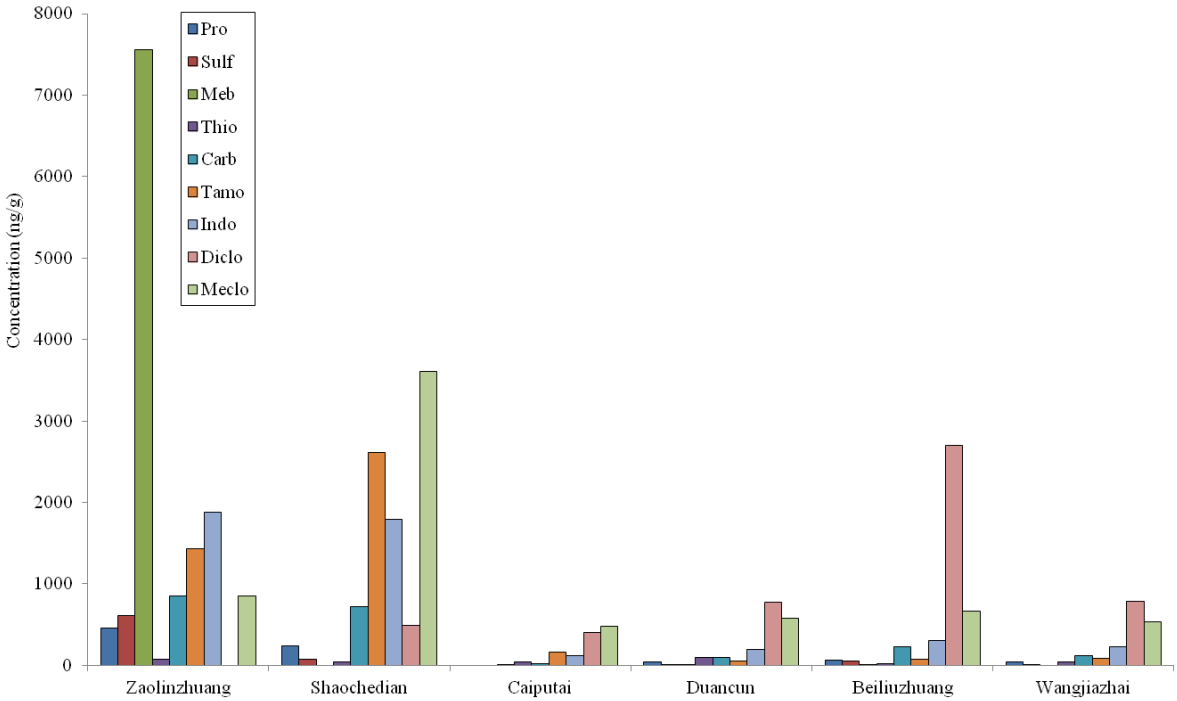

Figure 153 Concentration (ng/l) of target pharmaceutical compounds for November 2009 in water-release test samples for BYD Lake

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Zaolinzhuang	115.0	18.4	0.0	200.0	231.9	317.0	510.0	2983.3	1884.7
Duancun	232.2	102.2	5.0	326.2	882.3	430.7	1325.0	5443.5	3319.5
Beiliuzhuang	86.6	1.2	0.0	200.0	184.0	268.9	797.4	3102.7	2411.1

Table 48 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples in February 2010

All concentrations of target pharmaceutical compounds found in BYD Lake surface water samples from February are listed in Table 48. These are displayed graphically in Figure 154.

During February, a total of only three samples were collected for surface water samples only; Zaolinzhuang, Duancun and Beiliuzhuang. Out of these, there were two zero concentrations observed for Meb at the first and last location. The lowest observed concentration overall was 1.2 ng/l for Sulf at Beiliuzhuang. The highest overall observed concentration was 3319 ng/l for Meclo at Duancun.

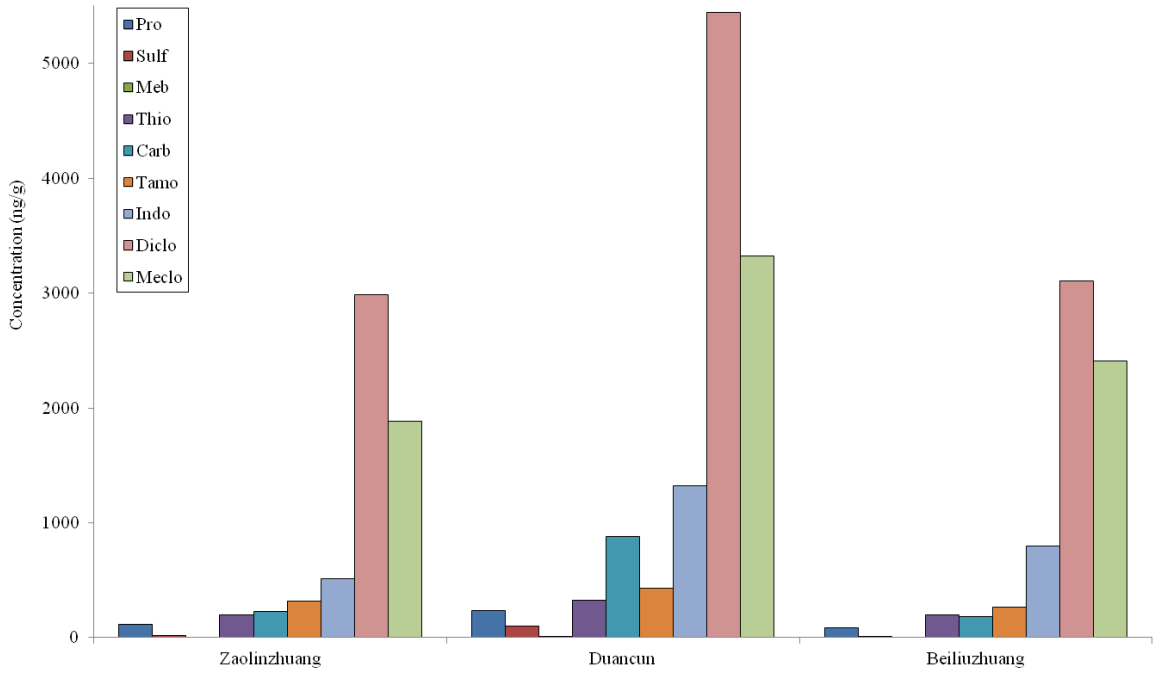

Figure 154 Concentration (ng/l) of target pharmaceutical compounds for February 2010 in Surface water samples for BYD Lake

5.3.3 Sediment samples from Beijing

5.3.3.1 Haihe River

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Daliangdukou	6.6	4.2	27.7	14.7	25.0	433.6	107.4	23.7	100.9
Xihe	10.5	27.6	177.6	12.2	20.1	452.2	114.4	44.0	8.0
Dengshangu	3.2	1.4	58.0	15.2	31.7	465.5	191.4	35.8	41.4
Jintanggaosu	5.2	4.5	3.4	8.2	20.4	482.9	176.5	29.5	26.6
Gegu	8.5	6.8	38.5	16.2	27.2	466.3	174.4	61.2	31.9
Tianjinzhan	12.7	1.0	25.2	10.2	34.2	547.2	169.7	61.9	45.7
Haijin Bridge	6.4	5.0	81.3	13.3	51.0	414.8	168.4	71.8	9.9
Zhangguizhuang	16.0	6.5	75.8	8.1	40.6	548.3	162.1	53.1	10.9
Dongyangchang	16.1	7.7	8.8	6.0	69.0	497.1	180.2	48.8	22.5
Fengkoucun	17.2	6.5	102.8	10.2	55.6	383.7	172.1	46.1	21.5
Niwocun	10.0	10.9	2.2	15.2	48.9	320.5	162.0	38.1	51.6
Majiacaiyuan	15.0	5.5	59.1	16.2	27.0	434.8	151.4	0.0	629.2
Caizhuangzi	13.9	3.3	81.4	12.0	31.8	341.2	95.9	3.1	5.2
Chaqian	12.1	2.5	59.4	12.3	27.0	420.9	128.3	62.0	79.2
Beicha	11.1	3.2	60.6	7.2	27.6	406.8	107.4	30.6	56.8
Nancha	13.8	7.5	155.5	14.2	56.5	387.8	108.2	13.7	27.0
Pangu	20.3	7.6	76.2	10.2	41.1	322.5	65.1	32.7	45.4
Dagu1	31.3	0.4	77.1	16.2	61.7	236.0	118.7	25.5	31.2
Dagu2	20.0	3.7	129.6	15.0	58.9	349.8	185.5	18.9	101.6
Dagu3	15.2	21.4	48.9	13.3	60.3	291.4	105.4	54.8	114.8
Haihe Bridge	32.0	4.0	155.6	9.3	53.6	267.0	150.4	39.8	0.0

Table 49 Concentration (ng/g) of pharmaceutical compounds found in Haihe River sediment samples in June 2009

During June 2009, 21 sample locations were analysed for the concentration of 9 target pharmaceutical compounds in sediment samples from Haihe River. These data are found in Table 49. Following data analysis, these were presented graphically in Figure 155 - 156. Due to the nature of these data for Tamo and Meclo, graphs were made giving a more normalized representation in Figure 157 - 159.

The maximum overall concentration was observed for Meclo at Majiacaiyuan with a concentration of 629 ng/g. There were two zero concentrations observed; one for Diclo

and the other for Meclo. Disregarding these, the lowest overall observed concentration was for Sulf at Dagu1 with a concentration of only 0.4 ng/g. Both Sulf and Thio had low overall concentrations of only 0.4 ng/g to 27.6 ng/g and 6.0 ng/g and 16.2 ng/g respectively. Remaining sample range concentrations were as follows; 3.2 ng/g to 32.0 ng/g for Pro, 2.2 ng/g to 177.6 ng/g for Meb, 20.1 ng/g to 69.0 ng/g for Carb, 236.0 ng/g to 548.3 ng/g for Tamo, 65.1 ng/g to 191.4 ng/g for Indo, 3.1 ng/g to 71.8 ng/g (excluding 0.0 ng/g concentration) for Diclo and 5.2 ng/g to 629.2 ng/g for Meclo (again, excluding <LOD).

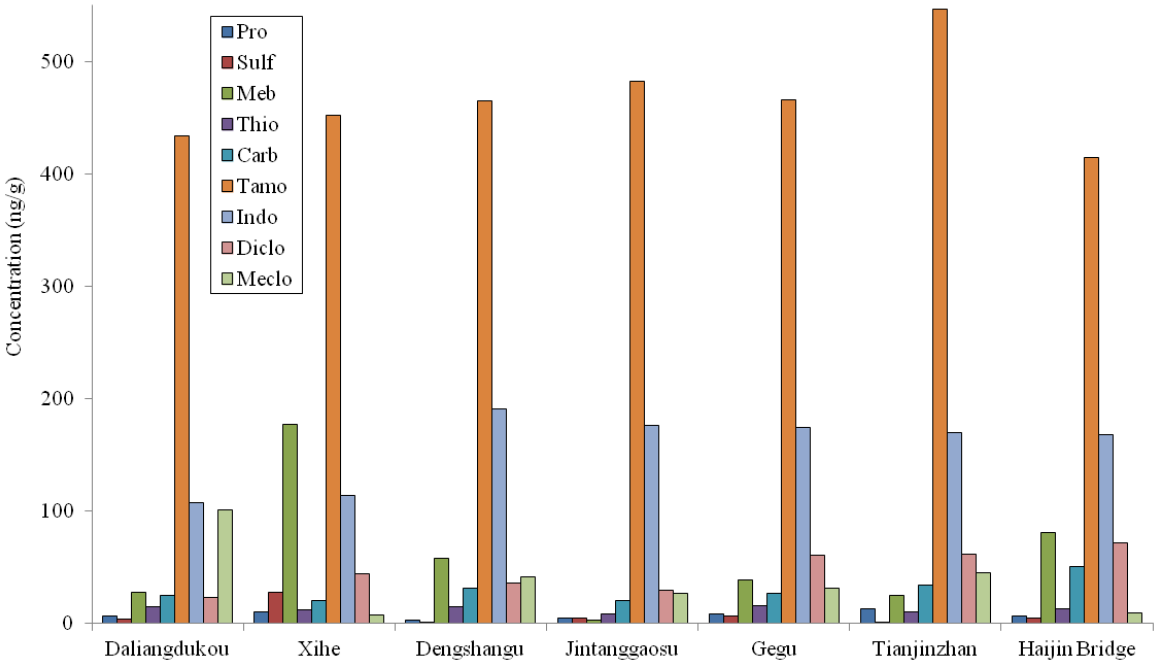

Figure 155 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the first seven sites in June 2009

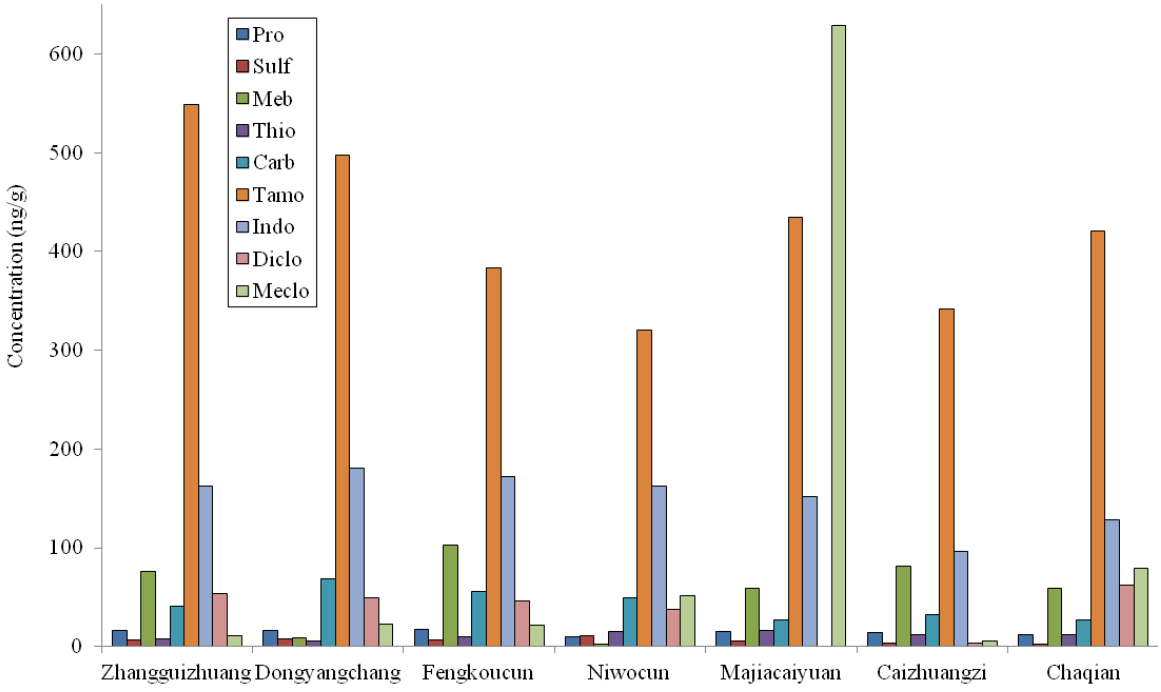

Figure 156 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the next seven sites in June 2009

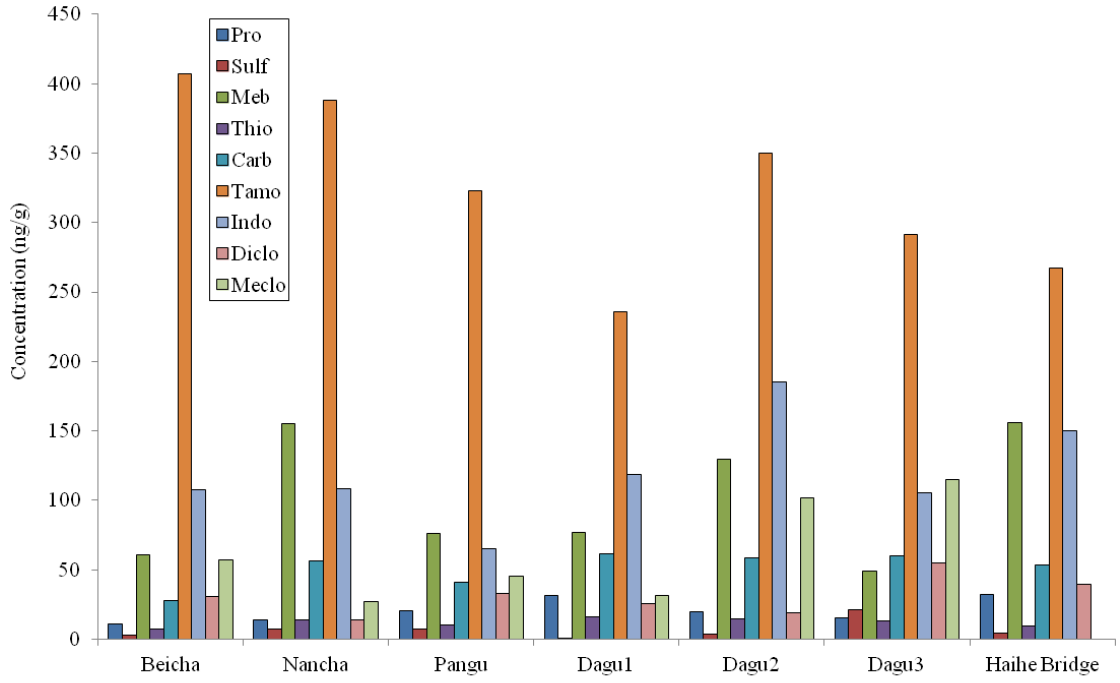

Figure 157 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the last seven sites in June 2009

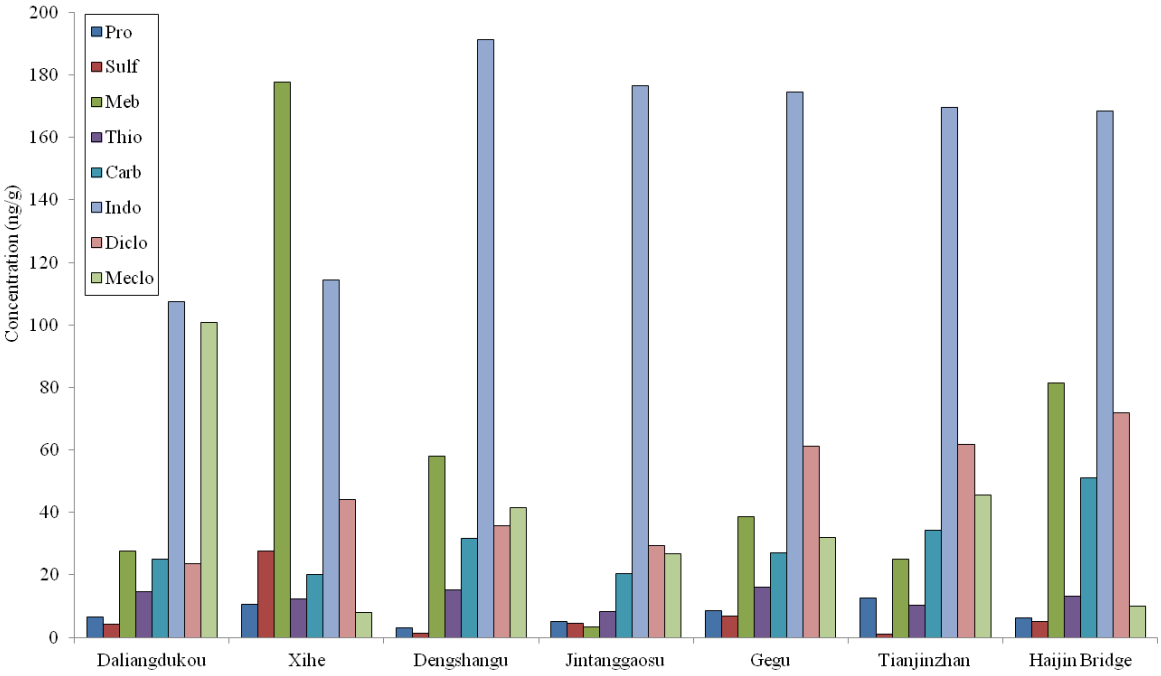

Figure 158 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the first seven sites in June 2009 (normalized results)

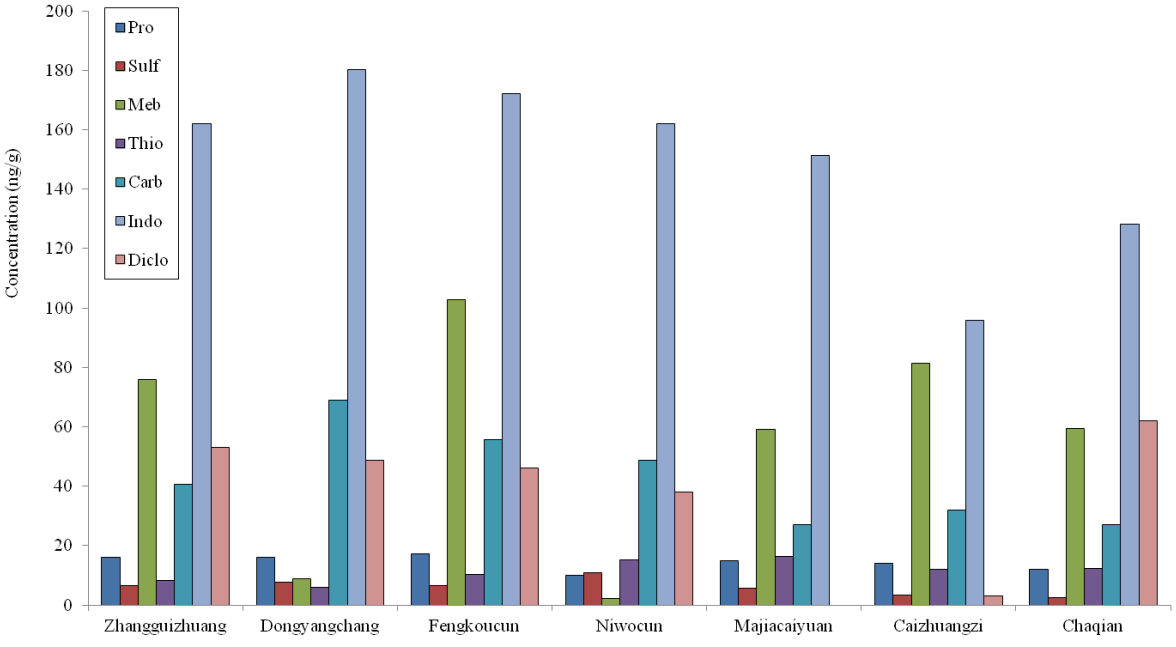

Figure 159 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the next seven sites in June 2009 (normalized results)

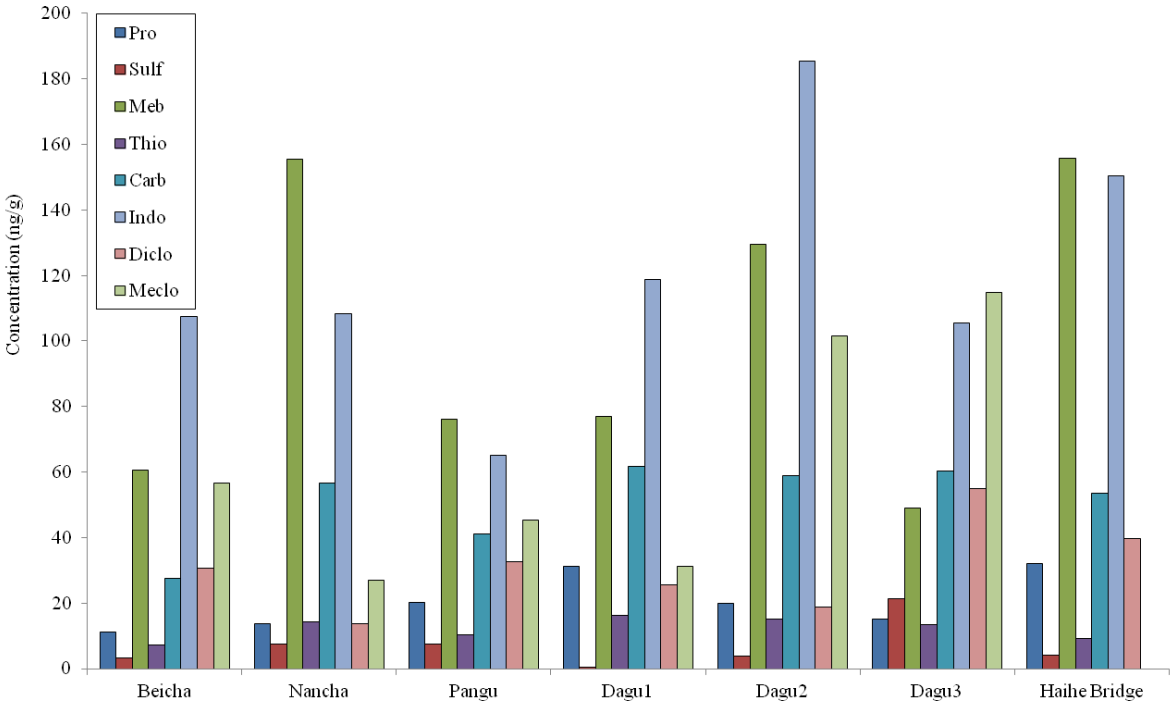

Figure 160 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the last seven sites in June 2009 (normalized results)

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Daliangdukou	55.3	3.1	68.7	10.3	71.9	341.0	93.9	75.2	0.0
Xihe	28.9	3.3	1276.9	14.2	34.6	234.9	82.3	27.8	0.0
Dengshangu	35.6	68.5	22.6	5.3	94.9	413.2	218.1	21.3	41.1
Jintanggaosu	84.2	0.0	39.4	6.2	130.0	483.0	173.1	183.1	225.5
Gegu	27.0	1.4	288.8	18.2	140.3	430.6	265.9	181.4	48.4
Tianjinzhan	49.8	1.9	7.3	13.3	55.5	291.7	92.3	102.2	29.2
Haijin Bridge	46.4	0.0	842.1	4.2	75.6	332.1	245.2	0.0	7.0
Zhangguizhuang	38.7	5.4	163.0	23.3	61.6	262.8	176.6	38.5	0.0
Dongyangchang	67.5	12.8	277.7	34.3	51.9	396.3	237.0	91.2	27.3
Fengkoucun	83.1	10.6	363.7	6.2	131.9	260.0	94.4	151.7	219.0
Niwocun	23.0	0.0	1975.0	4.2	97.2	270.3	122.4	0.0	2.2
Majiacaiyuan	109.9	3.8	1279.8	11.0	218.9	667.9	253.8	0.0	41.6
Caizhuangzi	56.0	0.0	3594.7	10.2	108.2	663.2	199.1	0.0	291.3
Chaqian	11.8	40.1	7.8	7.2	73.0	221.1	111.6	8.4	2.6
Beicha	77.7	20.8	370.4	13.2	178.0	387.9	266.6	12.2	0.0
Nancha	6.3	0.0	386.8	39.3	54.8	469.6	145.1	55.8	16.6
Pangu	19.3	570.8	60.1	12.4	86.0	268.5	96.4	62.9	497.8
Dagu1	16.0	0.0	29.6	41.2	69.0	382.9	425.9	102.0	0.0
Dagu2	20.0	0.0	305.7	12.4	139.5	578.7	379.4	919.0	0.0
Dagu3	15.7	85.7	11.9	4.3	76.6	396.0	176.7	141.0	57.6
Haihe Bridge	80.1	0.8	74.8	6.2	72.1	380.6	237.1	39.1	126.8

Table 50 Concentration (ng/g) of pharmaceutical compounds found in Haihe River sediment samples in November 2009

Table 50 details the concentration of pharmaceutical compounds found in sediment samples from the Haihe River in November 2009. Figure 160, figure 161 and figure 162 graphically show these data from Table 50. Due to the high concentration nature of Meb and Tamo, these were removed for normalisation purposes of results shown graphically, in Figure 163 – 165. Meb and Tamo have the highest overall concentrations observed, with a range of 7.3 ng/g to 3595 ng/g and 221.1 ng/g to 667.9 ng/g respectively. Meb has the highest overall observed value of 3595 ng/g at Caizhuangzi. A zero concentration was observed on 16 occasions for compounds Sulf, Diclo and Meclo. Aside from these, the lowest recorded concentration was observed at the sample location Haihe Bridge for Sulf of 0.8 ng/g.

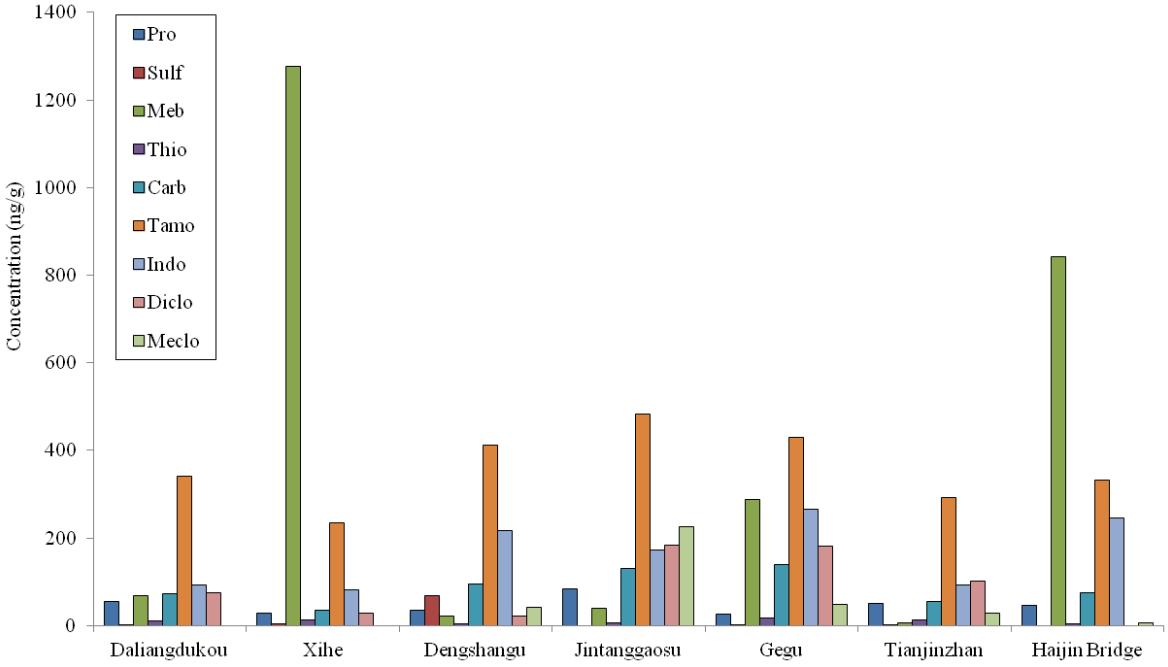

Figure 161 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the first seven sites in November 2009

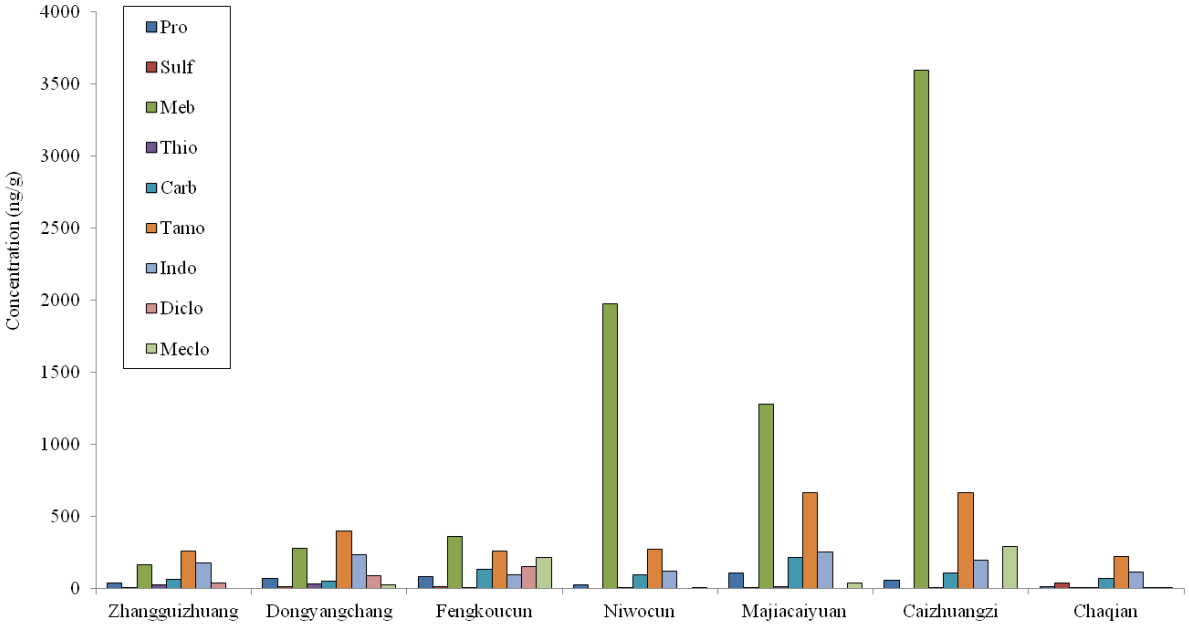

Figure 162 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the next seven sites in November 2009

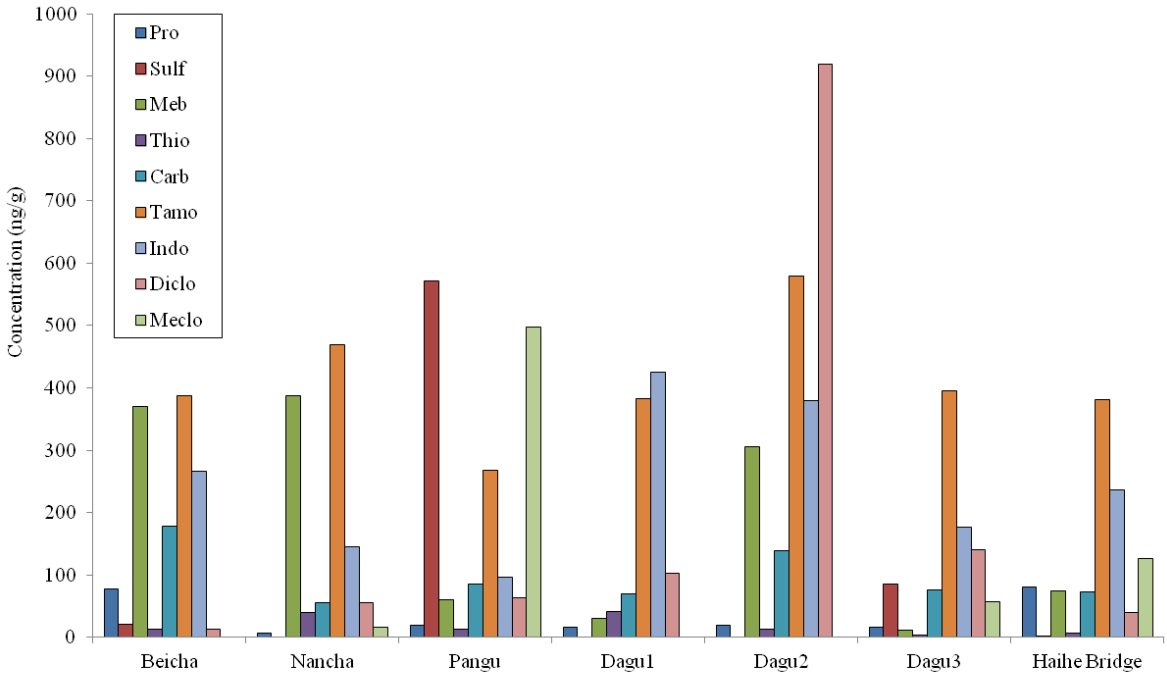

Figure 163 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the last seven sites in November 2009

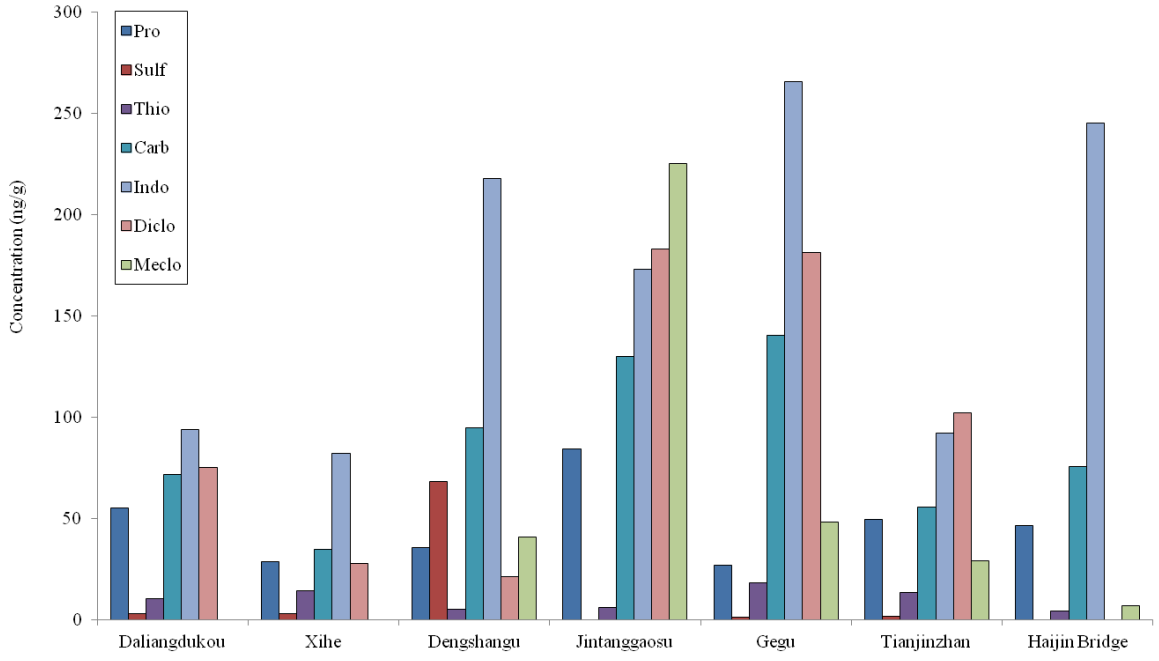

Figure 164 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the first seven sites in November 2009 (normalized results)

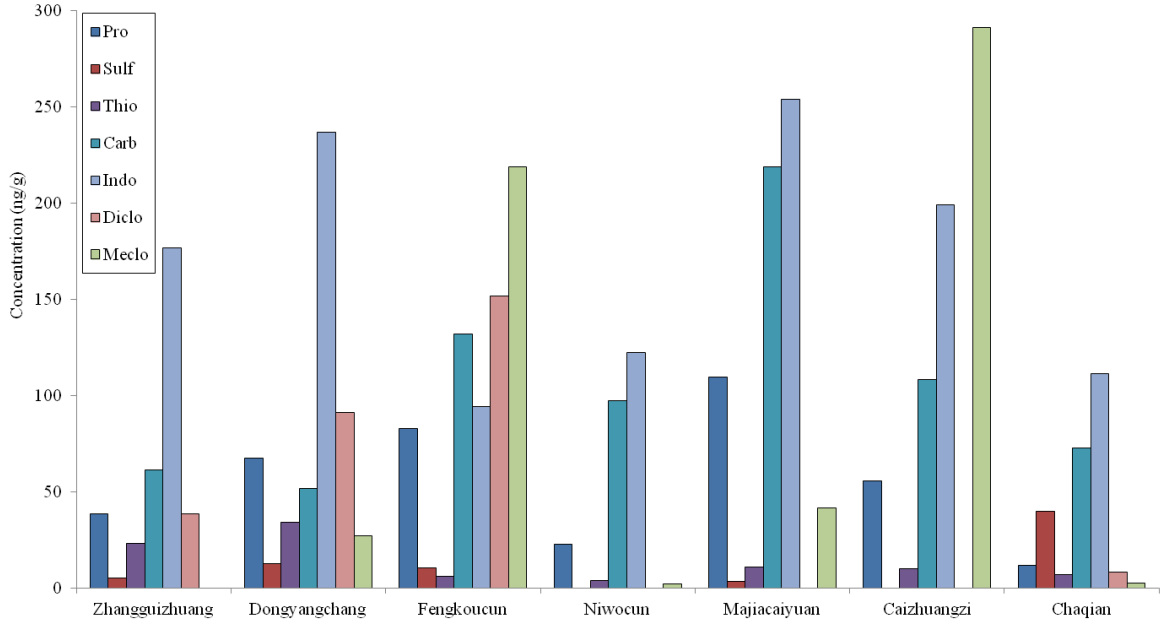

Figure 165 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the next seven sites in November 2009 (normalized results)

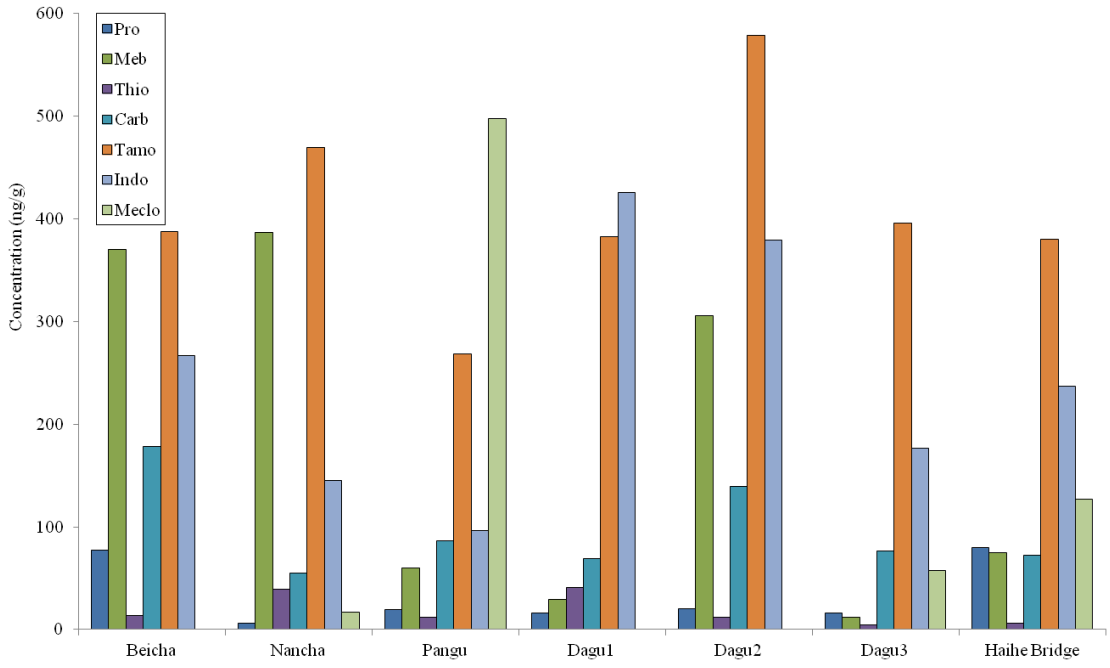

Figure 166 Concentration (ng/g) of target pharmaceutical compounds from Haihe river sediment samples for the last seven sites in November 2009 (normalized results)

5.3.3.2 Bohai Bay

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Shagouzi	37.7	5.9	139.7	4.3	135.6	245.5	99.3	68.5	39.1
Huangligang	92.9	9.5	42.6	5.2	92.7	222.3	104.9	72.6	58.7
Xujiabao	64.7	8.9	113.3	9.2	49.0	255.9	216.2	35.6	244.6
Nanpaihe	41.2	2.3	139.7	7.2	52.6	262.5	98.1	19.7	52.1
Ziyaxinhe	82.3	28.5	111.3	10.2	78.0	324.1	153.2	32.9	17.1
Duliujianhe	82.2	10.4	11.7	15.2	58.5	297.3	350.6	35.0	118.6
Lvjugou	46.9	30.1	529.3	13.2	53.3	284.9	118.2	1.1	8.2
Tianjinlingang	72.8	41.9	86.3	16.2	116.1	423.5	224.5	0.0	19.1
Tianjinlgang	52.8	7.7	121.4	18.2	66.6	224.5	98.5	17.7	15.1
Tianjin Harbor	66.2	18.0	35.2	21.2	74.6	317.1	144.8	0.0	25.7
Do ngjianggang	126.4	0.0	78.0	20.5	109.8	417.0	77.1	2.7	62.4
Guanlanlu	39.7	0.0	95.9	4.3	77.4	383.3	60.9	39.7	9.6
Luanhekou	48.9	8.2	79.0	5.2	155.4	257.7	90.8	4.4	0.0
Letinggongyeyuan	32.7	84.4	49.2	6.2	89.0	314.2	43.4	23.2	19.2
Xihekou	15.1	0.0	75.0	4.2	65.2	288.7	215.5	94.1	94.5
Caofeidian	15.5	16.5	24.3	11.2	82.4	286.0	110.7	14.9	37.3
Heiyanzi	67.2	5.4	5.8	17.2	81.3	271.4	123.0	28.7	4.0
Beitangkou	31.6	0.0	70.8	13.2	76.7	480.6	147.8	41.6	326.5

Table 51 Concentration (ng/g) of pharmaceutical compounds found in Bohai Bay sediment samples in June 2009

All eighteen sample locations from Bohai Bay analysed for sediment samples in June 2009 are detailed in Table 51. These are displayed graphically in Figure 167 - 168.

Tamo and Indo were removed for

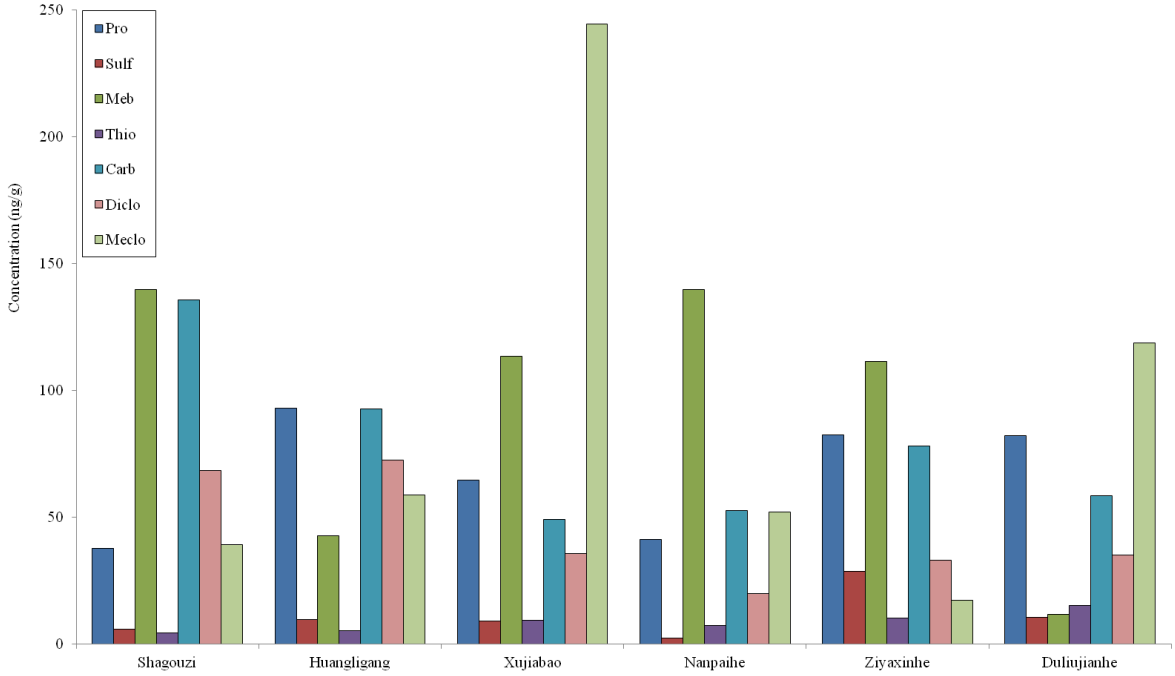

Figure 170 – 171 for the normalisation of results. The maximum observed concentration was observed for Meb at Lvjugou with 529.3 ng/g. Zero values were identified for three compounds; Sulf, Diclo and Meclo on five occasions. Other than these, the lowest observed concentration identified was 1.1 ng/l for Diclo at Lvjugou. Thio gave the lowest overall concentrations with a range between 4.2 ng/g and 21.2 ng/g, only a difference of 17.0 ng/g. The highest overall range observed was for Meb of 523.5 ng/g, with concentrations ranging between 5.8 ng/g and 529.3 ng/g.

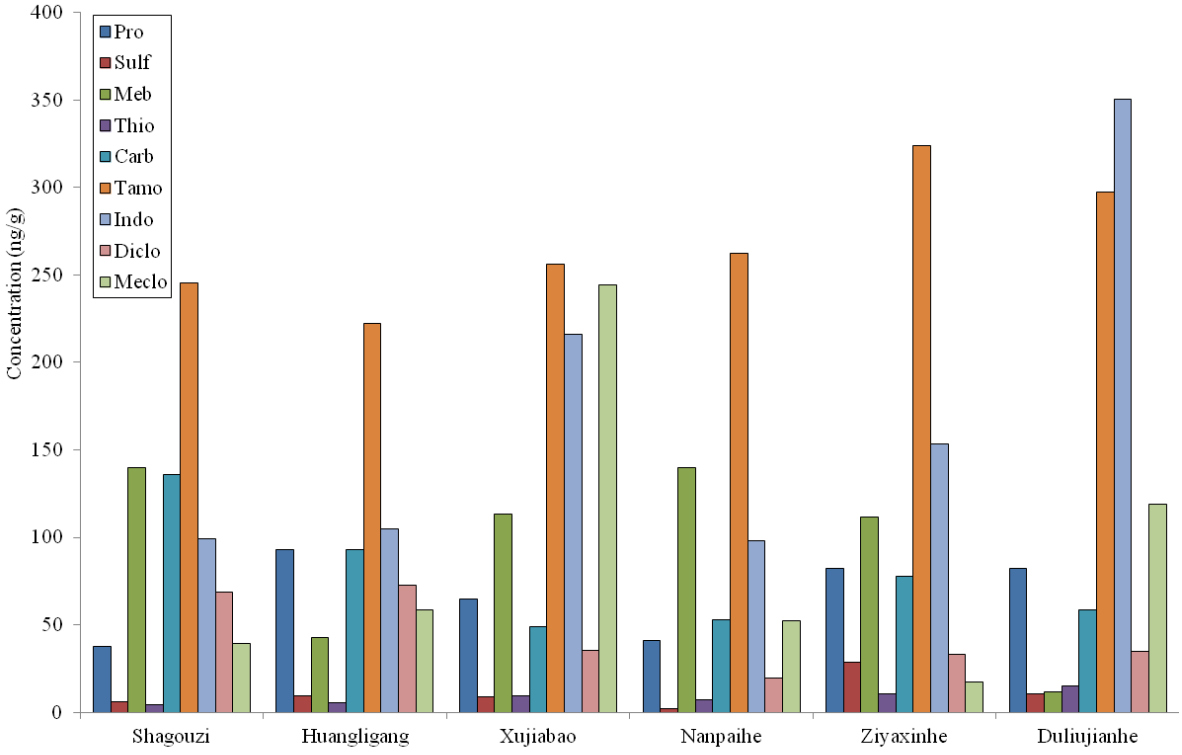

Figure 167 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the first six sites in June 2009

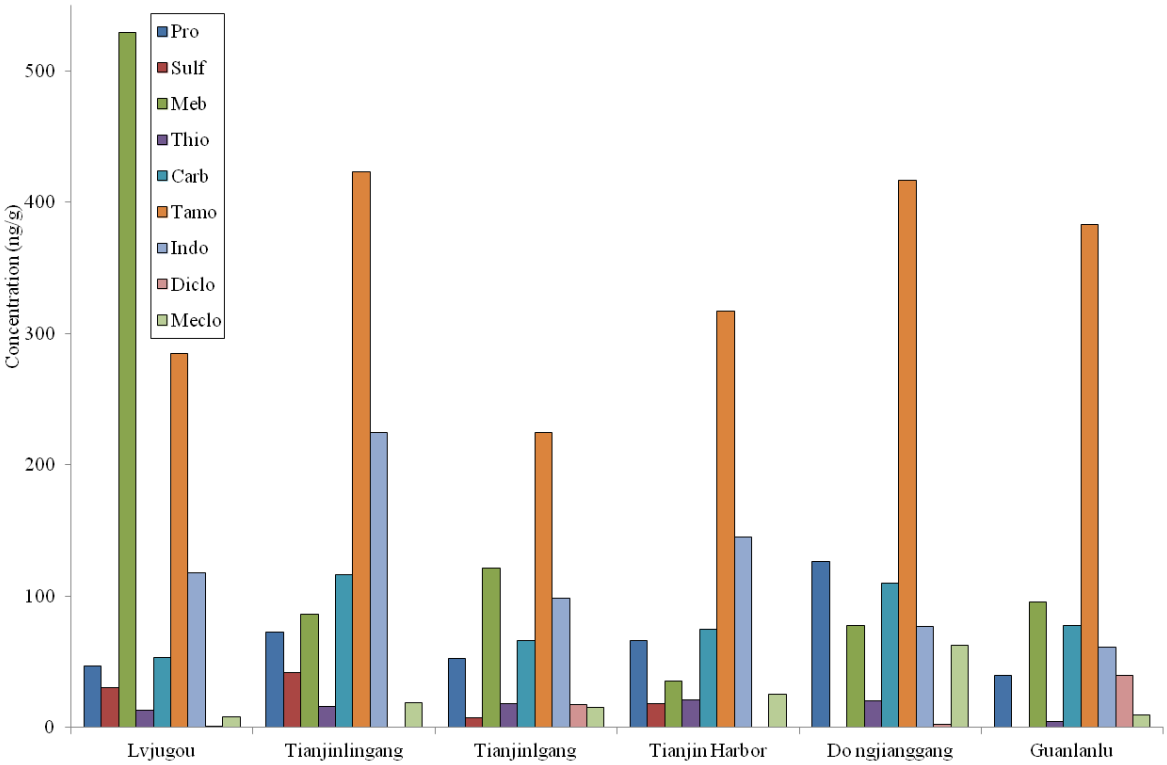

Figure 168 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the next six sites in June 2009

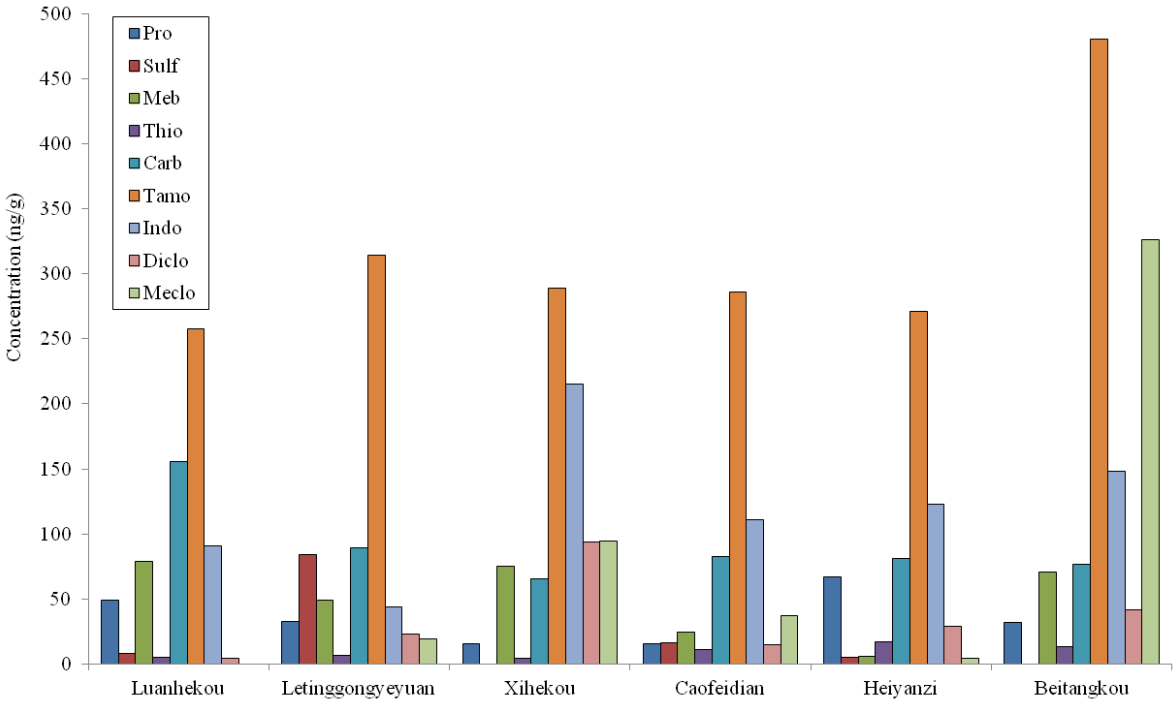

Figure 169 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the last six sites in June 2009

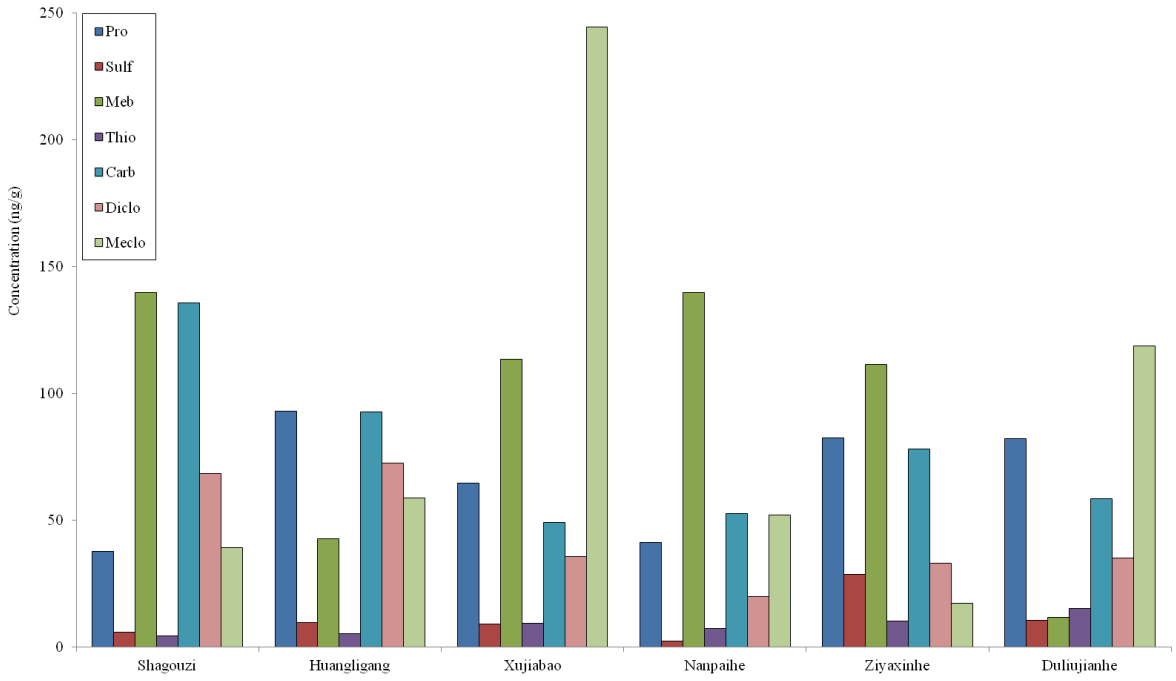

Figure 170 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the first six sites in June 2009 (normalized results)

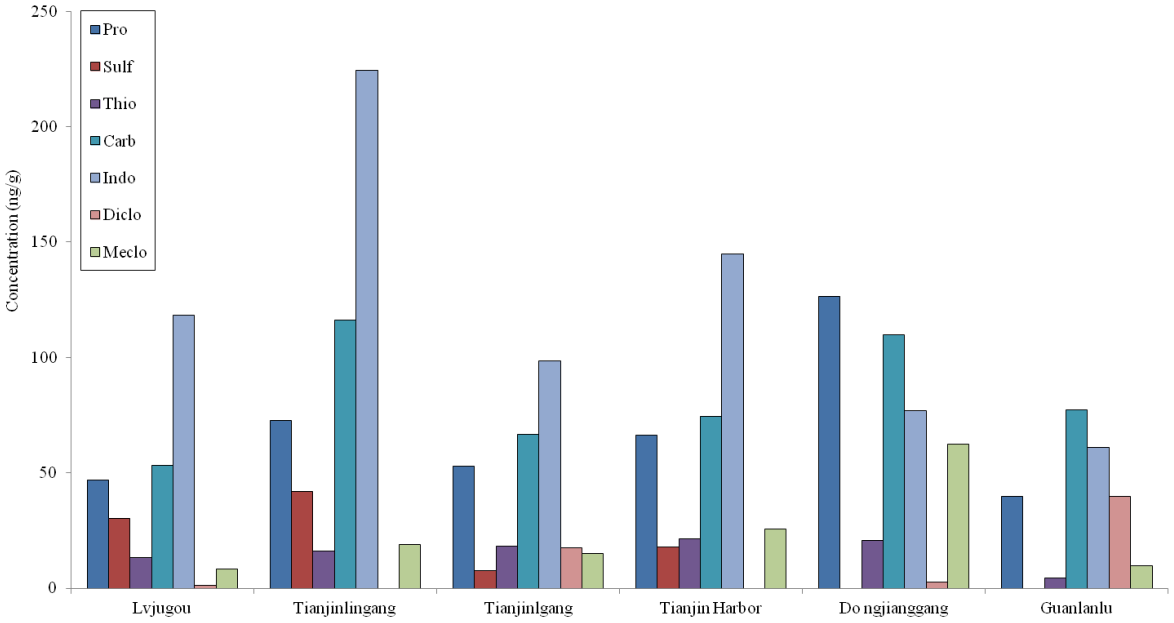

Figure 171 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the next six sites in June 2009 (normalized results)

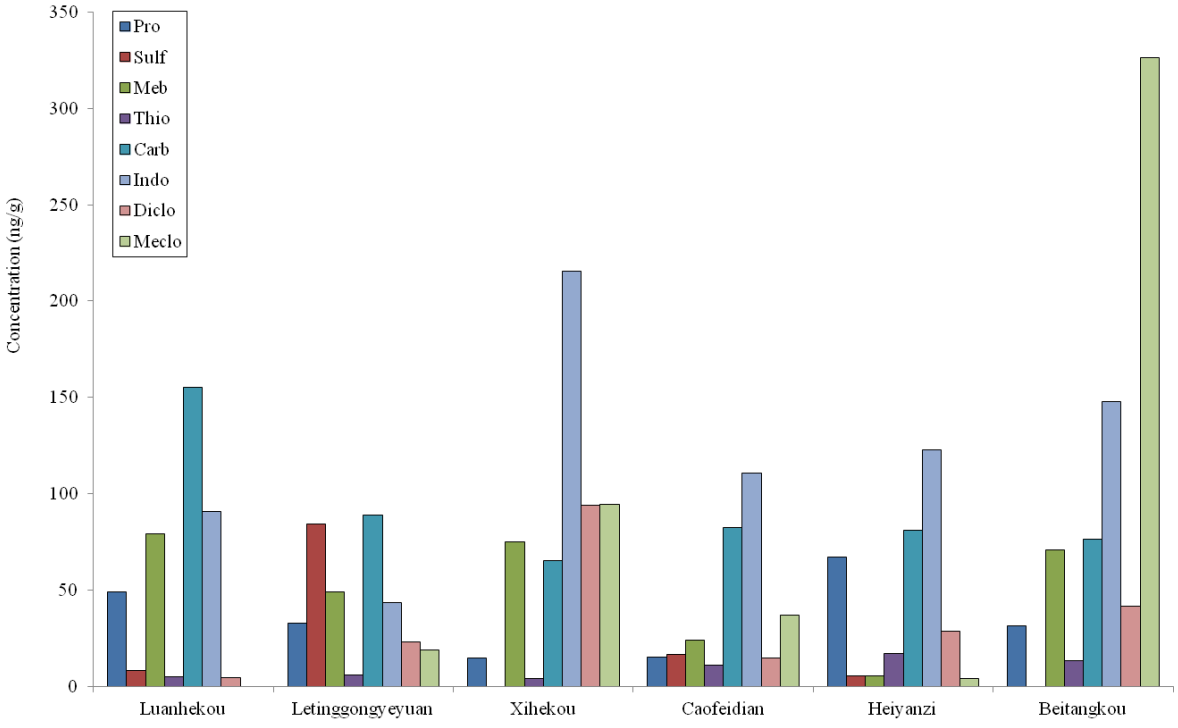

Figure 172 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the last six sites in June 2009 (normalized results)

	Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
Shagouzi	33.5	30.5	47.9	8.4	61.3	359.3	130.2	155.4	365.8
Huangligang	27.1	1.2	79.5	13.1	92.0	276.4	152.0	381.5	27.6
Xujiabao	106.9	8.9	311.9	15.2	137.2	340.5	108.2	0.0	0.0
Nanpaihe	74.0	65.7	76.5	21.2	81.1	301.6	176.9	10.4	49.6
Ziyaxinhe	42.5	58.5	1275.0	3.2	150.6	412.4	189.5	19.5	11.5
Duliujianhe	97.9	189.4	742.8	16.2	128.5	412.6	342.5	0.0	37.9
Lvjugou	51.8	730.0	550.7	53.2	176.2	363.6	225.7	7.1	78.8
Tianjinlingang	76.7	1.7	7.9	8.2	179.1	370.9	102.2	0.0	38.3
Tianjinlgang	180.5	30.8	0.0	9.2	378.2	258.7	387.7	1069.1	797.2
Tianjin Harbor	195.3	12.6	248.7	16.3	138.5	300.1	361.8	253.2	116.8
Do ngjianggang	102.8	3.6	351.6	12.3	101.8	191.0	157.4	6.9	29.8
Guanlanlu	46.4	42.2	318.9	13.2	79.5	273.5	120.3	50.4	0.0
Luanhekou	72.4	0.0	51.8	10.2	114.9	212.2	89.5	77.1	8.5
Letinggongyeyuan	85.6	7.6	1959.9	7.5	138.0	349.4	106.9	15.6	0.0
Xihekou	110.3	154.3	650.0	22.2	215.3	544.6	191.5	212.3	28.0
Caofeidian	76.9	17.9	4647.7	21.4	124.8	285.5	143.9	95.9	76.1
Heiyanzi	49.2	27.8	44.4	8.5	122.6	247.4	72.1	5.2	30.7
Beitangkou	93.2	3.0	535.1	5.2	80.3	522.2	185.9	77.7	33.9

Table 52 Concentration (ng/g) of pharmaceutical compounds found in Bohai Bay sediment samples in November 2009

Sediment samples taken from Bohai Bay in November are listed in Table 52 and displayed in Figure 173 - 174. The compound with concentrations of the highest overall range was Meb, with a range from <LOD to 1960 ng/g. The compound with the lowest range was Thio with only 50.0 ng/g, with the minimum and maximum values of 3.2 ng/g and 53.2 ng/g. The highest observed concentration for Meb was also the highest overall for this data set and was found at the location Latinggongyeyuang. <LOD were found for the compounds Sulf, Meb, Diclo and Meclo for a total of seven occasions. Other than these, the lowest observed concentration was for Suld at Huangligang with 1.2 ng/g. Normalised results are given in Figures 175 – 177.

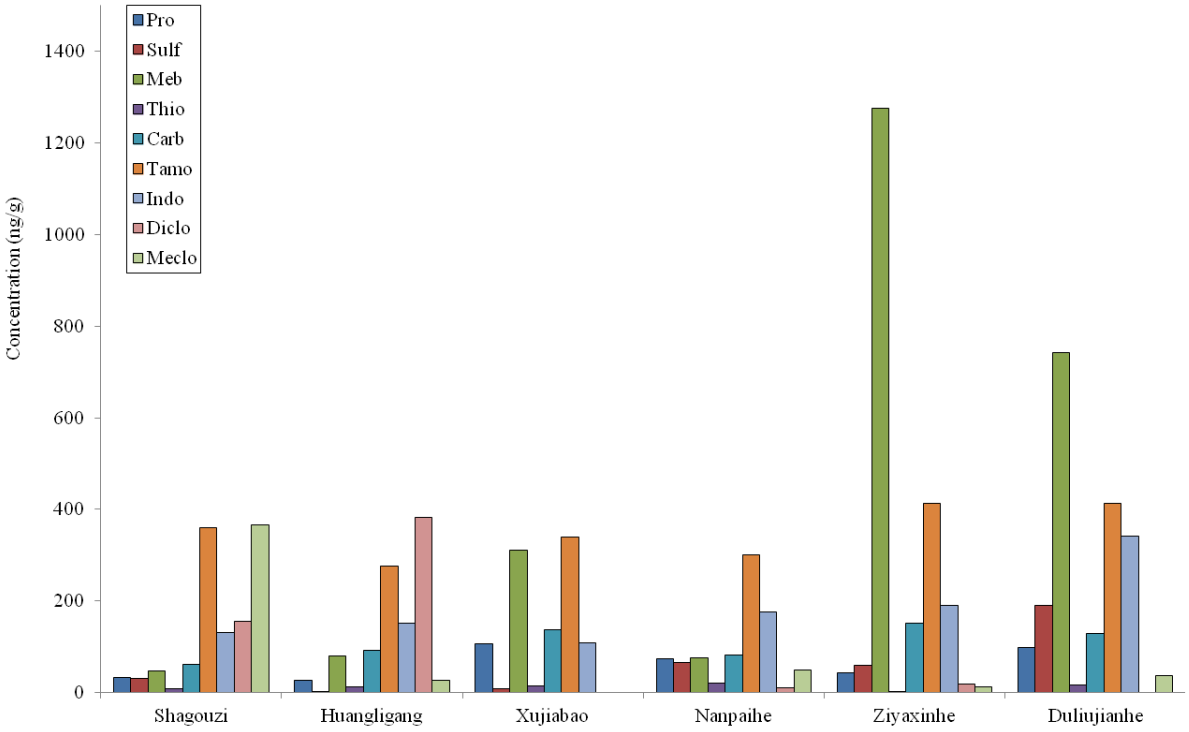

Figure 173 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the first six sites in November 2009

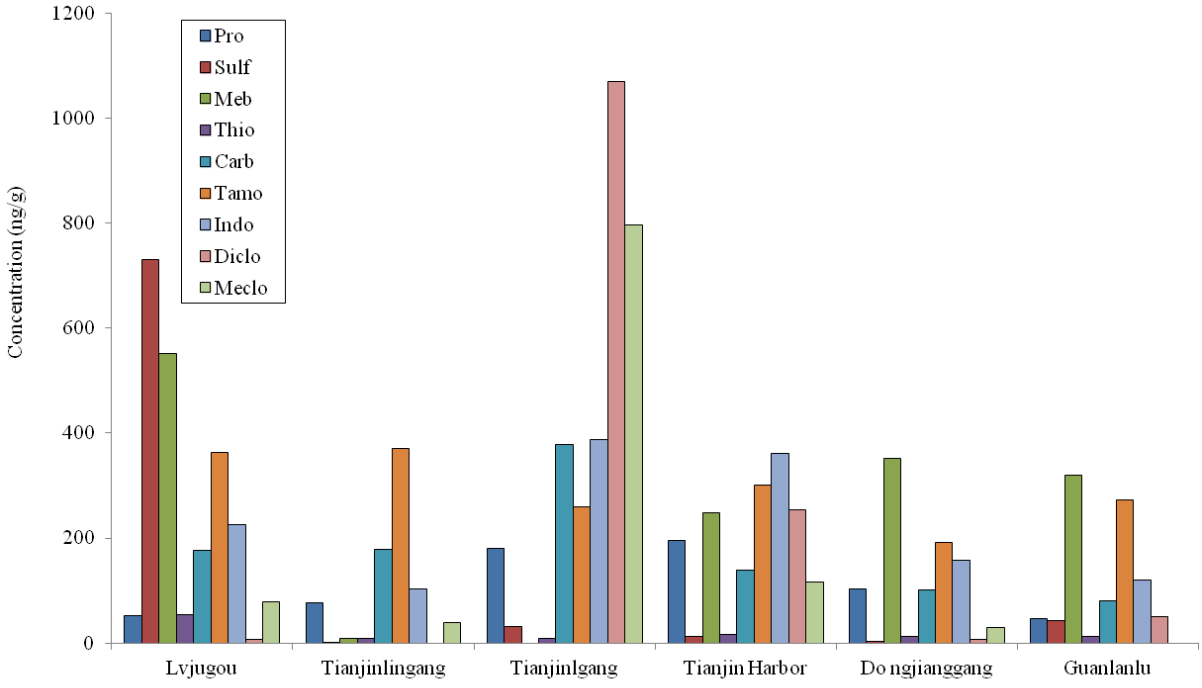

Figure 174 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the next six sites in November 2009

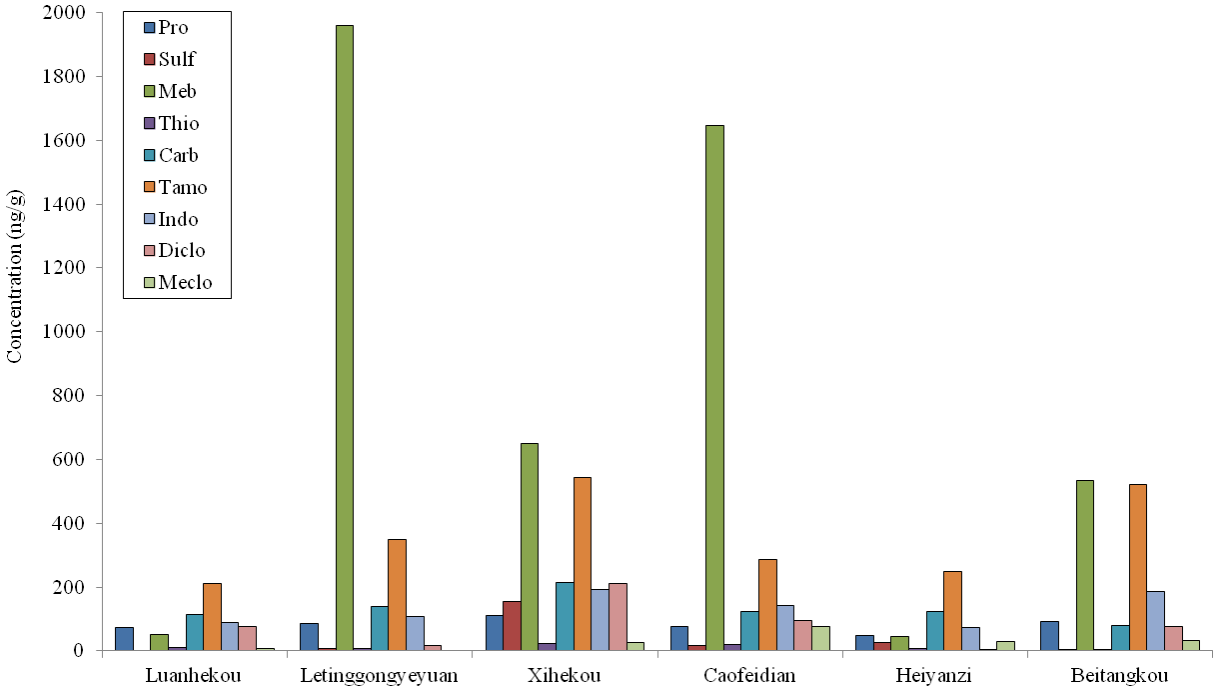

Figure 175 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the last six sites in November 2009

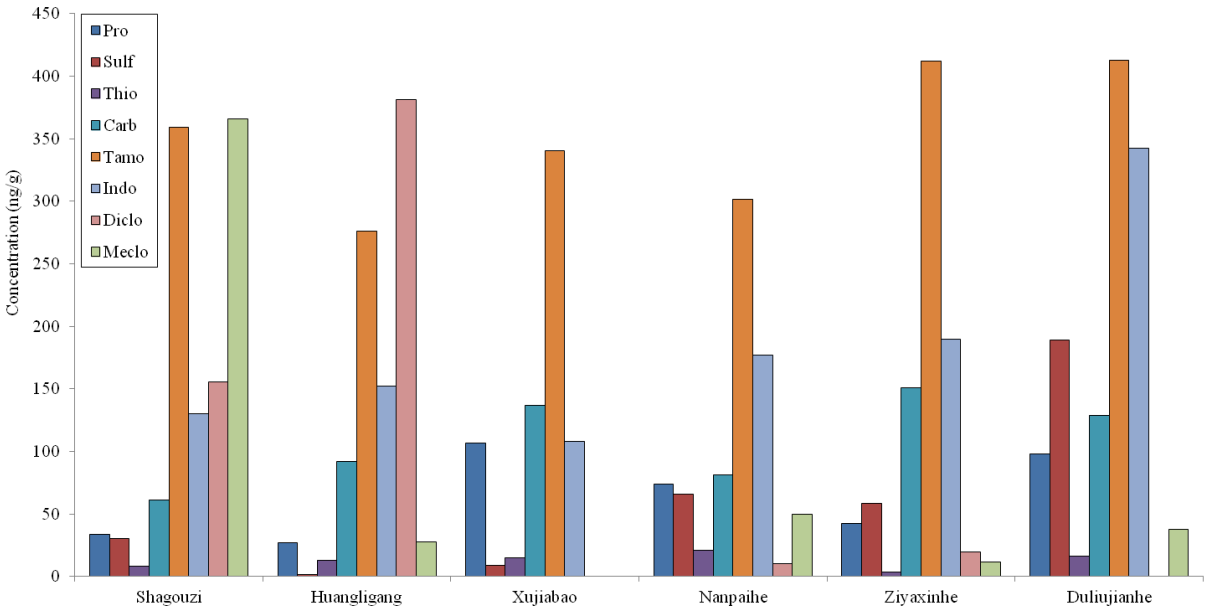

Figure 176 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the first six sites in November 2009 (normalized results)

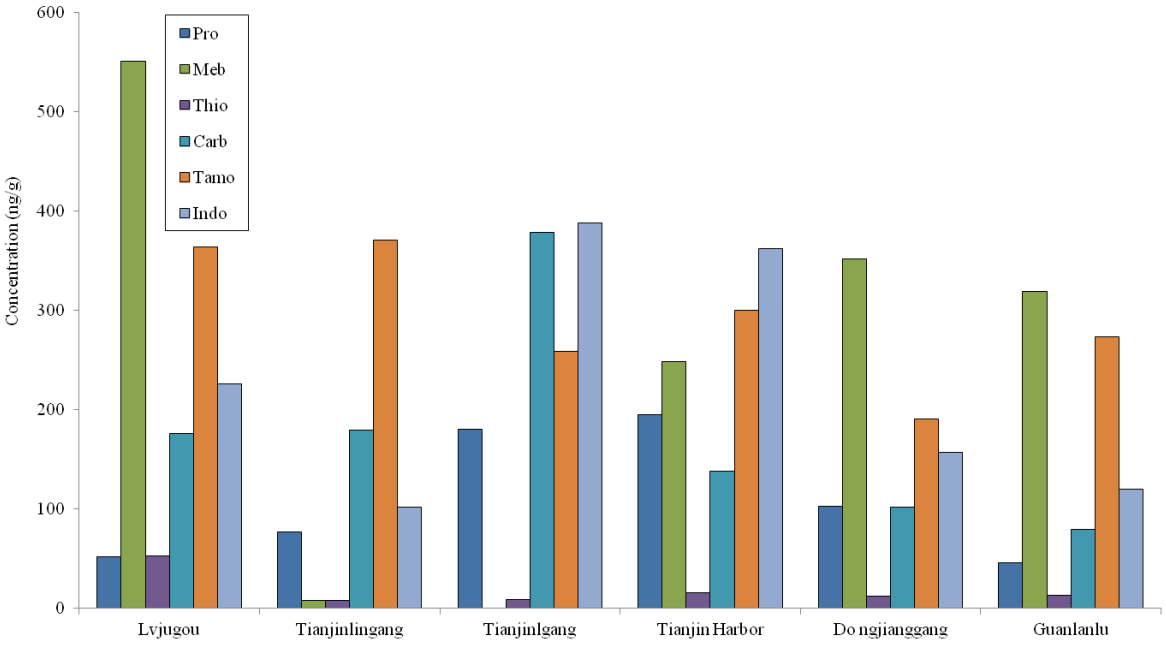

Figure 177 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the next six sites in November 2009 (normalized results)

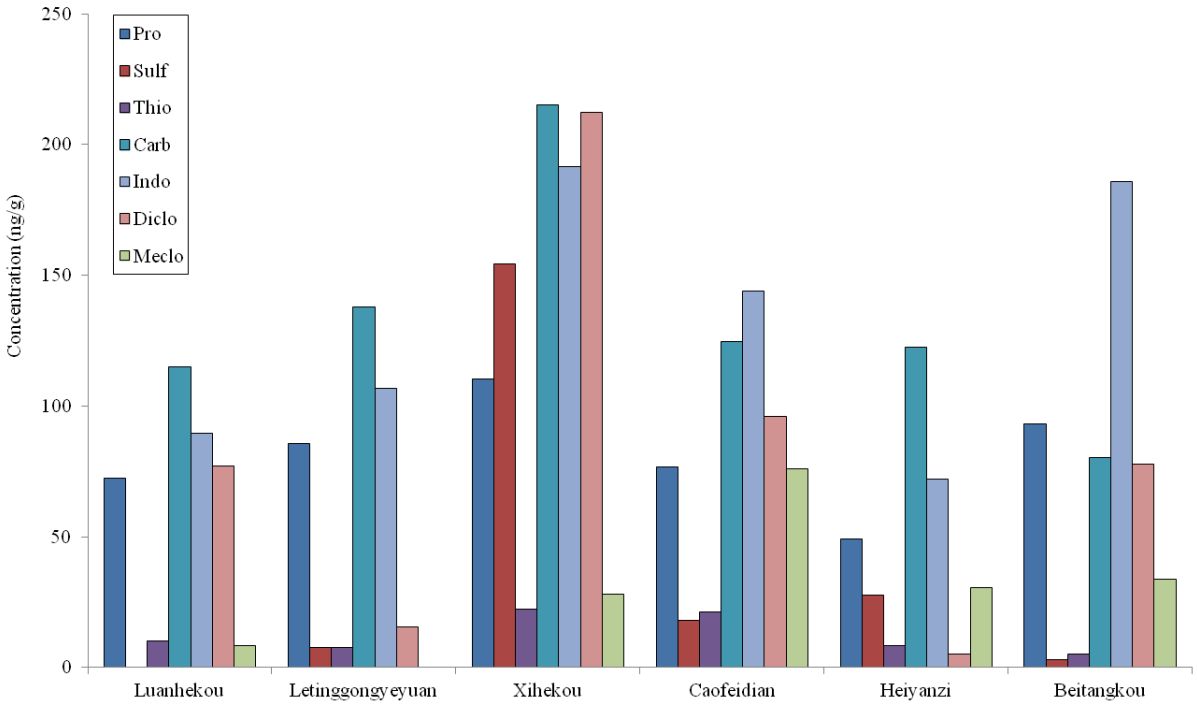

Figure 178 Concentration (ng/g) of target pharmaceutical compounds from Bohai Bay sediment samples for the last six sites in November 2009 (normalized results)

5.3.3.3 BYD Lake

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
March	Zaolinzhuang	66.6	1.1	117.1	323.9	105.0	395.8	96.4	55.0	50.7
	Shaochedian	22.4	24.1	9.8	235.2	53.4	192.1	62.3	0.0	410.5
	Caiputai	99.8	677.5	11.6	153.2	109.6	406.4	274.7	12.6	20.9
	Duancun	58.8	1.3	30.1	432.2	79.5	287.5	150.0	59.9	76.2
	Beiliuzhuang	38.8	0.0	116.0	296.5	139.4	420.8	227.7	1.2	8.1
	Wangjiazhai	24.4	34.2	317.7	313.9	117.2	394.5	181.3	0.0	0.0
April	Zaolinzhuang	38.7	10.7	882.6	298.5	100.9	331.3	175.7	3.2	40.1
	Shaochedian	38.2	0.0	20.3	165.2	72.1	566.3	574.7	50.4	1093.0
	Caiputai	74.8	4.1	0.0	356.5	62.0	558.3	516.6	379.6	0.0
	Duancun	14.3	13.9	199.1	215.2	11.7	257.7	234.3	126.7	1081.5
	Beiliuzhuang	80.0	234.8	1054.5	175.2	177.2	667.7	452.3	414.4	32.8
	Wangjiazhai	41.9	0.0	33.6	291.1	118.3	501.5	252.9	82.4	69.3
May	Zaolinzhuang	4.1	49.4	0.0	223.7	63.3	328.3	199.1	0.0	69.7
	Shaochedian	48.4	0.0	333.9	204.2	175.2	779.7	629.8	378.0	760.9
	Caiputai	14.5	16.3	224.5	185.2	51.0	609.9	361.9	66.6	1593.1
	Duancun	73.9	16.2	141.9	143.2	83.3	334.0	167.2	0.0	276.7
	Beiliuzhuang	14.4	159.3	3352.7	196.3	80.0	533.5	250.3	203.7	1792.1
	Wangjiazhai	32.7	12.3	834.9	200.7	110.7	340.9	258.0	0.0	48.0
June	Zaolinzhuang	0.0	22.8	539.7	163.2	122.4	210.9	326.5	0.0	54.8
	Shaochedian	36.2	1362.4	514.6	95.6	125.1	290.6	155.5	85.7	0.0
	Caiputai	1.4	46.5	24.9	128.2	139.8	349.5	336.9	466.2	2055.2
	Duancun	1.8	28.1	454.2	108.3	103.3	296.9	270.9	18.9	10.9
	Beiliuzhuang	72.5	46.5	0.0	268.3	46.0	313.2	99.1	53.2	19.3
	Wangjiazhai	5.8	4.0	0.0	342.3	62.9	387.8	130.9	0.0	73.9
July	Zaolinzhuang	10.2	0.0	10.6	196.3	43.3	413.2	329.3	279.3	17.3
	Shaochedian	36.4	581.4	442.0	172.0	180.5	253.3	423.9	1616.1	234.2
	Caiputai	70.3	47.1	105.4	196.3	64.1	361.5	209.8	12.9	574.8
	Duancun	6.4	2.3	1194.0	365.3	34.1	205.3	152.4	59.2	88.3
	Beiliuzhuang	1.5	207.4	1067.3	265.2	201.3	439.5	446.6	1996.6	1162.0
	Wangjiazhai	108.9	350.1	699.9	185.2	46.4	369.7	206.4	86.4	1907.0
September	Zaolinzhuang	2.0	7.2	3414.6	285.3	211.7	340.3	127.8	79.8	1217.8
	Shaochedian	17.6	37.5	633.7	149.2	42.3	185.8	139.2	314.3	3625.6
	Caiputai	4.1	571.3	4447.7	235.6	22.5	324.2	251.9	0.0	0.0
	Duancun	54.7	43.1	1794.7	168.3	87.5	451.1	291.5	3050.7	82.2
	Beiliuzhuang	29.8	11.5	172.2	326.4	34.0	403.1	320.6	59.1	134.7
	Wangjiazhai	28.3	6.2	759.4	126.3	61.2	348.4	214.1	3295.2	0.0
October	Zaolinzhuang	3.4	8.1	0.0	75.3	25.7	493.8	247.0	136.6	99.4

	Shaochedian	3.7	2.9	11.7	120.2	25.5	502.2	177.7	0.0	3730.3
	Caiputai	23.1	165.6	4355.2	86.3	27.2	643.3	157.7	28.3	89.0
	Duancun	17.3	0.0	0.0	96.2	7.9	600.7	489.7	167.9	52.8
	Beiliuzhuang	0.4	0.0	455.4	43.2	31.2	374.0	348.4	72.8	0.0
	Wangjiazhai	2.0	0.0	5.8	161.2	0.5	604.4	279.5	0.0	429.2
	Zaolinzhuang	66.6	3.6	3152.9	152.245.	65.3	569.0	325.2	41.7	88.7
	Shaochedian	17.4	18.1	76.7	85.3	53.4	692.6	297.5	84.2	2534.9
November	Caiputai	14.1	0.3	10.0	76.3	1.8	578.3	364.2	70.3	1797.9
	Duancun	0.5	0.5	622.2	101.0	30.4	721.3	306.3	138.2	147.6
	Beiliuzhuang	0.9	0.0	367.7	146.3	3.4	910.0	547.0	65.5	0.0
	Wangjiazhai	4.4	9.2	31.6	136.2	22.1	458.6	205.1	20.0	163.0
	Zaolinzhuang	22.4	9.7	60.5	143.2	35.6	461.0	222.2	3.5	21.6
Dec	Duancun	8.6	96.1	229.7	63.2	40.0	539.6	226.2	128.5	0.0
	Wangjiazhai	5.1	7.6	630.9	166.2	57.6	656.8	281.7	4.0	22.6

Table 53 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples between March to December 2009 (excluding August)

Table 53 details all concentrations of target pharmaceutical compounds from sediment samples of BYD Lake between March to December, excluding August as this month was not investigated due to logistical reasons.

During March, concentrations of pharmaceuticals were fairly low in comparison to other months in this set of observations (Figure 179). The maximum observed concentration was 677.5 ng/g for Sulf at Caiputai, and the lowest (excluding zero concentrations) was 1.1 ng/g for Sulf at Zaolinzhuang. Duancan also had a low concentration for Sulf of 1.3 ng/g. Beiliuzhuang had a concentration of only 1.2 ng/g for Diclo. <LOD were observed on four occasions; one for Sulf, two for Diclo and one for Meclo.

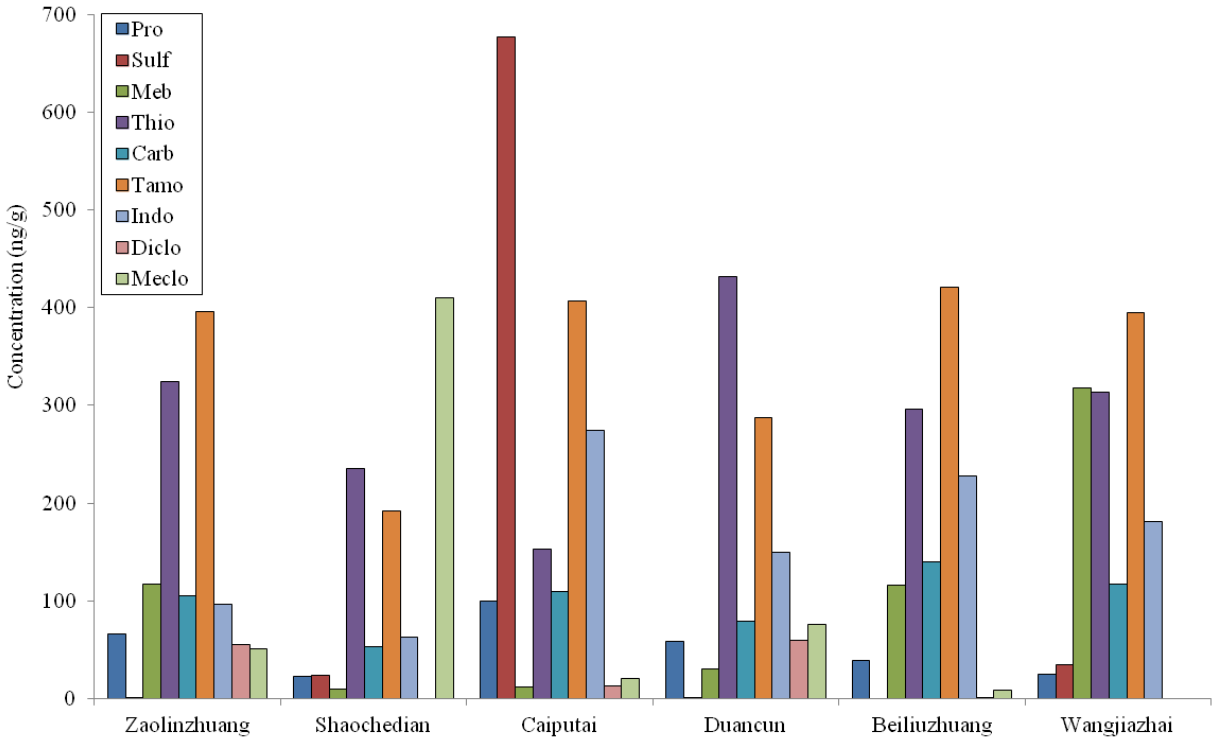

Figure 179 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during March 2009

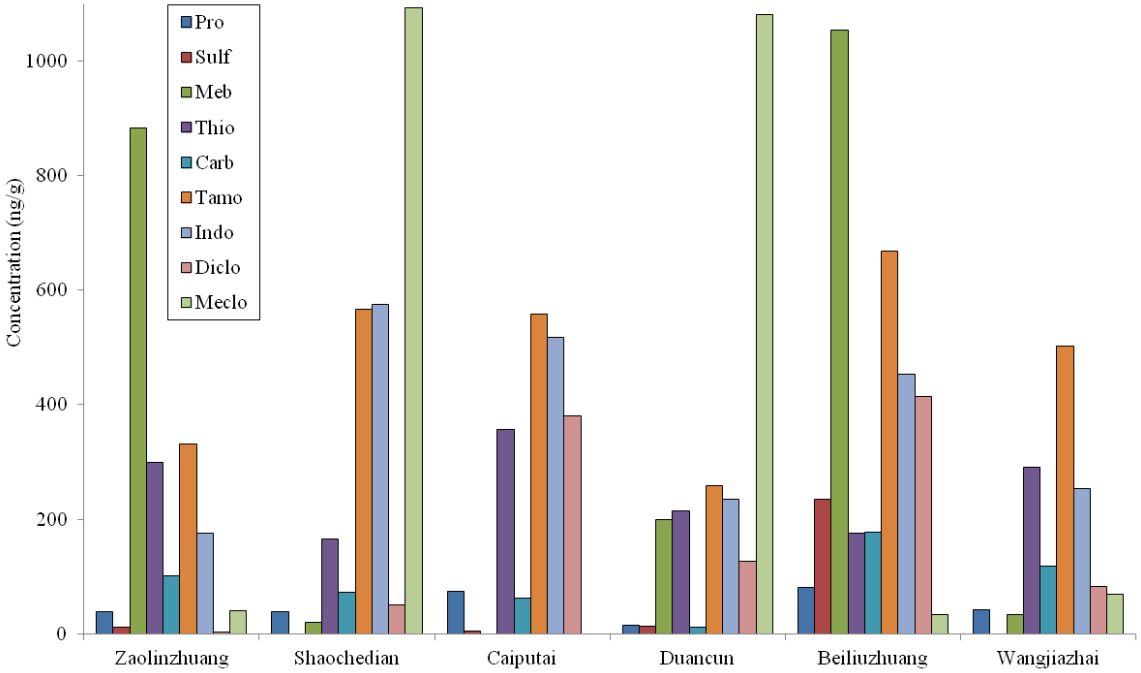

Figure 180 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during April 2009

The maximum observed concentration during April was 1093 ng/g for Meclo at Shaochedian. This was closely followed by 1081.5 at Duancun for the same compound. <LOD were observed on three occasions; Shaochedian and Wangjiazhai for Sulf, and Caiputai for Meb. Excluding these, the lowest observed concentration was for Diclo of 3.2 ng/g at Zaolinzhuang (Figure 180).

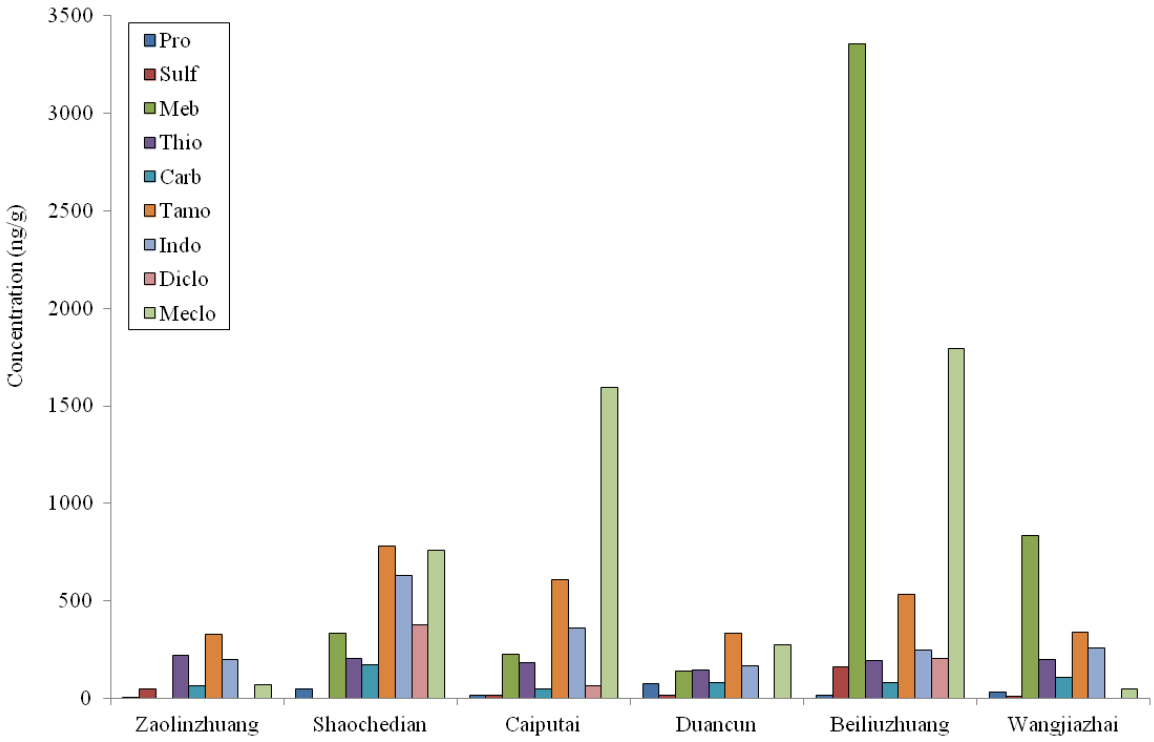

Figure 181 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during May 2009

The sample ranges for each site during May 2009 sediment samples for BYD lake were as follows; <LOD (Meb and Diclo) to 328.3 ng/g (Tamo) for Zaolinzhuang, <LOD (Sulf) to 779.7 ng/g (Tamo) for Shaochedian, 16.3 ng/g (Sulf) to 1593 ng/g (Meclo) for Caiputai, <LOD (Diclo) to 334 ng/g (Tamo) for Duancun, 14.4 ng/g (Pro) to 3352.7 ng/g (Meb) for Beiliuzhuang and <LOD (Diclo) to 835 ng/g (Meb) at Wangjiazhai (Figure 181). Concentrations were generally high during May 2009.

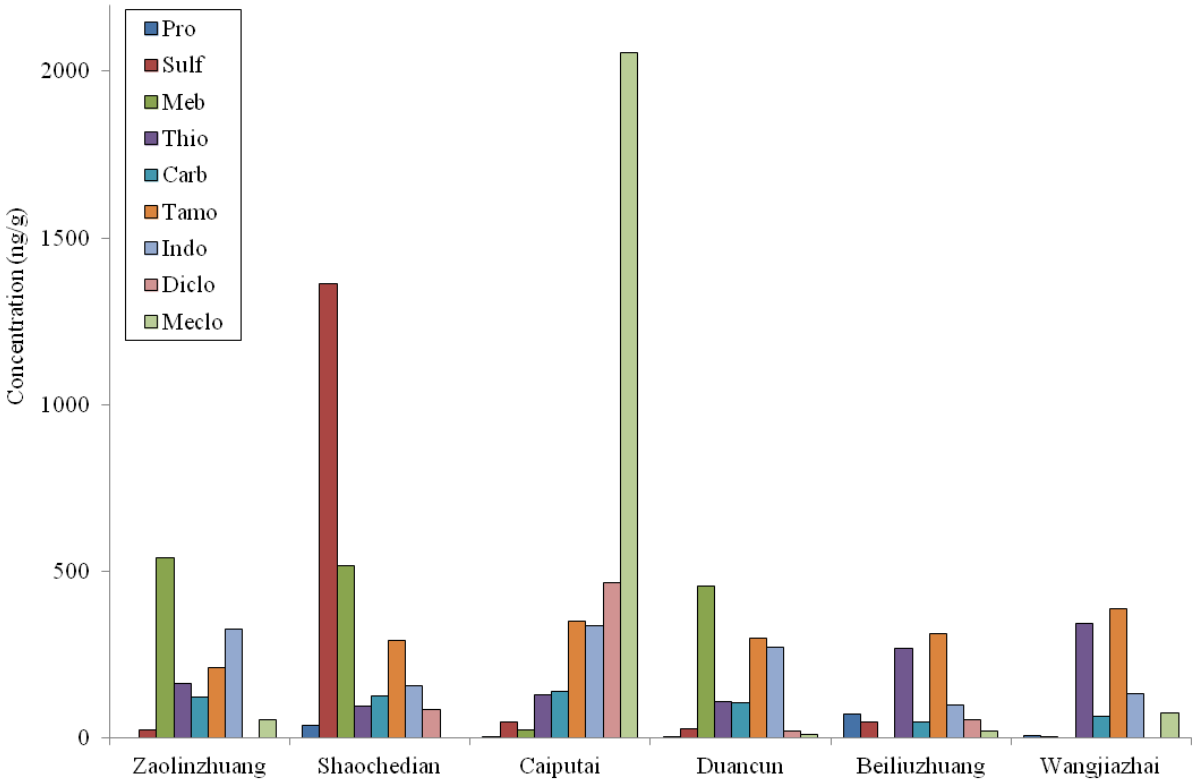

Figure 182 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during June 2009

It is immediately apparent that the highest concentration observed for BYD Lake sediment samples during June 2009 is at Caiputai for Meclo, with a value of 2055 ng/g. The second highest is at Shaochedian for Sulf with a concentration of 1362 ng/g (Figure 182). <LOD were observed on six occasions for the compounds Pro, Meb, Diclo and Meclo.

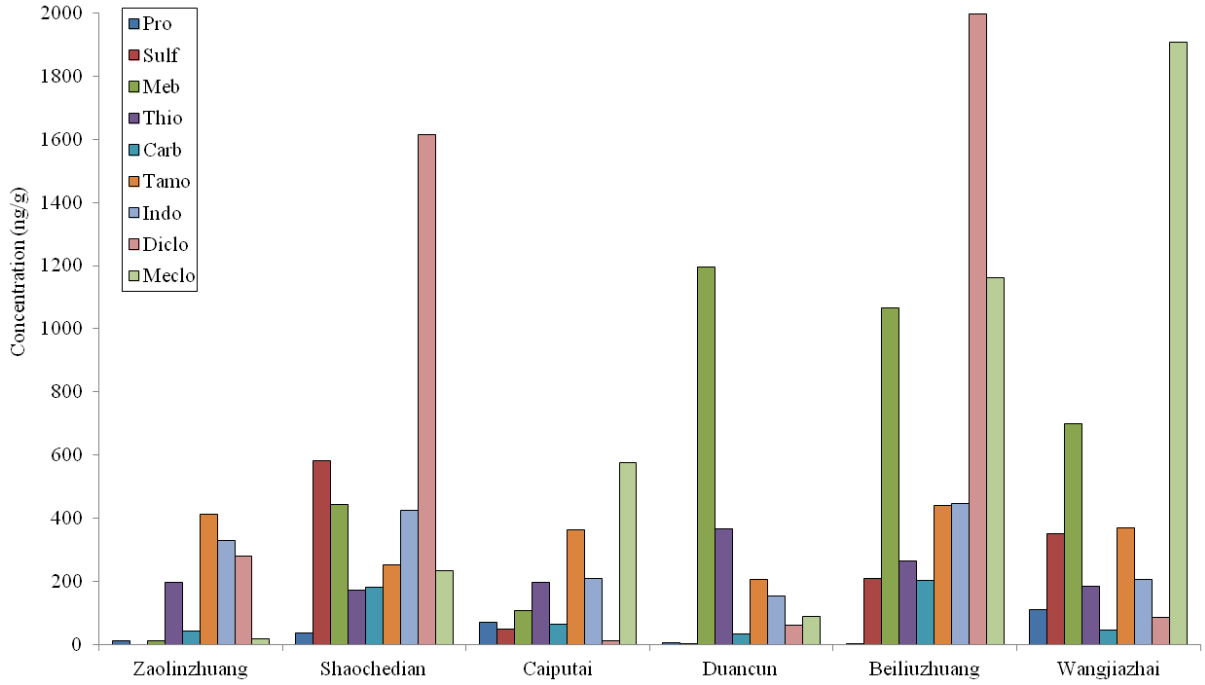

Figure 183 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during July 2009

During July, the compound Diclo presented two instances of high observed concentrations at Shaochedian and Beiliuzhuang of 1616 ng.g and 1997 ng/g respectively (Figure 183). High concentrations were also observed for Meclo at Wangjiazhai and Beiliuzhuang with values of 1907 ng/g and 1162 ng/g respectively. Meb also gave relatively high concentrations on three occasions at Duancun (1194 ng/g), Beiliuzhuang (1067 ng/g) and Wangjiazhai (700 ng/g). The lowest observed concentration was 1.5 ng/g at Beiliuzhuang for Pro.

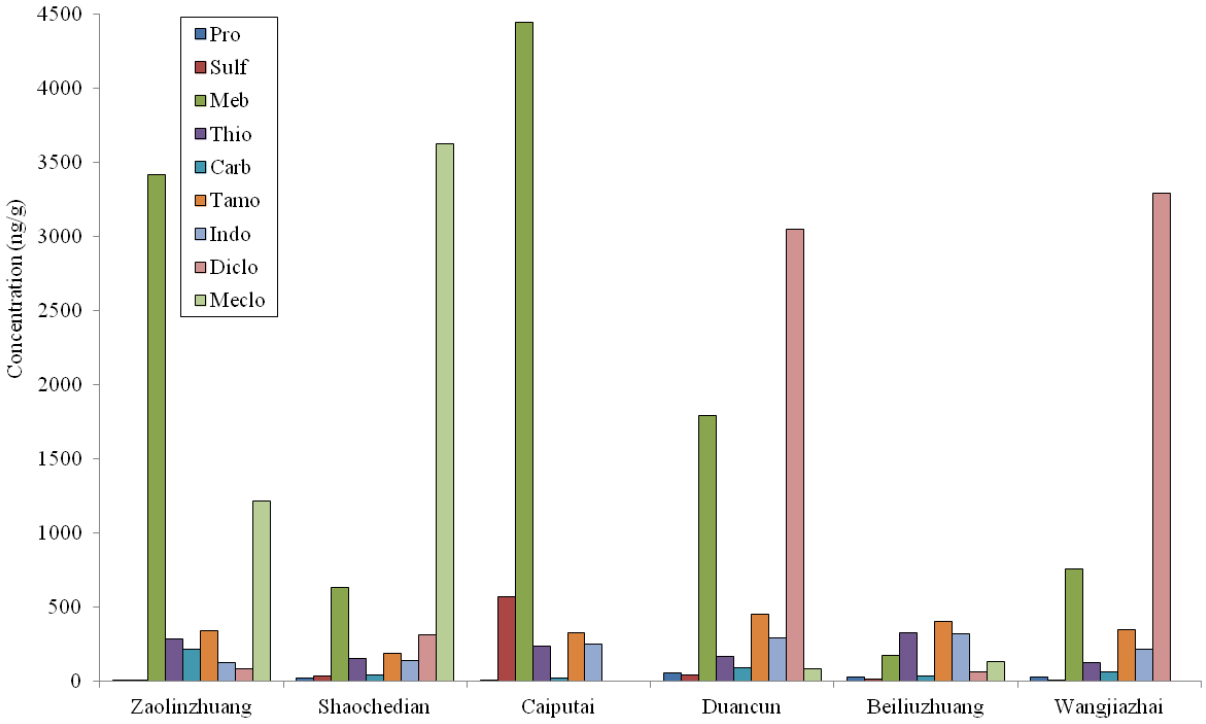

Figure 184 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during September 2009

The concentration of target pharmaceutical compounds observed at BYD Lake in September 2009 are illustrated in Figure 184. Some concentrations during September are considerably high; Meb at Caiputai is the highest overall observed with 4448 ng/g. The next being Meclo at Shaochedian with a concentration of 3626 ng/g, Pro at Zaolinzhuang with 3414 ng/g, Diclo at Wangjiazhai with a concentration of 3295 ng/g and lastly at Duancun of 3051 ng/g. Excluding these, concentrations ranged from <LOD on three occasions to 1795 ng/g (Meb at Duancun).

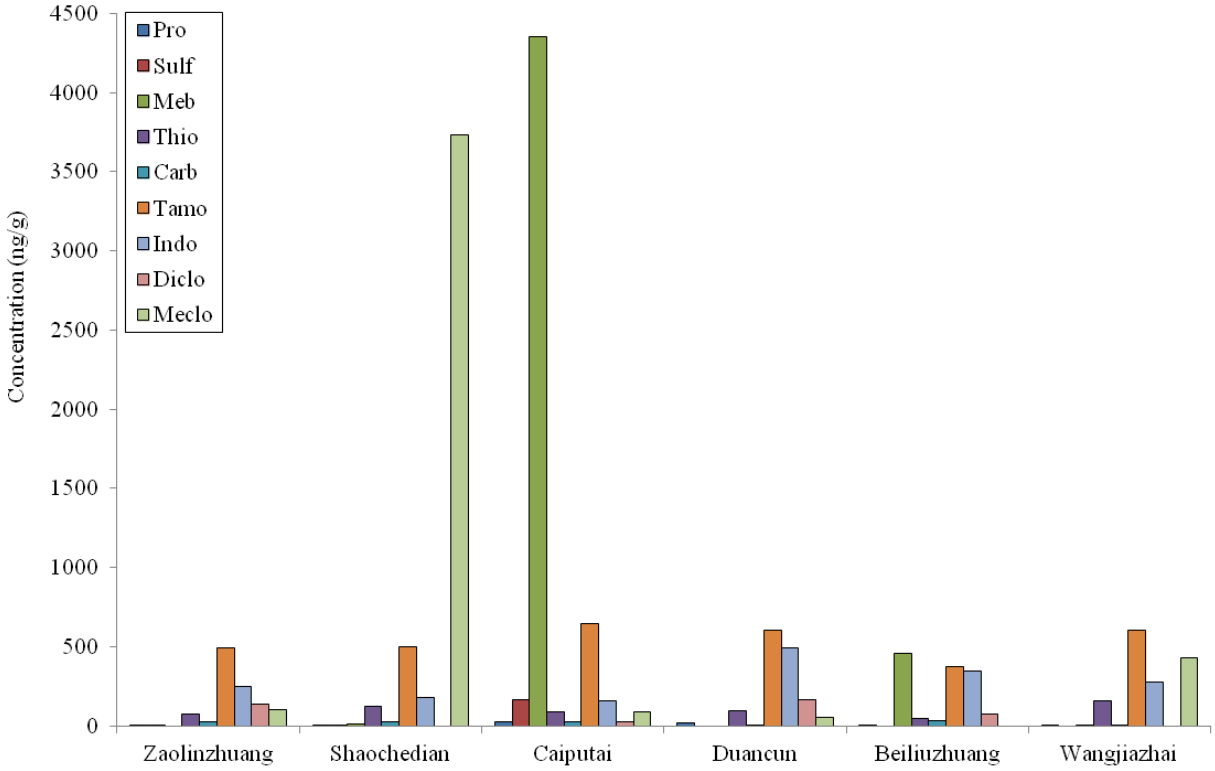

Figure 185 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during October 2009

Figure 184 displays the concentration of target pharmaceutical compounds found in BYD Lake sediment samples during October 2009. These concentrations are fairly low across all compounds excluding two; Meclo at Shaochedian with a concentration of 3730 ng/g and Meb at Caiputai with 4355.2 ng/g, also being the highest overall observed. The lowest concentration excluding <LOD was for 0.4 ng/g at Beiliuzhuang for Pro, closely followed by 0.5 ng/g at Wangjiazhai for Carb. <LOD concentrations were identified on eight different occasions. Pro gave the lowest overall concentration range of only 22.7 ng/g, with values ranging between 0.4 ng/g and 23.1 ng/g.

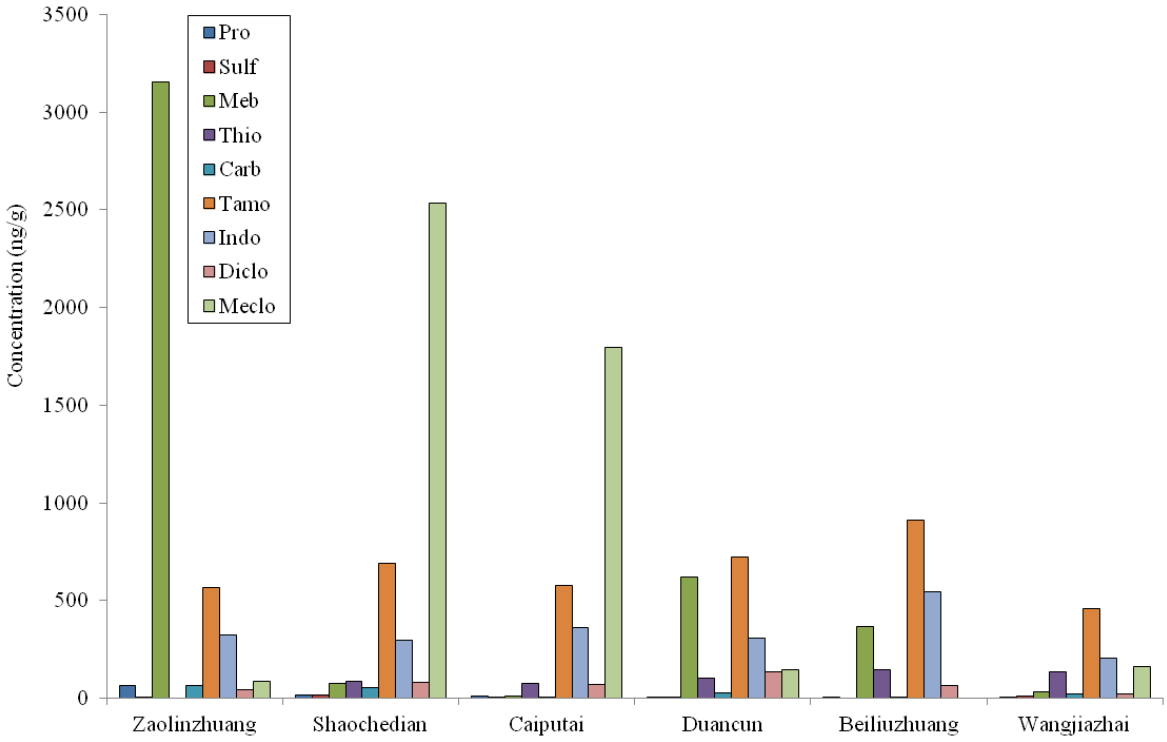

Figure 186 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during November 2009

The highest overall concentration observed during November 2009 for sediment samples at BYD Lake was 3153 ng/g for Meb at Zaolinzhuang (Figure 186). Excluding this and two other significantly high concentrations observed for Meclo at Shaochedian and Caiputai of 2535 ng/g and 1798 ng/g respectively, concentrations were fairly stable and all below 910 ng/g (Tamo at Beiliuzhuang). On more than one occasion concentrations below 1.0 ng/g were observed; 0.5 ng/g and 0.9 ng/g for Pro, 0.3 ng/g and 0.5 ng/g for Sulf, and zero concentrations at Sulf and Meclo.

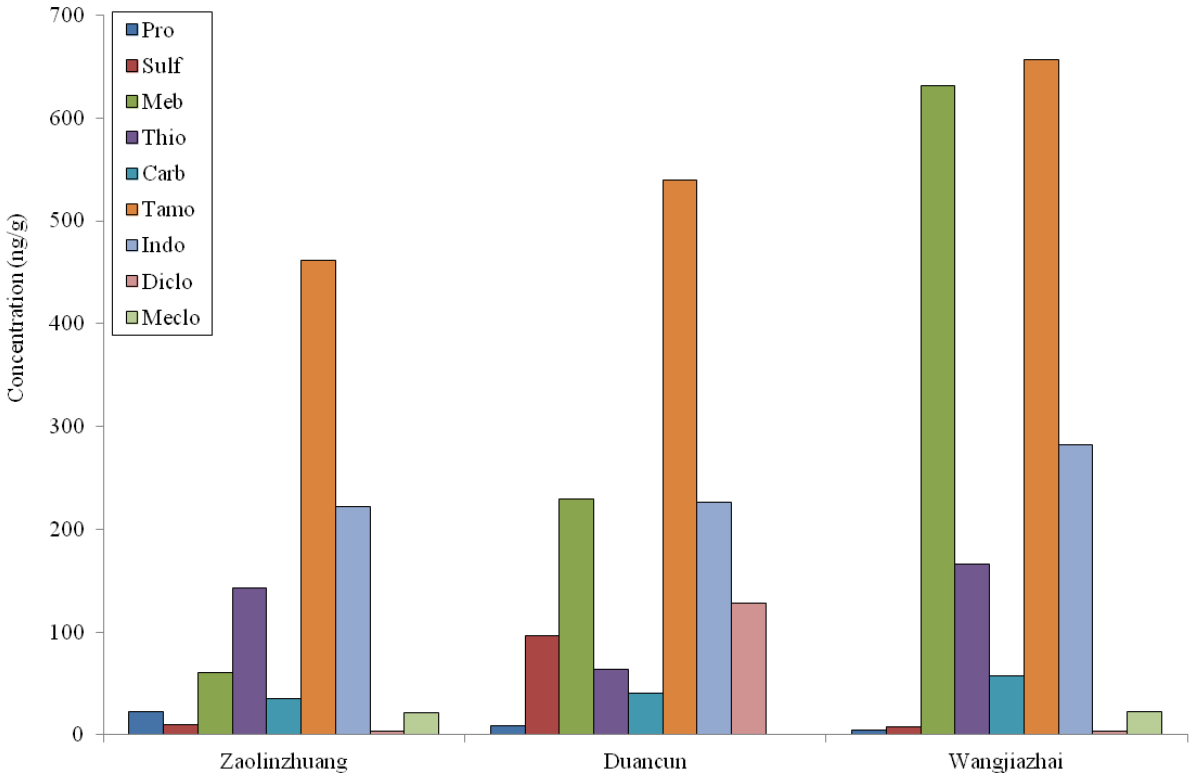

Figure 187 Concentration (ng/g) of pharmaceutical compounds found in BYD Lake sediment samples during December 2009

Figure 187 is the last in this series of sediment sampling results from BYD Lake in 2009. Only three sampling locations were investigated due to logistical reasons. A concentration of <LOD was only observed on one occasion for Meclo at Duancun. The highest concentration observed was for Indo of 657 ng/g for Tamo at Wangjiazhai. This was closely followed with a concentration of 631 ng/g for Meb at the same sampling location. Two other relatively high concentrations were observed during December; 461 ng/g and 540 ng/g both for Tamo at Zaolinzhuang and Duancun respectively.

5.4 Shanghai sample analysis

5.4.1 Sampling location

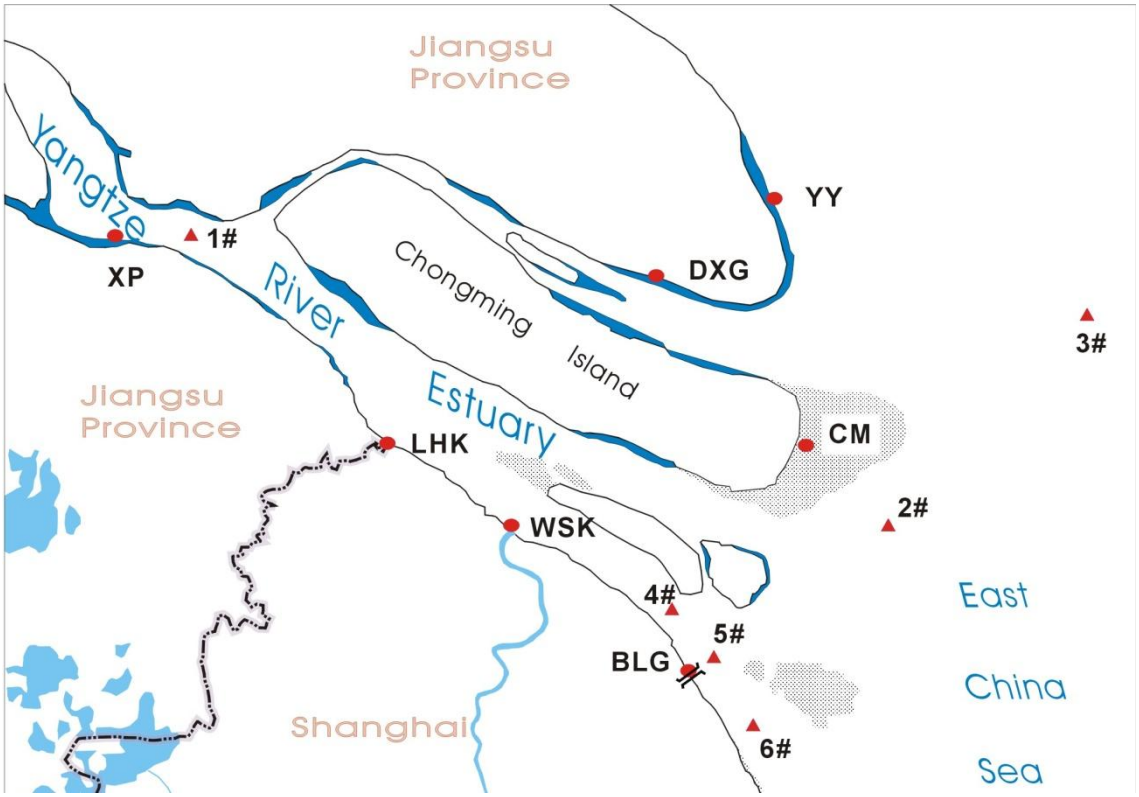

Figure 188 Map detailing sampling locations of onshore locations from the Huangpu River in Shanghai

The sampling locations of those from the Huangpu River in Shanghai are detailed in Figure 188. Due to incomplete information from the sampling locations related to the samples sent from Shanghai, it was not possible to match samples to their exact locations seen in figures to follow.

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
SPM EDCs		0.82	6.45	55.03	283.00	10.88	177.56	387.16	2.96	296.01
		0.60	0.00	59.11	248.25	7.05	131.52	173.54	12.34	67.48
		0.42	1.65	137.99	282.90	0.00	126.39	174.77	0.00	62.76
		0.99	4.69	0.00	268.30	0.00	116.70	175.03	0.00	173.10
		51.73	1.34	430.89	285.75	45.10	612.94	639.14	98.35	573.32
		2.47	0.00	346.14	261.50	3.35	621.81	15.02	0.00	0.00
Sediments	ng/g	0.43	0.06	93.12	99.40	1.18	84.11	54.31	5.00	24.49
		0.36	0.22	91.77	55.40	0.83	67.39	46.60	6.31	33.80
		0.02	0.00	89.08	30.80	0.01	112.19	26.76	1.50	14.64
		0.18	0.13	44.40	32.90	0.07	59.98	29.26	0.00	16.63
		4.72	0.14	231.70	75.62	6.81	45.20	123.76	11.91	67.68
		0.13	0.51	153.06	68.30	1.08	207.40	57.01	3.56	35.68
		0.37	0.48	58.44	15.90	2.46	249.59	163.85	15.09	99.19
Onshore locations		0.06	0.00	40.45	25.22	1.52	430.97	12.41	2.06	0.00
		0.25	0.15	63.07	53.85	0.90	258.23	130.34	18.39	63.89
		0.14	0.22	62.06	3.00	0.55	290.48	71.92	0.00	24.90
		2.04	0.10	414.66	18.95	0.56	287.96	114.69	0.00	52.64
		0.20	1.00	18.60	7.85	0.59	323.94	61.89	2.26	30.38
		0.95	0.39	67.40	18.14	3.93	372.60	135.36	35.12	75.58
		0.77	0.01	126.87	1.39	0.00	212.03	105.61	0.00	43.90
IS		0.02	0.11	2.10	3.05	0.00	1.30	2.90	0.00	2.66

Table 54 Concentration data for the analysis of pharmaceutical compounds in samples from Shanghai

		Pro	Sulf	Meb	Thio	Carb	Tamo	Indo	Diclo	Meclo
SPM EDCs	ng/g	1.16	0.77	3.07	20.51	0.40	16.05	76.79	0.23	7.90
		0.24	0.00	79.91	54.94	0.91	7.87	4.07	0.96	2.57
		0.59	2.34	73.01	0.71	0.00	1.25	28.51	0.00	69.34
		0.14	0.55	0.00	13.72	0.00	26.44	6.06	0.00	25.51
		14.43	0.20	36.79	19.30	17.67	32.89	13.40	15.77	10.50
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sediments		0.26	0.08	15.37	0.57	0.79	4.08	5.57	1.27	8.18
		0.31	0.32	3.23	5.23	0.04	0.21	6.53	0.57	6.93
		0.03	0.00	1.33	1.84	0.01	25.30	1.04	0.42	2.60
		0.19	0.19	10.28	12.45	0.05	4.38	11.14	0.00	2.30
		0.74	0.20	14.57	6.17	1.28	11.74	7.41	2.14	0.74
		0.13	0.00	19.16	4.10	0.29	2.83	0.88	0.94	18.70
		0.53	0.20	12.88	4.67	0.53	35.16	6.99	3.24	3.89
Onshore locations		0.09	0.00	7.56	5.49	0.45	80.51	0.60	0.93	0.00
		0.01	0.21	2.99	21.00	0.33	58.00	15.99	1.68	7.18
		0.15	0.32	4.76	0.14	0.25	28.25	16.60	0.00	2.54
		0.24	0.14	11.93	1.06	0.26	90.96	14.69	0.00	7.84
		0.28	0.23	2.13	2.05	0.41	87.19	5.68	0.22	4.21
		0.12	0.16	4.95	4.47	1.03	76.31	63.83	3.42	4.92
		0.61	0.02	3.58	0.22	0.00	22.66	26.47	0.00	28.78
IS		0.03	0.07	0.85	0.07	0.00	0.28	1.27	0.00	0.05

Table 55 Standard deviation for the analysis of pharmaceutical compounds in samples from Shanghai

5.4.2 Sediment Samples

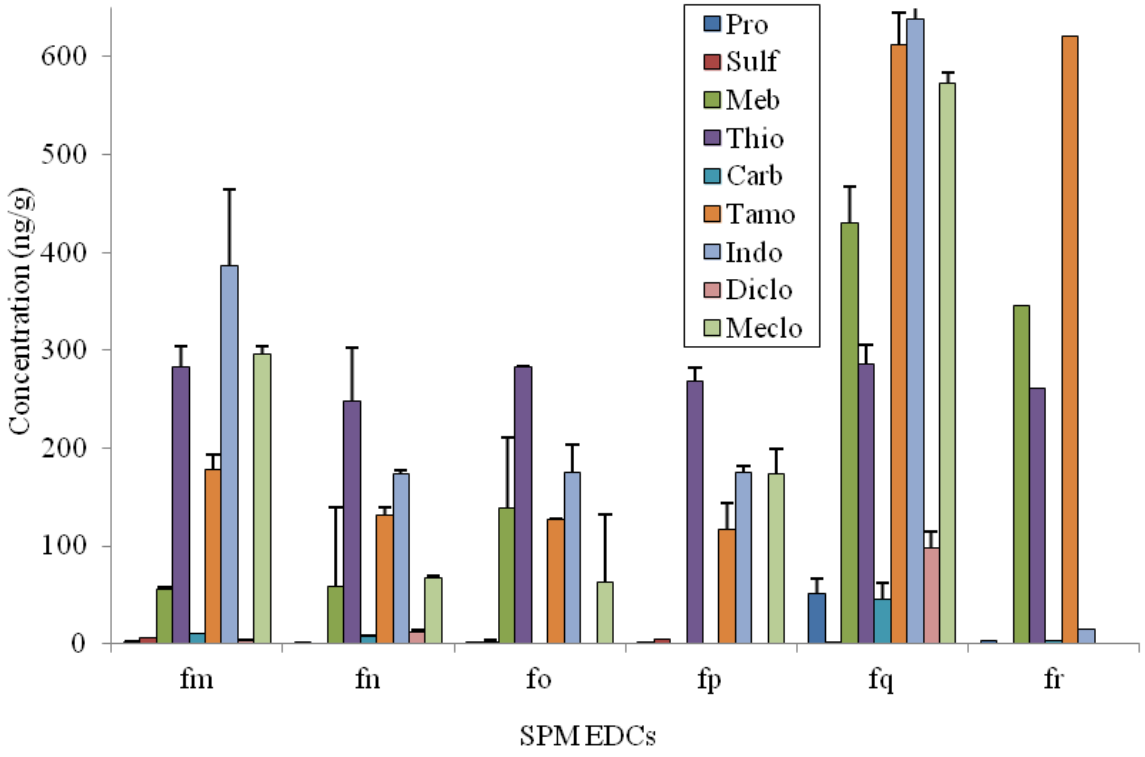

Figure 189 Concentration of pharmaceutical compounds in SPM samples from Shanghai

Testing samples for SPM EDCs still showed surprisingly high concentrations of pharmaceuticals (Figure 189). Nine recordings of zero concentrations were identified for compounds Sulf, Carb, Diclo and Meclo, shown in Table 54 and 55. Location fq had the highest overall contamination with values ranging from a minimum of 1.34 ng/g for Sulf and 639.14 ng/l for Indo, also the highest overall identified concentration value. Thio gave relatively similar values for all sites, with a range of only 262 – 286 ng/g.

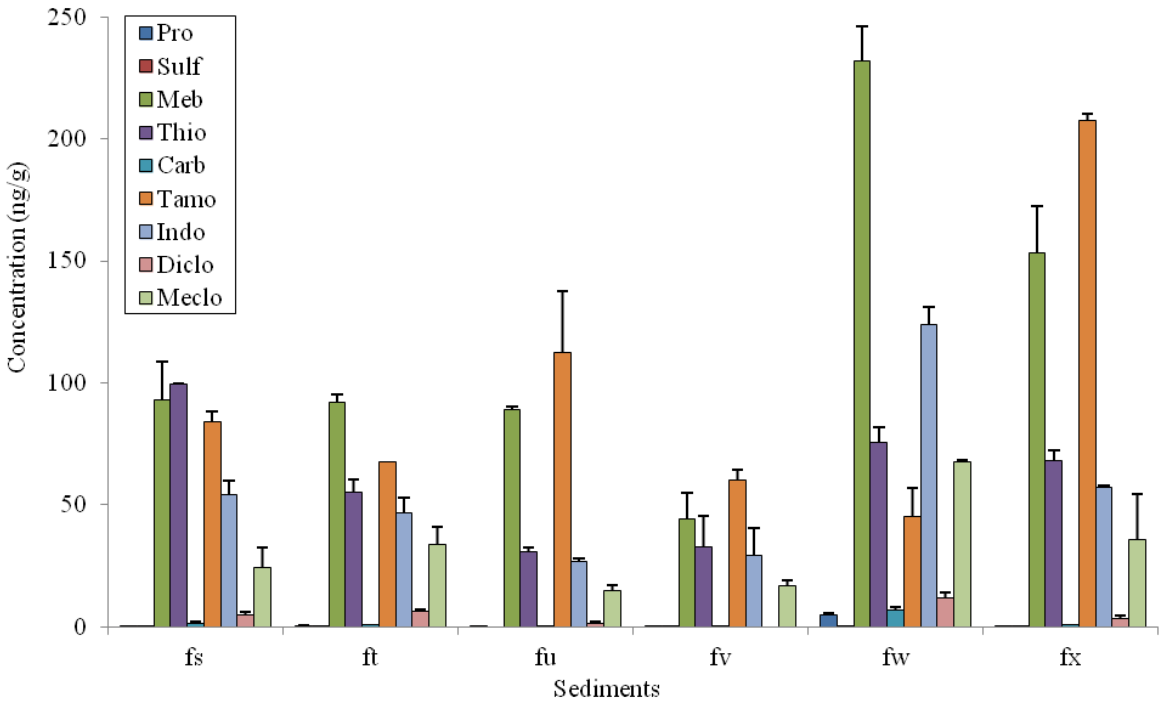

Figure 190 Concentration of pharmaceutical compounds in sediment samples from Shanghai

Figure 190 represents the concentration of selected pharmaceutical compounds identified in sediment samples from Shanghai. Concentrations ranged from <LOD to 232 ng/g (Table 54). Concentration range for each compound was as follows, with the minimum and maximum site noted respectively; Pro 0.02 – 4.72 ng/g (fu anf fw), Sulf <LOD – 0.51 ng/g (fu and fx), Meb 44.40 – 232 ng/g (fv and fw), Thio 30.80 – 99.40 ng/g (fu and fs), Carb 0.01 – 6.81 ng/g (fu and fw), Tamo 45.20 – 207 ng/g (fw and fx), Indo 26.76 – 124 ng/g (fu and fw), Diclo <LOD – 11.91 ng/g (fv and fw), and Meclo 14.64 – 67.68 ng/g (fu and fw). Fw harbours the highest concentration for six of the nine compounds. The opposite is true for fu, which records the lowest for six of the nine compounds.

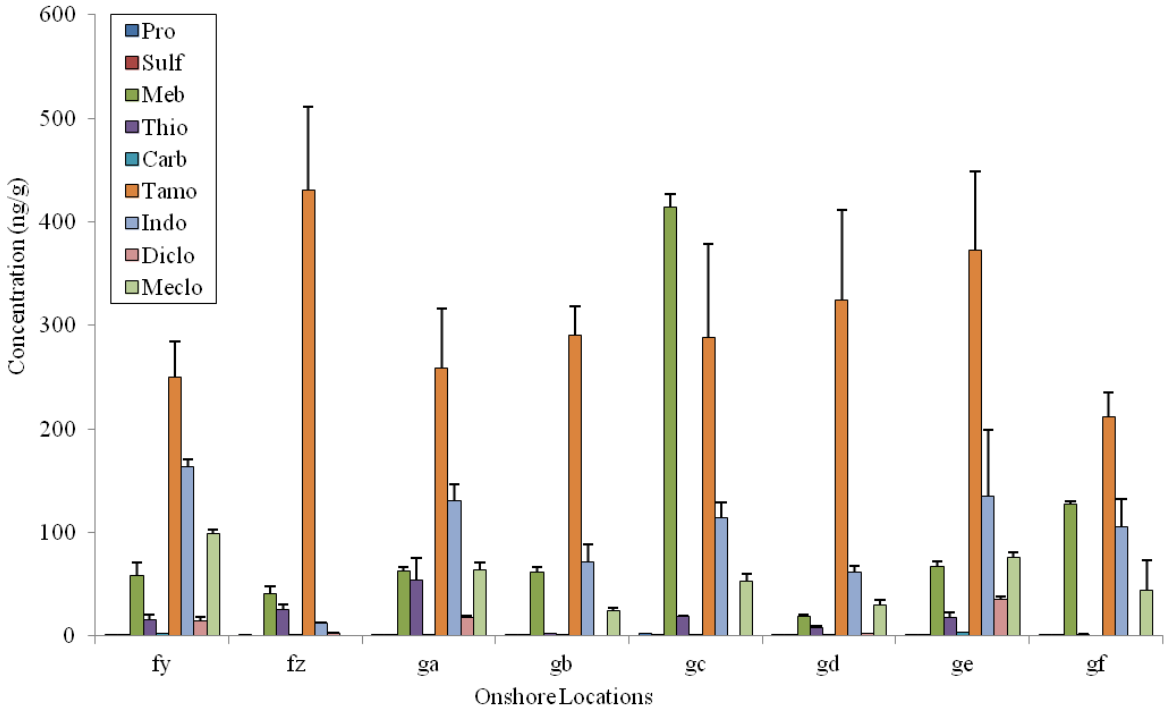

Figure 191 Concentration of pharmaceutical compounds from onshore locations in Shanghai

Eight onshore locations in Shanghai were analyzed for the concentration of pharmaceutical compounds (Figure 191). Excluding gc, Tamo is the highest concentration identified for all sites with a range between 212. – 431 ng/g (gf and fz respectively). At gc, the highest concentration identified was for Meb with 415 ng/g. Concentration range for each site was as follows; fy 0.37 – 250 ng/g, fz <LOD – 431 ng/g , ga 0.15 – 258 ng/g, gb 0.00 – 291 ng/g, gc <LOD – 415 ng/g, gd 0.20 – 324 ng/g, ge 0.39 – 373 ng/g, and finally gf <LOD – 212. ng/g.

5.5 Conclusion

Overall, pharmaceutical concentrations of samples from Shanghai were higher in water than sediment samples, with results regularly over 10,000 ng/l. This is particularly the case for concentrated fluid samples. Maximum concentration for sediment samples rarely exceeded 300 ng/g, with maximum results just over 600 ng/g for just three individual cases. Some <LOD concentrations were observed, but this is perfectly viable.

In comparison to Chapter 4, samples from Shanghai demonstrated the same patterns; higher in water than sediment as a comparison. The maximum concentration of pharmaceuticals in the River Medway water samples was 14,573.12 ng/l. Perhaps more surprising, is the concentration of pharmaceutical compounds in sediment comparison. Throughout the sampling of sediment between December 2009 and June 2010, the minimum and maximum concentrations observed from the River Medway were 0.47 ng/g and 6156 ng/g, compared to a maximum of just over 600 ng/g in Shanghai, with most concentrations rarely exceeding 300 ng/g.

Chapter 6 - Conclusions

6.1 Development of an LC-MS/MS method for the extraction of pharmaceutical compounds from sediment samples

Through a series of method development, a method of sediment extraction for pharmaceuticals was successfully developed. The developed method used a dry and homogenized sample mass of 2 g, followed by the use of methanol as the solvent of choice. Extraction using Ultrasonication (U) was then used followed by Waters Oasis SPE cartridges for the clean-up process. Standard practice reduction of samples using N_2 blow down followed, and then finally analysis with a previously tried and tested method using LC-MS/MS for final quantification.

Experiments were conducted at each stage of the method development process and gave excellent results. A sample mass of 2 g was concluded to be the optimal to use following recovery results (%) of these and sample masses of 1 g and 5 g. The recovery range for the sample masses were as follows; 0.01 – 58.37 % for 1g, 4.54 – 78.28 % for 2 g and 0.02 – 25.30 % for 5 g (Figure 14). The sample mass of 2 g had the highest overall recovery average of 52.67 % compared to 13.48 % for 1 g and 13.71 % for 5 g (Figure 15).

Among the various solvents tested including Methanol, Acetonitrile, Hexane and Ethyl Acetate, it was concluded that methanol was the best solvent to use for the extraction process. Overall methanol produced the highest recoveries for the majority of compounds, with the majority over 70 % (Figure 16). It is fair to say that other solvents out performed methanol on some occasions for a few compounds, however this was a fair and accepted trade-off to be able to use one solvent for the extraction process, as all compounds tested using methanol had good recoveries. Figure 17 clearly highlights that methanol is the best overall solvent to use with a recovery average (%) outperforming any other solvent or solvent mixture.

Although there was extensive testing of the use of MAE for method development, this was also compared to using U (Figure 11). MAE produced the best recoveries at 110 °C for 15 min using methanol with results between 4.5 % (Thio) to 84.6 % (Pro), however U for 30 mins at room temperature outperformed the MAE method with results between

19.3 % (Thio) to 97.6 % (Pro), with seven out of nine compounds achieving a recovery above 60 %. Therefore, U was selected for further method development and testing.

The effect of changing the cleanup method on extraction was considered to enhance the sensitivity and overall quality of analysis for pharmaceuticals. Tested were laboratory made columns of silica, alumina, silica: alumina (1:1), silica: alumina (1:3), silica: alumina (3:1), SPE Oasis and SPE Supelco, with results illustrated in

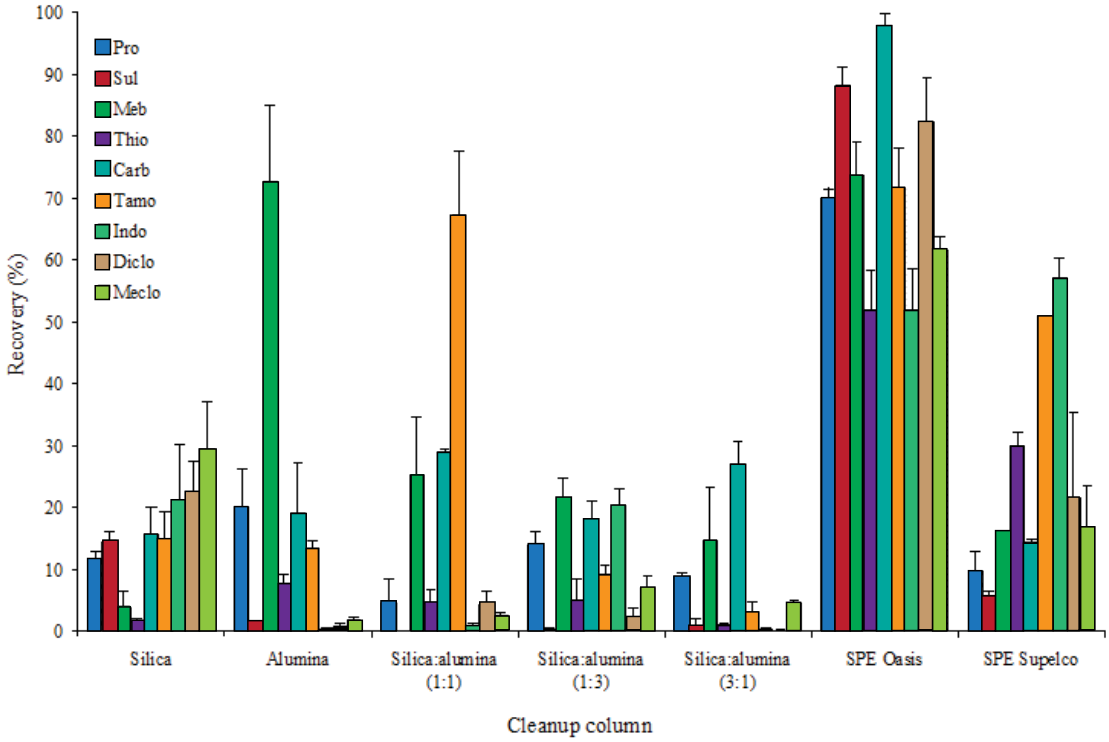

Figure 10. It is clearly noticeable that SPE Oasis far outperforms any other cleanup method with results between 50.99 % (Indo) to 98.35 % (Carb) and so was chosen for continued use in this method development process.

A flow diagram to illustrate the new developed method for the simultaneous determination of nine pharmaceutical compounds is displayed in Figure 18.

6.2 Validation of the developed method by application to environmental samples

Following a successful pilot study on samples from the River Ouse (East Sussex, UK), the developed method was then used to conduct a formal spatial and temporal variability report of the nine target pharmaceutical compounds in the River Medway (Kent, UK) at locations at and around Motney Hill sewage treatment works.

Temporal variability was recorded across 7 seasons for a total of 4 occasions; December 2009, February 2010, April 2010 and June 2010. Concentrations of pharmaceuticals at the River Medway during December 2009 ranged from 0.6 ng/g to 1194.2 ng/g for Sulf upstream and Tamo at the effluent site respectively (Table 22). During February 2010, pharmaceutical compounds at the chosen sampling locations ranged from 0.47 ng/g for Sulf at the effluent site to 239.42 ng/g for Tamo downstream from the effluent site. During April the concentration range across all sites and compounds was between 11.03 ng/g for Sulf and 2080.75 ng/g for Tamo. Concentrations of pharmaceuticals at the River Medway in June 2010 ranged from 11.57 ng/g for Sulf downstream and 6165 ng/g for Diclo at the effluent site. For all observed months Sulf had the lowest observed concentrations.

The month of February in 2009 showed the overall lowest target pharmaceutical concentration whereas the month of June in 2010 saw the highest concentration (concentrations previously stated). This suggests that this is due to seasonal variability of water temperature having an effect on the remobilization and presence of compounds remaining in the sediment. However the discharge of pharmaceutical compounds from STWs is also expected to be seasonal. During Summer (warmer) months, the water is more likely to be less agitated and therefore more of the target pharmaceutical compounds remain in the sediment. For example it is proposed that in Winter months when waters are more agitated due to bad weather and winds that there may be a higher remobilization of the compounds from the sediments into the water and therefore leaving the sediment with a lower compound concentration than in the surrounding waters. There may also be an increased concentration of pharmaceutical compounds in the sediment at this time of year as there may be an increase in these compounds released into the water (and subsequently becomes dormant in the sediment) due to increased river activity (ie recreational boating) in these warmer Summer months. Another contributing factor may be the effect of water dilution with rainfall and increased mobilization between solids and water.

6.3 Application of the developed and validated method to different environmental matrices.

The developed method was successfully applied to a variety of different matrices including samples of water, vegetation (seaweed, grass root, grass shoot, rosehip, dandelion, slow buds and sloe leaves), biological (tissue) samples (lug worm and crab) and crustacean samples (clam shell and snails).

The method was very successful and confirmed the further assessment of fate of pharmaceutical compounds in the aquatic environment by giving a snap shot into the scale of the concentrations. Success in this instance is the recovery of spiked compounds; at this stage of the method development, any recovery for these set of experiments is deemed successful. All samples were treated the same as sediment samples previously and then subjected to the same developed analytical method. Recoveries ranged from 2.51 % (Sulf for rosehip) to 536.44 % (Tamo for dandelion). Overall Meb had particularly high recoveries, with the highest of 520.37 % found in the crab sample. Results suggest a high degree of bio accumulation and magnification through the food chain, which is considered in 6.5. In the instance of these experiments however, it is the recovery (%) which is of more importance than the target pharmaceutical content (ng/g).

During sampling at locations aforementioned in the River Medway, seaweed samples were taken for the months of February, April and June of 2010 and so analysed using the developed method to give an indication of pharmaceutical concentration, temporal variability and bioaccumulation. Seaweed was chosen as it was the only available sample to be collected on all occasions. Other examples such as crabs were available, however they may not have always originated at these locations.

The seaweed samples had increasingly high concentrations as you travel from the upstream to effluent to downstream sampling locations, in particular for February and April. The concentrations did not show this pattern as strongly in June for all compounds, although the maximum concentrations during this month were almost ten fold greater than the previous observations. February 2010 was by far the month where seaweed concentrations were the overall lowest ranging between 0.06 ng/g for Pro at the upstream site and 622.22 ng/g for Diclo at the downstream site. For April 2010 the concentration range was between 20.55 ng/g to 4712 ng/g at upstream and downstream sites respectively both for Indo. The concentrations in June 2010 were significantly

higher with a range bwtween 239.53 ng/g for Meb at the effluent site and 43987.78 ng/g for Meclo.

6.4 Completion of aims and objectives

6.4.1 Aim 1 – Pharmaceutical analysis and monitoring

6.4.1.1 Objective 1 – Extraction and clean-up method

It was proposed to identify the best extraction and cleanup method of pharmaceutical compounds from a solid sample matrix. This included selecting the optimum solvent, sample size, extraction conditions and cleanup method.

This aim was successfully completed as a method was developed and tested with excellent results. It was concluded that the best solvent to use is methanol with a (dry) sample size of 2 g. The extraction method of choice is U for 30 mins at room temperature followed by cleanup using SPE Oasis columns.

6.4.1.2 Objective 2 – LC-MS/MS method

Objective 2 described how a LC-MS/MS method would be used for the simultaneous extraction, separation and quantitative analysis of a group of nine pharmaceutical

compounds from river sediment samples. After initial investigations it was concluded that an existing tried and tested (and published) analytical LC-MS/MS technique was to be used as described by Zhang and Zhou (2007).

6.4.1.3 Objective 3 – Environmental samples

As described in objective three, the developed method from objectives one and two were used to quantify the concentration of target pharmaceuticals in the River Ouse and River Medway. The samples were taken at locations which would determine spatial analysis as required. Using samples from the River Medway, a temporal and spatial variability report was successfully conducted as well as finally applying the successful method to samples from China.

6.4.2 Aim 2 – Assessing the quality of analysis

6.4.2.1 Objective 4 – Precision and bias of sampling

To assess the precision and bias of sampling, triplicate sampling was conducted ex situ (in the field). Once back in the laboratory, each replicate sample was processed in triplicate once again to further increase precision and bias. At the final stage of analysis, LC-MS/MS, each sample was analysed three times (triple injection). Therefore objective four was fulfilled successfully at every possible opportunity.

To further assess quality of the results, experiments were conducted to analyse the inter- and intra-day variability of the LC-MS/MS equipment. Although there was slight variation in results taken from the same sample, these were not significant to analysis and so continued as normal.

6.4.2.2 Objective 5 – Analysis of blank samples

As required by objective five, a blank sample was analysed to minimize any effect of background concentration of the nine target pharmaceutical compounds. It was concluded that there was no significant effect as concentrations were either below the LOD or very low and close to this.

6.4.2.3 Objective 6 – Sample storage

To be able to identify the best possible way to store samples and elucidate any significant differences in doing so, four different conditions were tested; in a freezer, in a fridge, at room temperature and at room temperature uncovered. Results for each compound were conclusive over a six week period that storing the samples in a fridge was the best option.

6.4.2.4 Objective 7 - Matrix effect

It was important to consider if there was any bias once the validated method was successful and applied to environmental samples with a solid matrix. To assess this, the extraction method was tested on samples with no matrix and a solid soil matrix. Results were conclusive across all compounds that there was no significant matrix effect from the use of ultrasonication (U), and that MAE experiments showing extracts had significant interference in the response of compounds upon analysis. Similar results were found for the other compounds, suggesting that MAE is more destructive than U in causing the dislodging of sediment compounds.

6.4.3 Aim 3 – Applications of the developed method

6.4.3.1 Objective 8 – Biological samples

A variety of different biological samples were taken from the River Medway as stated in objective eight and the recovery of pharmaceuticals following spiking experiments were determined. It can be concluded that the method is extremely successful in application to all of the biological samples tested and is applicable to a wider variety of matrices. This makes the method more widely useable. On reflection, non-spiked as well as spiked samples should have been analysed to further validate the developed methodology, however time and sample volume constraints prohibited this.

6.4.3.2 Objective 9 – Water samples

To test whether the developed method can be applied successfully to water samples, they were taken from the River Medway for analysis. Target pharmaceuticals were

successfully identified in samples rendering the developed method suitable for both sediment and water sample analysis with good high recoveries.

6.4.3.3 Objective 10 – Geochemistry of contaminants

It was proposed that the pH, salinity and dissolved Oxygen were to be analysed from water samples used in objective nine to assess the geochemistry of contaminants of the nine selected pharmaceutical compound concentrations. This unfortunately was the only objective which was not conducted due to logistical and practical reasons concerning the equipment. However after consideration post experimentation, this objective was not entirely relevant to the rest of the studies as it only assessed these from water samples and the work was predominantly focused on sediment samples.

6.5 Further work

Although my work has shown the successful development, validation and application of a simultaneous and reliable method to detect a group of nine selected pharmaceutical compounds, and my original objectives have all been achieved, there are still areas for consideration, concern and further remediation as follows;

- Organisms in water and sediment at certain levels and selected compounds will be affected
- There is no immediate or direct threat to human health
- Concentrations found are relatively high; bioaccumulation in sea life through recreational activities is of indirect dancer to human health
- Water should be treated before use by humans and as a general rule should be avoided by consumption even after treatment as a safety precaution.
- PPCPs found in sediment are less of a threat to humans. However sediment of this nature (busy river) are likely to be significantly high due to remobilization of PPCPs from sediment due to activity on and in the waters which will disturb the sediment.
- PPCP levels should be routinely monitored as a precautionary measure due to the potential threats.

Sampling was collected once a month for four months over a total of seven. These were in December, February, April and June. In future work, more continuous monitoring should be practiced to include a more detailed insight into seasonal variability and increased temporal monitoring to minimize limitations of point sampling.

Comparisons of total PPCP concentrations from different studies should be treated with caution as different numbers of parent PPCP compounds were analysed from different studies and so compounds measured may differ. Different methods are likely to have been used as this project focuses on a new methodology, and also variables such as time of year mean that comparisons cannot be made directly without considerations. This is especially true as it appears that PPCPs show a tendency to be higher in Winter compared to Summer months. The simple numeric predicament of altering units must be treated with caution.

Further data collection could include particle size analysis routinely of each of the original samples before analysis. This may determine how well sediments retain contaminants and prove a vital variable for consideration. It may also determine how easily these contaminants are decomposed or remobilized and even have an effect on sediment pH and salinity.

It is important to assess the pH and salinity of samples which was an objective which was unable to be considered in this study for logistical reasons. These were that the equipment were not available at the time I required and it was needed for these to be collected at each sampling opportunity. This is something that must be considered in further work.

Measuring water turbidity may prove beneficial as there may be a relationship for example; in Summer months there is an increased water turbidity due to increased recreational activity on the river and therefore leads to increased PPCP concentration in water samples compared to sediment samples at this time of year. Installing a flow meter at each sampling location to measure constantly which would increase temporal variation in results. Higher flow data collection will increase knowledge of how PPCPs move through the water column and how easily they move within a small area.

Processes which affect the distribution of PPCPs should be considered. For example how easily degradable they are in sediment once settled. It would be interesting to

conduct a study of the selected pharmaceutical compounds on a sample core from each of the sample sites. Sedimentation rate would determine the timescale of this deposition with an insight further into temporal variability.

Future concentrations in sediment (and water) in the locations considered in this investigation cannot be inferred. They are dynamic in nature and a considerable number of variables determine their distribution and fate.

Lastly but most importantly, if the study were to take one further direction for this work, I would consider developing a method for the successful removal of these nine target pharmaceutical compounds simultaneously from sediment samples. I feel that now these can be successfully and reliably detected and measured in sediment samples, the next logical step would be to develop a way to effectively remove these from the environment. This would in turn ensure and enable certain aquatic areas to be deemed safe for activities such as aquaculture and not pose a threat to marine (or in turn human) life.

Chapter 7 - References

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Chapter 8 - Appendices

8.1 Appendix 1

Particle size analysis of River Ouse samples using a HORIBA particle size analyzer. These begin on the following page.

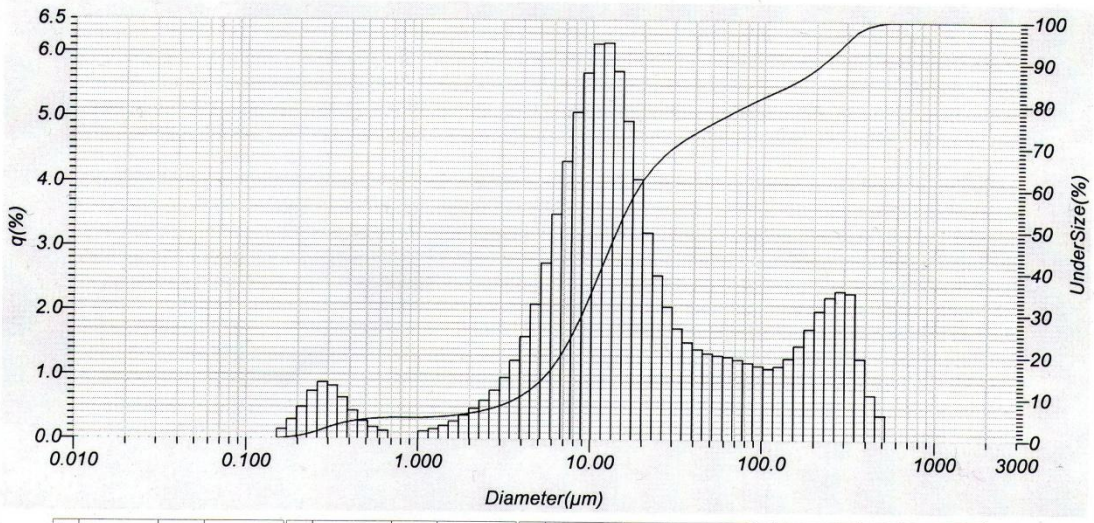
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Down1
Material :
Lot Number :
Source :
ID# : 200912151516790
Transmittance(R) : 72.6(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 14.10429(μm)
Mean Size : 57.71250(μm)
Diameter on Cumulative % : (2)10.00 (%)- 3.8670(μm)
: (9)90.00 (%)- 216.9107(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 85.454(%)
: (7)106.0 (μm)- 82.433(%)
: (8)75.00 (μm)- 79.444(%)
: (9)53.00 (μm)- 76.161(%)
: (10)38.00 (μm)- 72.769(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.818	3.347	51	10.097	5.684	35.076	76	300.518	2.316	95.344
2	0.013	0.000	0.000	27	0.389	0.636	3.983	52	11.565	6.134	41.210	77	344.206	2.287	97.631
3	0.015	0.000	0.000	28	0.445	0.431	4.415	53	13.246	6.150	47.359	78	394.244	1.271	98.902
4	0.017	0.000	0.000	29	0.510	0.274	4.689	54	15.172	5.710	53.069	79	451.556	0.706	99.608
5	0.020	0.000	0.000	30	0.584	0.176	4.865	55	17.377	4.941	58.010	80	517.200	0.392	100.000
6	0.022	0.000	0.000	31	0.669	0.123	4.988	56	19.904	4.047	62.057	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.000	4.988	57	22.797	3.216	65.273	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.000	4.988	58	26.111	2.549	67.823	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.000	4.988	59	29.907	2.063	69.885	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.115	5.103	60	34.255	1.727	71.612	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.150	5.253	61	39.234	1.514	73.126	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.203	5.456	62	44.938	1.408	74.534	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.275	5.730	63	51.471	1.345	75.878	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.365	6.095	64	58.953	1.310	77.188	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.472	6.568	65	67.523	1.292	78.480	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.598	7.166	66	77.339	1.246	79.726	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	0.752	7.918	67	88.583	1.199	80.925	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	0.950	8.868	68	101.460	1.151	82.075	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	1.219	10.087	69	116.210	1.110	83.185				
20	0.150	0.000	0.000	45	4.472	1.589	11.676	70	133.103	1.148	84.334				
21	0.172	0.143	0.143	46	5.122	2.089	13.765	71	152.453	1.272	85.606				
22	0.197	0.297	0.440	47	5.867	2.733	16.498	72	174.616	1.468	87.073				
23	0.226	0.491	0.931	48	6.720	3.500	19.998	73	200.000	1.727	88.801				
24	0.259	0.732	1.663	49	7.697	4.319	24.317	74	229.075	2.005	90.806				
25	0.296	0.866	2.530	50	8.816	5.074	29.392	75	262.376	2.222	93.028				

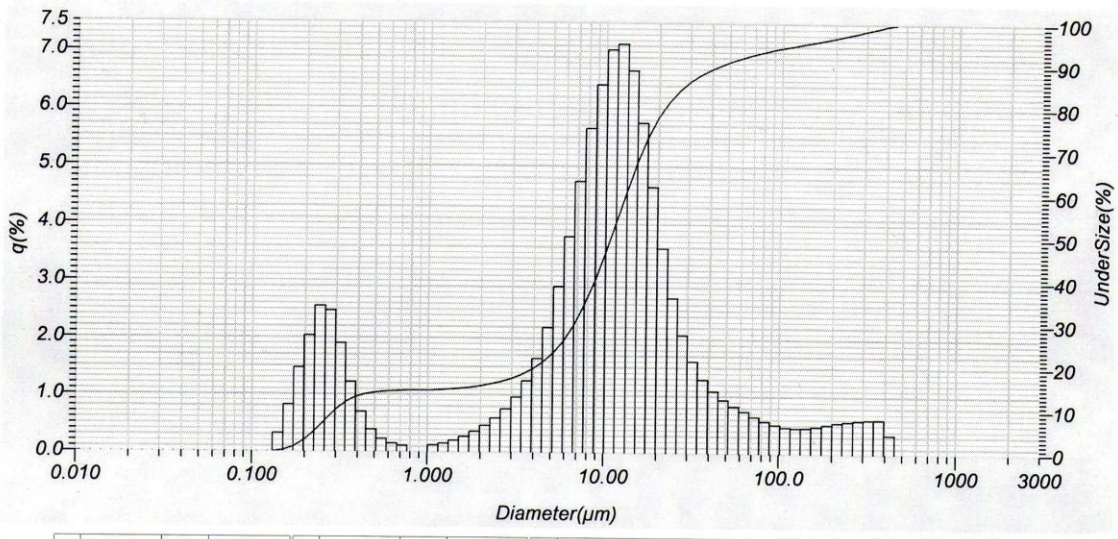
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Down2
Material :
Lot Number :
Source :
ID# : 200912151521791
Transmittance(R) : 60.7(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 10.76222(μm)
Mean Size : 25.91017(μm)
Diameter on Cumulative % : (2)10.00 (%)- 0.3040(μm)
: (9)90.00 (%)- 43.6741(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 95.743(%)
: (7)106.0 (μm)- 94.580(%)
: (8)75.00 (μm)- 93.216(%)
: (9)53.00 (μm)- 91.338(%)
: (10)38.00 (μm)- 88.842(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	1.895	11.532	51	10.097	6.412	46.697	76	300.518	0.581	98.468
2	0.013	0.000	0.000	27	0.389	1.212	12.743	52	11.565	7.027	53.724	77	344.206	0.596	99.064
3	0.015	0.000	0.000	28	0.445	0.692	13.436	53	13.246	7.130	60.854	78	394.244	0.602	99.666
4	0.017	0.000	0.000	29	0.510	0.383	13.819	54	15.172	6.659	67.514	79	451.556	0.334	100.000
5	0.020	0.000	0.000	30	0.584	0.222	14.041	55	17.377	5.744	73.258	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.143	14.184	56	19.904	4.636	77.894	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.108	14.292	57	22.797	3.579	81.473	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.000	14.292	58	26.111	2.713	84.186	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.000	14.292	59	29.907	2.068	86.254	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.117	14.409	60	34.255	1.605	87.859	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.150	14.559	61	39.234	1.286	89.145	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.200	14.758	62	44.938	1.083	90.228	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.269	15.028	63	51.471	0.933	91.161	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.358	15.386	64	58.953	0.822	91.983	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.465	15.850	65	67.523	0.736	92.718	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.593	16.443	66	77.339	0.643	93.361	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	0.751	17.195	67	88.583	0.565	93.926	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	0.959	18.154	68	101.460	0.504	94.430	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	1.244	19.398	69	116.210	0.465	94.895				
20	0.150	0.315	0.315	45	4.472	1.642	21.040	70	133.103	0.448	95.343				
21	0.172	0.813	1.128	46	5.122	2.188	23.228	71	152.453	0.454	95.797				
22	0.197	1.464	2.593	47	5.867	2.904	26.131	72	174.616	0.476	96.272				
23	0.226	2.030	4.623	48	6.720	3.776	29.907	73	200.000	0.508	96.780				
24	0.259	2.546	7.169	49	7.697	4.734	34.641	74	229.075	0.541	97.321				
25	0.296	2.468	9.637	50	8.816	5.645	40.285	75	262.376	0.566	97.887				

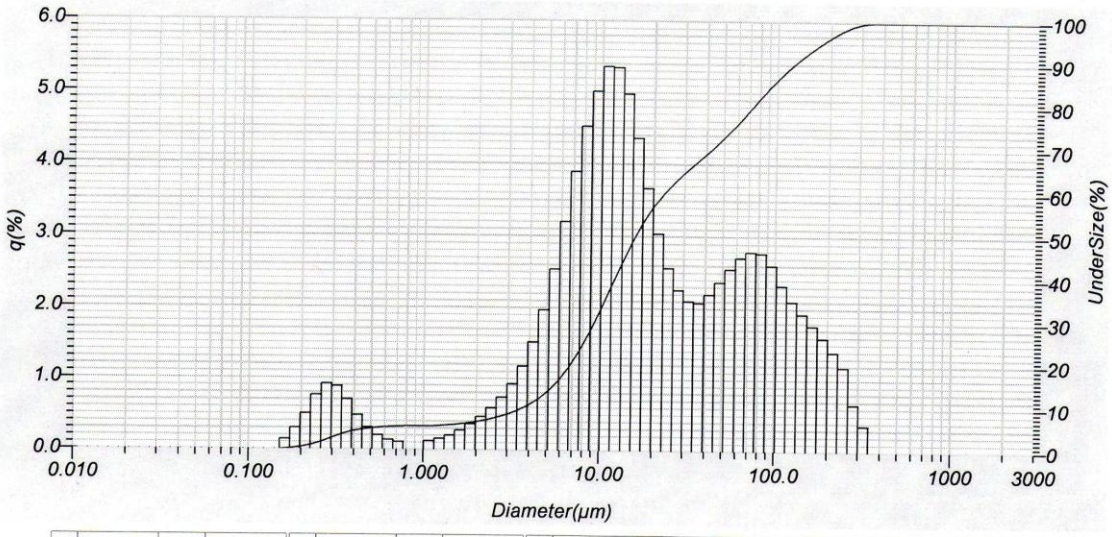
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Down3
Material :
Lot Number :
Source :
ID# : 200912151523792
Transmittance(R) : 73.7(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 15.80077(μm)
Mean Size : 42.98937(μm)
Diameter on Cumulative % : (2)10.00 (%)- 3.7318(μm)
: (9)90.00 (%)- 123.7168(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 92.828(%)
: (7)106.0 (μm)- 87.461(%)
: (8)75.00 (μm)- 80.710(%)
: (9)53.00 (μm)- 73.850(%)
: (10)38.00 (μm)- 68.250(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.881	3.487	51	10.097	5.010	33.024	76	300.518	0.657	99.635
2	0.013	0.000	0.000	27	0.389	0.697	4.184	52	11.565	5.355	38.379	77	344.206	0.365	100.000
3	0.015	0.000	0.000	28	0.445	0.479	4.663	53	13.246	5.344	43.723	78	394.244	0.000	100.000
4	0.017	0.000	0.000	29	0.510	0.307	4.969	54	15.172	4.973	48.696	79	451.556	0.000	100.000
5	0.020	0.000	0.000	30	0.584	0.197	5.166	55	17.377	4.356	53.052	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.136	5.303	56	19.904	3.661	56.713	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.106	5.409	57	22.797	3.035	59.748	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.000	5.409	58	26.111	2.561	62.309	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.000	5.409	59	29.907	2.255	64.564	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.118	5.527	60	34.255	2.097	66.662	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.151	5.679	61	39.234	2.078	68.740	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.202	5.881	62	44.938	2.196	70.936	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.271	6.152	63	51.471	2.364	73.300	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.359	6.511	64	58.953	2.548	75.848	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.463	6.974	65	67.523	2.706	78.554	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.586	7.560	66	77.339	2.787	81.341	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	0.734	8.294	67	88.583	2.770	84.111	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	0.923	9.217	68	101.460	2.602	86.713	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	1.175	10.392	69	116.210	2.318	89.031				
20	0.150	0.000	0.000	45	4.472	1.516	11.907	70	133.103	2.100	91.132				
21	0.172	0.139	0.139	46	5.122	1.967	13.875	71	152.453	1.926	93.058				
22	0.197	0.296	0.435	47	5.867	2.536	16.411	72	174.616	1.760	94.817				
23	0.226	0.498	0.933	48	6.720	3.198	19.609	73	200.000	1.587	96.404				
24	0.259	0.758	1.690	49	7.697	3.890	23.499	74	229.075	1.393	97.797				
25	0.296	0.915	2.606	50	8.816	4.515	28.014	75	262.376	1.182	98.979				

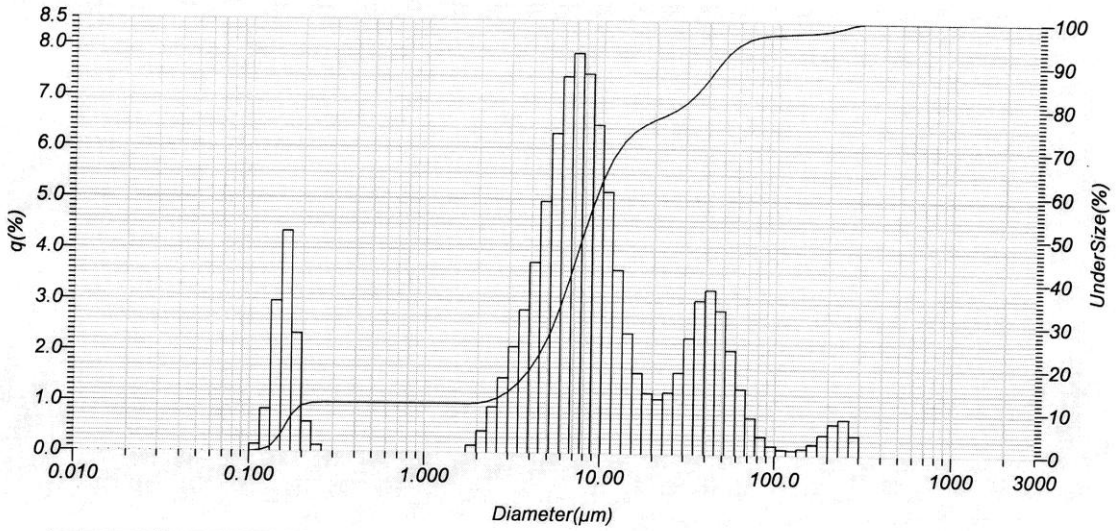
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Effluent1
Material :
Lot Number :
Source :
ID# : 200912151527793
Transmittance(R) : 56.0(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 7.77810(μm)
Mean Size : 19.36845(μm)
Diameter on Cumulative % : (2)10.00 (%)- 0.1903(μm)
: (9)90.00 (%)- 45.3876(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 97.623(%)
: (7)106.0 (μm)- 97.325(%)
: (8)75.00 (μm)- 96.569(%)
: (9)53.00 (μm)- 93.079(%)
: (10)38.00 (μm)- 85.829(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.000	11.308	51	10.097	6.455	63.336	76	300.518	0.397	100.000
2	0.013	0.000	0.000	27	0.389	0.000	11.308	52	11.565	5.144	68.480	77	344.206	0.000	100.000
3	0.015	0.000	0.000	28	0.445	0.000	11.308	53	13.246	3.621	72.101	78	394.244	0.000	100.000
4	0.017	0.000	0.000	29	0.510	0.000	11.308	54	15.172	2.383	74.484	79	451.556	0.000	100.000
5	0.020	0.000	0.000	30	0.584	0.000	11.308	55	17.377	1.599	76.083	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.000	11.308	56	19.904	1.201	77.285	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.000	11.308	57	22.797	1.087	78.372	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.000	11.308	58	26.111	1.217	79.589	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.000	11.308	59	29.907	1.617	81.206	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.000	11.308	60	34.255	2.301	83.507	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.000	11.308	61	39.234	3.037	86.545	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.000	11.308	62	44.938	3.247	89.792	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.000	11.308	63	51.471	2.842	92.633	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.136	11.444	64	58.953	2.066	94.699	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.423	11.868	65	67.523	1.302	96.001	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.902	12.769	66	77.339	0.733	96.735	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	1.478	14.247	67	88.583	0.367	97.101	92	2636.467	0.000	100.000
18	0.115	0.135	0.135	43	3.409	2.098	16.346	68	101.460	0.186	97.287	93	3000.000	0.000	100.000
19	0.131	0.835	0.970	44	3.905	2.822	19.167	69	116.210	0.117	97.404				
20	0.150	2.964	3.934	45	4.472	3.756	22.923	70	133.103	0.102	97.506				
21	0.172	4.339	8.273	46	5.122	4.951	27.875	71	152.453	0.133	97.639				
22	0.197	2.329	10.601	47	5.867	6.284	34.159	72	174.616	0.224	97.863				
23	0.226	0.581	11.183	48	6.720	7.401	41.560	73	200.000	0.405	98.268				
24	0.259	0.125	11.308	49	7.697	7.862	49.422	74	229.075	0.622	98.890				
25	0.296	0.000	11.308	50	8.816	7.459	56.880	75	262.376	0.714	99.603				

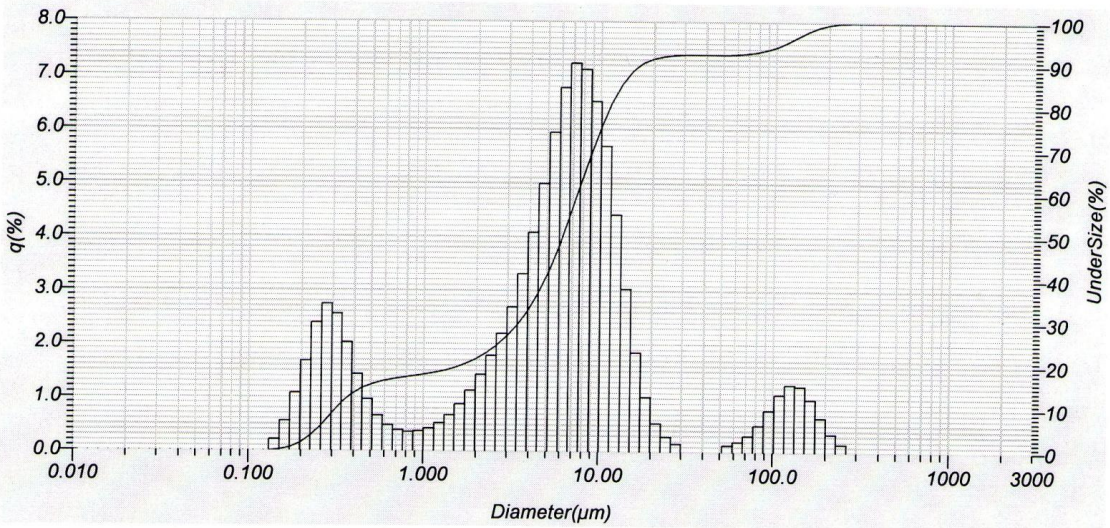
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Effluent2
Material :
Lot Number :
Source :
ID# : 200912151529794
Transmittance(R) : 90.1(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 6.12586(μm)
Mean Size : 15.58069(μm)
Diameter on Cumulative % : (2)10.00 (%)- 0.3186(μm)
: (9)90.00 (%)- 16.8346(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 97.738(%)
: (7)106.0 (μm)- 94.679(%)
: (8)75.00 (μm)- 92.986(%)
: (9)53.00 (μm)- 92.453(%)
: (10)38.00 (μm)- 92.425(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	2.553	11.180	51	10.097	6.511	75.464	76	300.518	0.000	100.000
2	0.013	0.000	0.000	27	0.389	2.018	13.198	52	11.565	5.681	81.145	77	344.206	0.000	100.000
3	0.015	0.000	0.000	28	0.445	1.427	14.625	53	13.246	4.413	85.558	78	394.244	0.000	100.000
4	0.017	0.000	0.000	29	0.510	0.962	15.587	54	15.172	3.028	88.586	79	451.556	0.000	100.000
5	0.020	0.000	0.000	30	0.584	0.658	16.245	55	17.377	1.846	90.432	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.481	16.726	56	19.904	1.022	91.453	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.390	17.116	57	22.797	0.535	91.988	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.358	17.474	58	26.111	0.280	92.269	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.370	17.844	59	29.907	0.156	92.425	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.423	18.267	60	34.255	0.000	92.425	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.522	18.789	61	39.234	0.000	92.425	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.671	19.459	62	44.938	0.000	92.425	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.873	20.333	63	51.471	0.000	92.425	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	1.128	21.461	64	58.953	0.128	92.553	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	1.429	22.890	65	67.523	0.196	92.749	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	1.778	24.668	66	77.339	0.306	93.056	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	2.187	26.854	67	88.583	0.499	93.555	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	2.684	29.539	68	101.460	0.779	94.334	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	3.306	32.844	69	116.210	1.069	95.403				
20	0.150	0.210	0.210	45	4.472	4.077	36.921	70	133.103	1.256	96.659				
21	0.172	0.551	0.761	46	5.122	4.984	41.906	71	152.453	1.225	97.884				
22	0.197	1.071	1.832	47	5.867	5.941	47.847	72	174.616	0.982	98.866				
23	0.226	1.672	3.504	48	6.720	6.767	54.614	73	200.000	0.640	99.506				
24	0.259	2.388	5.892	49	7.697	7.224	61.838	74	229.075	0.341	99.847				
25	0.296	2.735	8.627	50	8.816	7.115	68.953	75	262.376	0.153	100.000				

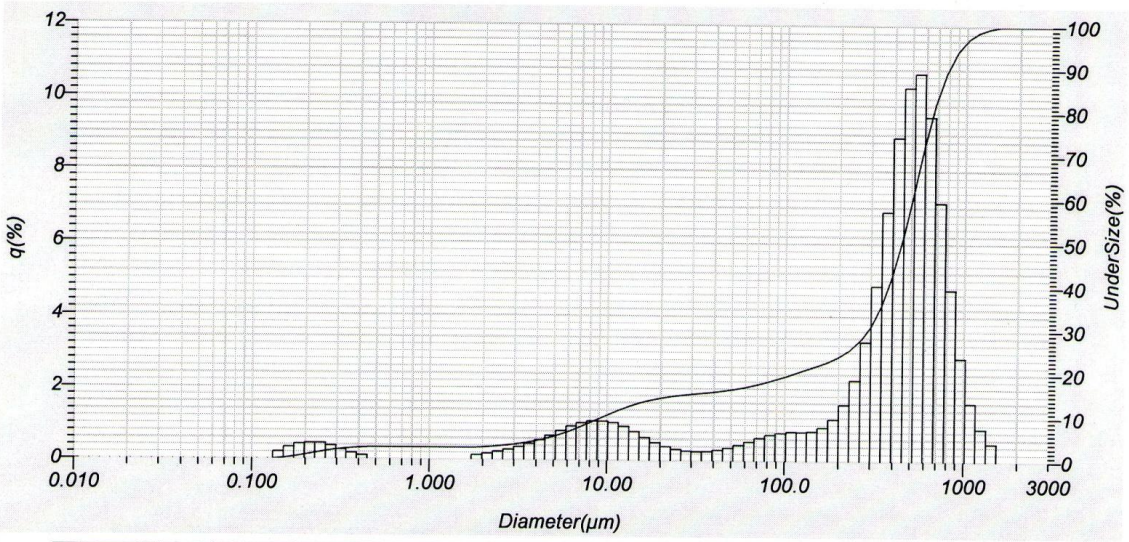
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Effluent3
Material :
Lot Number :
Source :
ID# : 200912151531795
Transmittance(R) : 86.5(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 439.15839(μm)
Mean Size : 430.47247(μm)
Diameter on Cumulative % : (2)10.00 (%)- 9.4967(μm)
: (9)90.00 (%)- 790.2200(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 21.925(%)
: (7)106.0 (μm)- 19.851(%)
: (8)75.00 (μm)- 17.958(%)
: (9)53.00 (μm)- 16.598(%)
: (10)38.00 (μm)- 15.785(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.265	2.454	51	10.097	1.076	10.486	76	300.518	3.293	31.195
2	0.013	0.000	0.000	27	0.389	0.174	2.628	52	11.565	1.036	11.522	77	344.206	4.842	36.037
3	0.015	0.000	0.000	28	0.445	0.110	2.738	53	13.246	0.933	12.456	78	394.244	6.873	42.910
4	0.017	0.000	0.000	29	0.510	0.000	2.738	54	15.172	0.789	13.245	79	451.556	8.919	51.829
5	0.020	0.000	0.000	30	0.584	0.000	2.738	55	17.377	0.633	13.878	80	517.200	10.295	62.124
6	0.022	0.000	0.000	31	0.669	0.000	2.738	56	19.904	0.494	14.372	81	592.387	10.682	72.806
7	0.026	0.000	0.000	32	0.766	0.000	2.738	57	22.797	0.385	14.756	82	678.504	9.491	82.297
8	0.029	0.000	0.000	33	0.877	0.000	2.738	58	26.111	0.311	15.067	83	777.141	7.121	89.417
9	0.034	0.000	0.000	34	1.005	0.000	2.738	59	29.907	0.268	15.335	84	890.116	4.738	94.155
10	0.039	0.000	0.000	35	1.151	0.000	2.738	60	34.255	0.251	15.586	85	1019.515	2.851	97.006
11	0.044	0.000	0.000	36	1.318	0.000	2.738	61	39.234	0.260	15.846	86	1167.725	1.605	98.611
12	0.051	0.000	0.000	37	1.510	0.000	2.738	62	44.938	0.299	16.145	87	1337.481	0.893	99.504
13	0.058	0.000	0.000	38	1.729	0.000	2.738	63	51.471	0.359	16.504	88	1531.914	0.496	100.000
14	0.067	0.000	0.000	39	1.981	0.131	2.869	64	58.953	0.438	16.942	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.172	3.041	65	67.523	0.530	17.472	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.222	3.263	66	77.339	0.628	18.100	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	0.282	3.545	67	88.583	0.718	18.818	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	0.354	3.899	68	101.460	0.772	19.590	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	0.443	4.343	69	116.210	0.809	20.398				
20	0.150	0.194	0.194	45	4.472	0.551	4.893	70	133.103	0.801	21.199				
21	0.172	0.330	0.524	46	5.122	0.675	5.568	71	152.453	0.824	22.023				
22	0.197	0.417	0.942	47	5.867	0.807	6.375	72	174.616	0.926	22.949				
23	0.226	0.441	1.382	48	6.720	0.931	7.306	73	200.000	1.152	24.101				
24	0.259	0.440	1.822	49	7.697	1.028	8.334	74	229.075	1.562	25.663				
25	0.296	0.367	2.189	50	8.816	1.076	9.410	75	262.376	2.239	27.902				

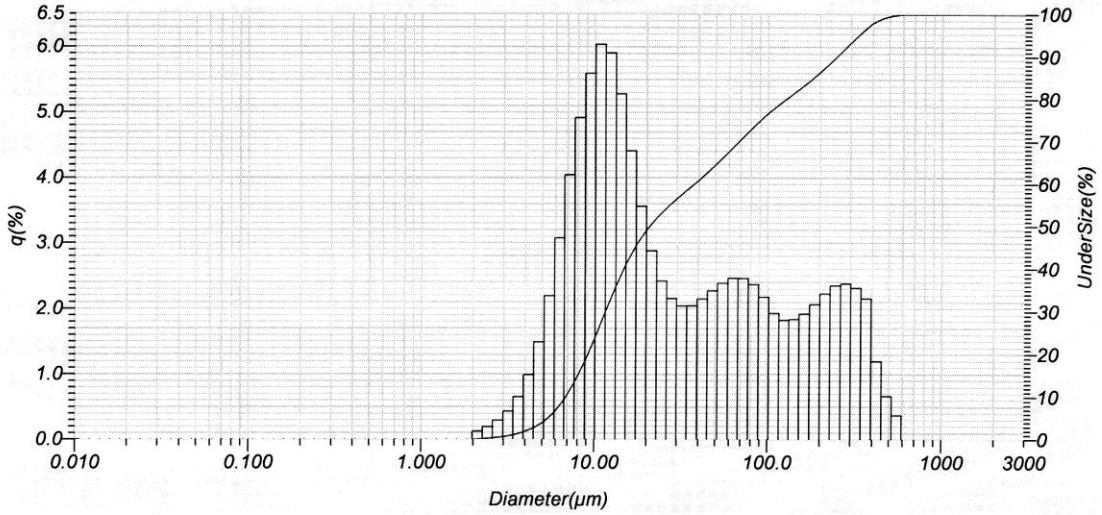
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Upstream1
Material :
Lot Number :
Source :
ID# : 200912151533796
Transmittance(R) : 77.7(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 20.76209(μm)
Mean Size : 75.71946(μm)
Diameter on Cumulative % : (2)10.00 (%)- 6.8575(μm)
: (9)90.00 (%)- 248.3082(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 82.197(%)
: (7)106.0 (μm)- 77.467(%)
: (8)75.00 (μm)- 71.758(%)
: (9)53.00 (μm)- 65.533(%)
: (10)38.00 (μm)- 60.130(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.000	0.000	51	10.097	5.591	23.934	76	300.518	2.372	93.323
2	0.013	0.000	0.000	27	0.389	0.000	0.000	52	11.565	6.036	29.970	77	344.206	2.310	95.634
3	0.015	0.000	0.000	28	0.445	0.000	0.000	53	13.246	5.902	35.871	78	394.244	2.145	97.779
4	0.017	0.000	0.000	29	0.510	0.000	0.000	54	15.172	5.275	41.146	79	451.556	1.192	98.970
5	0.020	0.000	0.000	30	0.584	0.000	0.000	55	17.377	4.406	45.552	80	517.200	0.662	99.632
6	0.022	0.000	0.000	31	0.669	0.000	0.000	56	19.904	3.554	49.106	81	592.387	0.368	100.000
7	0.026	0.000	0.000	32	0.766	0.000	0.000	57	22.797	2.873	51.979	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.000	0.000	58	26.111	2.412	54.391	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.000	0.000	59	29.907	2.147	56.539	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.000	0.000	60	34.255	2.033	58.572	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.000	0.000	61	39.234	2.038	60.610	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.000	0.000	62	44.938	2.142	62.752	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.000	0.000	63	51.471	2.267	65.019	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.000	0.000	64	58.953	2.382	67.402	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.120	0.120	65	67.523	2.458	69.860	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.189	0.309	66	77.339	2.453	72.313	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	0.288	0.597	67	88.583	2.363	74.676	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	0.431	1.028	68	101.460	2.172	76.847	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	0.649	1.677	69	116.210	1.921	78.768				
20	0.150	0.000	0.000	45	4.472	0.984	2.661	70	133.103	1.818	80.586				
21	0.172	0.000	0.000	46	5.122	1.485	4.146	71	152.453	1.829	82.416				
22	0.197	0.000	0.000	47	5.867	2.184	6.330	72	174.616	1.913	84.329				
23	0.226	0.000	0.000	48	6.720	3.067	9.397	73	200.000	2.057	86.386				
24	0.259	0.000	0.000	49	7.697	4.034	13.431	74	229.075	2.222	88.608				
25	0.296	0.000	0.000	50	8.816	4.912	18.343	75	262.376	2.344	90.952				

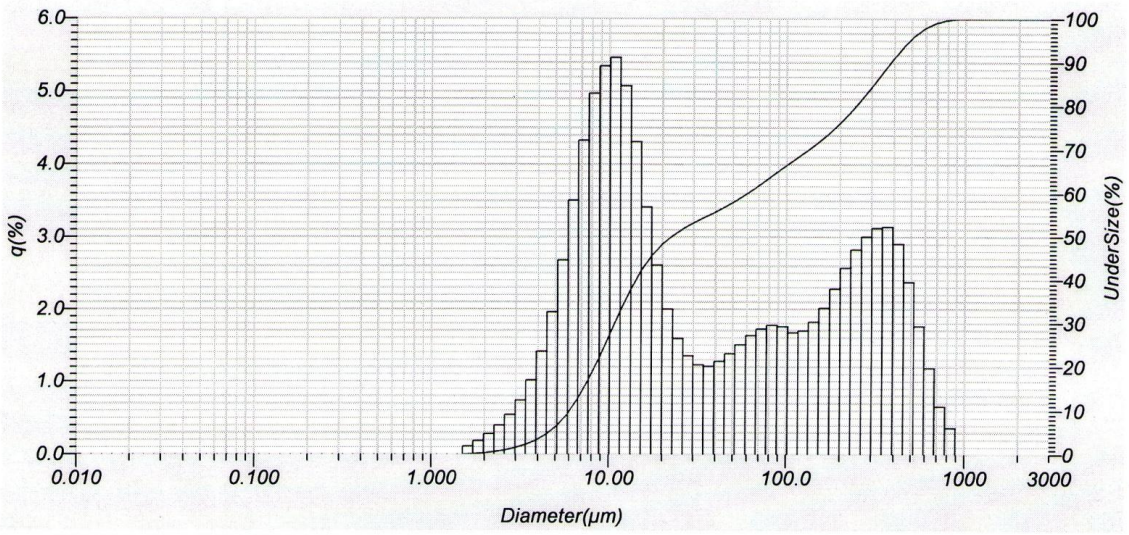
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Upstream2
Material :
Lot Number :
Source :
ID# : 200912151538797
Transmittance(R) : 77.3(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 22.37116(μm)
Mean Size : 119.31291(μm)
Diameter on Cumulative % : (2)10.00 (%)- 6.0323(μm)
: (9)90.00 (%)- 381.3455(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 71.621(%)
: (7)106.0 (μm)- 67.200(%)
: (8)75.00 (μm)- 62.747(%)
: (9)53.00 (μm)- 58.612(%)
: (10)38.00 (μm)- 55.357(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.000	0.000	51	10.097	5.345	27.426	76	300.518	3.000	84.513
2	0.013	0.000	0.000	27	0.389	0.000	0.000	52	11.565	5.463	32.889	77	344.206	3.118	87.631
3	0.015	0.000	0.000	28	0.445	0.000	0.000	53	13.246	5.071	37.960	78	394.244	3.138	90.769
4	0.017	0.000	0.000	29	0.510	0.000	0.000	54	15.172	4.305	42.265	79	451.556	2.902	93.671
5	0.020	0.000	0.000	30	0.584	0.000	0.000	55	17.377	3.412	45.677	80	517.200	2.372	96.043
6	0.022	0.000	0.000	31	0.669	0.000	0.000	56	19.904	2.606	48.283	81	592.387	1.758	97.801
7	0.026	0.000	0.000	32	0.766	0.000	0.000	57	22.797	1.994	50.277	82	678.504	1.179	98.981
8	0.029	0.000	0.000	33	0.877	0.000	0.000	58	26.111	1.586	51.863	83	777.141	0.655	99.636
9	0.034	0.000	0.000	34	1.005	0.000	0.000	59	29.907	1.346	53.209	84	890.116	0.364	100.000
10	0.039	0.000	0.000	35	1.151	0.000	0.000	60	34.255	1.227	54.436	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.000	0.000	61	39.234	1.205	55.641	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.000	0.000	62	44.938	1.272	56.913	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.109	0.109	63	51.471	1.375	58.289	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.183	0.292	64	58.953	1.500	59.789	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.278	0.570	65	67.523	1.628	61.417	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.395	0.964	66	77.339	1.720	63.136	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	0.541	1.505	67	88.583	1.772	64.908	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	0.735	2.240	68	101.460	1.753	66.662	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	1.010	3.250	69	116.210	1.669	68.330				
20	0.150	0.000	0.000	45	4.472	1.405	4.654	70	133.103	1.692	70.023				
21	0.172	0.000	0.000	46	5.122	1.956	6.610	71	152.453	1.815	71.838				
22	0.197	0.000	0.000	47	5.867	2.672	9.282	72	174.616	2.010	73.848				
23	0.226	0.000	0.000	48	6.720	3.504	12.786	73	200.000	2.275	76.123				
24	0.259	0.000	0.000	49	7.697	4.327	17.113	74	229.075	2.567	78.690				
25	0.296	0.000	0.000	50	8.816	4.968	22.081	75	262.376	2.823	81.512				

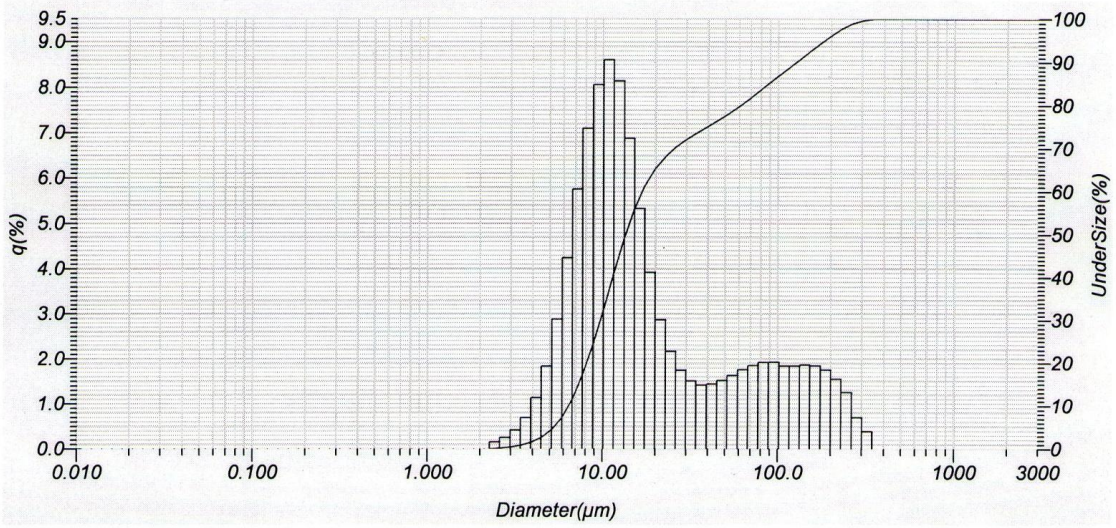
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : Upstream3
Material :
Lot Number :
Source :
ID# : 200912151540798
Transmittance(R) : 85.6(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 13.45583(μm)
Mean Size : 40.31256(μm)
Diameter on Cumulative % : (2)10.00 (%)- 6.3911(μm)
: (9)90.00 (%)- 126.5706(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 92.319(%)
: (7)106.0 (μm)- 87.602(%)
: (8)75.00 (μm)- 82.752(%)
: (9)53.00 (μm)- 78.291(%)
: (10)38.00 (μm)- 74.662(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.000	0.000	51	10.097	8.054	32.465	76	300.518	0.693	99.615
2	0.013	0.000	0.000	27	0.389	0.000	0.000	52	11.565	8.604	41.070	77	344.206	0.385	100.000
3	0.015	0.000	0.000	28	0.445	0.000	0.000	53	13.246	8.135	49.205	78	394.244	0.000	100.000
4	0.017	0.000	0.000	29	0.510	0.000	0.000	54	15.172	6.874	56.078	79	451.556	0.000	100.000
5	0.020	0.000	0.000	30	0.584	0.000	0.000	55	17.377	5.322	61.400	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.000	0.000	56	19.904	3.917	65.317	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.000	0.000	57	22.797	2.861	68.178	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.000	0.000	58	26.111	2.162	70.340	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.000	0.000	59	29.907	1.740	72.079	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.000	0.000	60	34.255	1.505	73.585	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.000	0.000	61	39.234	1.409	74.994	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.000	0.000	62	44.938	1.434	76.428	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.000	0.000	63	51.471	1.512	77.940	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.000	0.000	64	58.953	1.625	79.565	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.000	0.000	65	67.523	1.756	81.321	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.149	0.149	66	77.339	1.850	83.171	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	0.251	0.399	67	88.583	1.919	85.090	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	0.414	0.814	68	101.460	1.920	87.010	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	0.683	1.497	69	116.210	1.835	88.845				
20	0.150	0.000	0.000	45	4.472	1.126	2.623	70	133.103	1.836	90.681				
21	0.172	0.000	0.000	46	5.122	1.832	4.455	71	152.453	1.860	92.541				
22	0.197	0.000	0.000	47	5.867	2.873	7.329	72	174.616	1.841	94.382				
23	0.226	0.000	0.000	48	6.720	4.237	11.566	73	200.000	1.747	96.129				
24	0.259	0.000	0.000	49	7.697	5.752	17.318	74	229.075	1.545	97.674				
25	0.296	0.000	0.000	50	8.816	7.094	24.411	75	262.376	1.248	98.922				

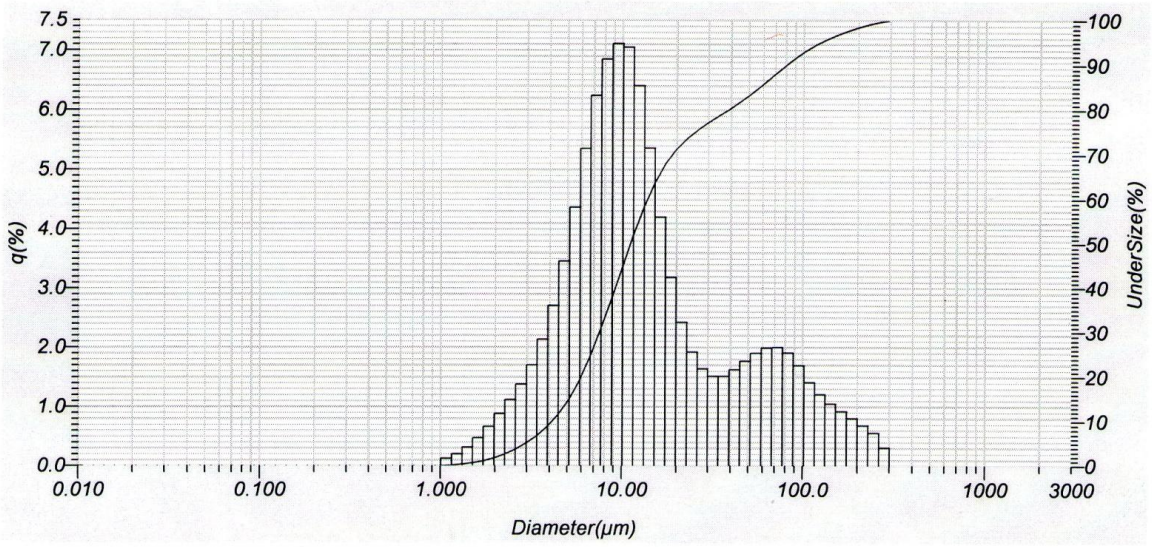
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : 500mUpstream1
Material :
Lot Number :
Source :
ID# : 200912151543799
Transmittance(R) : 63.2(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 11.11624(μm)
Mean Size : 28.50386(μm)
Diameter on Cumulative % : (2)10.00 (%)- 4.1180(μm)
: (9)90.00 (%)- 80.2750(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 96.613(%)
: (7)106.0 (μm)- 93.530(%)
: (8)75.00 (μm)- 89.025(%)
: (9)53.00 (μm)- 84.003(%)
: (10)38.00 (μm)- 79.845(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.000	0.000	51	10.097	7.104	45.010	76	300.518	0.309	100.000
2	0.013	0.000	0.000	27	0.389	0.000	0.000	52	11.565	7.044	52.054	77	344.206	0.000	100.000
3	0.015	0.000	0.000	28	0.445	0.000	0.000	53	13.246	6.403	58.457	78	394.244	0.000	100.000
4	0.017	0.000	0.000	29	0.510	0.000	0.000	54	15.172	5.358	63.815	79	451.556	0.000	100.000
5	0.020	0.000	0.000	30	0.584	0.000	0.000	55	17.377	4.201	68.017	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.000	0.000	56	19.904	3.181	71.198	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.000	0.000	57	22.797	2.418	73.616	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.000	0.000	58	26.111	1.920	75.537	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.000	0.000	59	29.907	1.637	77.174	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.122	0.122	60	34.255	1.513	78.687	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.196	0.319	61	39.234	1.515	80.202	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.310	0.629	62	44.938	1.626	81.827	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.468	1.097	63	51.471	1.766	83.593	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.661	1.758	64	58.953	1.900	85.493	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.877	2.635	65	67.523	1.987	87.480	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	1.110	3.746	66	77.339	1.997	89.477	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	1.373	5.119	67	88.583	1.907	91.383	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	1.697	6.816	68	101.460	1.693	93.076	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	2.125	8.941	69	116.210	1.408	94.484				
20	0.150	0.000	0.000	45	4.472	2.701	11.642	70	133.103	1.204	95.688				
21	0.172	0.000	0.000	46	5.122	3.454	15.096	71	152.453	1.050	96.738				
22	0.197	0.000	0.000	47	5.867	4.367	19.464	72	174.616	0.919	97.657				
23	0.226	0.000	0.000	48	6.720	5.351	24.814	73	200.000	0.800	98.457				
24	0.259	0.000	0.000	49	7.697	6.242	31.056	74	229.075	0.680	99.136				
25	0.296	0.000	0.000	50	8.816	6.849	37.906	75	262.376	0.555	99.691				

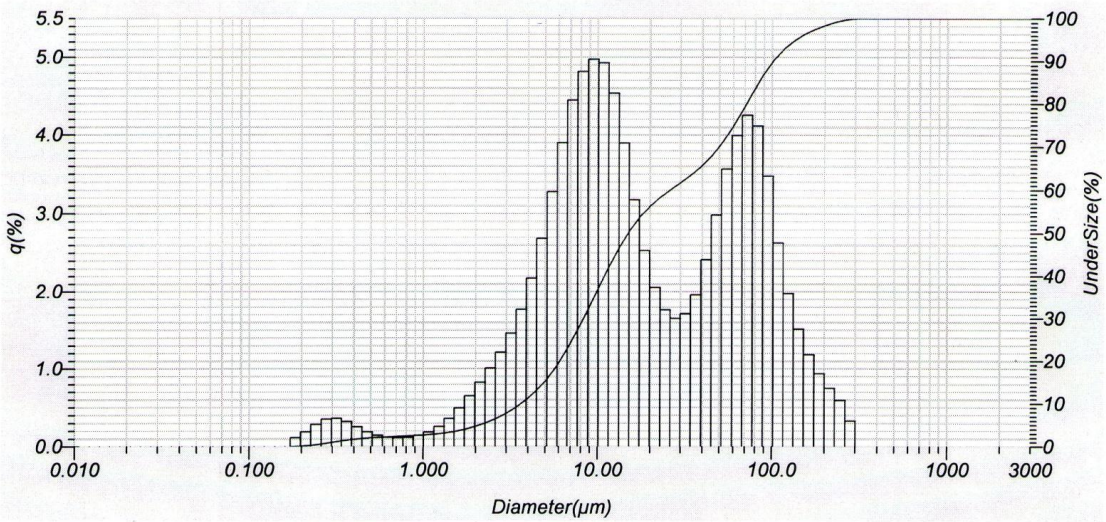
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : 500mUpstream2
Material :
Lot Number :
Source :
ID# : 200912151545800
Transmittance(R) : 69.0(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 14.91604(μm)
Mean Size : 39.06691(μm)
Diameter on Cumulative % : (2)10.00 (%)- 3.6491(μm)
: (9)90.00 (%)- 101.0210(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 96.047(%)
: (7)106.0 (μm)- 90.959(%)
: (8)75.00 (μm)- 81.561(%)
: (9)53.00 (μm)- 71.486(%)
: (10)38.00 (μm)- 64.860(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	0.361	1.295	51	10.097	4.971	37.140	76	300.518	0.327	100.000
2	0.013	0.000	0.000	27	0.389	0.318	1.613	52	11.565	4.923	42.063	77	344.206	0.000	100.000
3	0.015	0.000	0.000	28	0.445	0.250	1.863	53	13.246	4.532	46.595	78	394.244	0.000	100.000
4	0.017	0.000	0.000	29	0.510	0.188	2.051	54	15.172	3.893	50.488	79	451.556	0.000	100.000
5	0.020	0.000	0.000	30	0.584	0.144	2.196	55	17.377	3.174	53.662	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.119	2.315	56	19.904	2.530	56.192	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.111	2.426	57	22.797	2.051	58.243	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.117	2.544	58	26.111	1.761	60.004	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.140	2.684	59	29.907	1.651	61.655	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.184	2.868	60	34.255	1.710	63.364	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.255	3.123	61	39.234	1.957	65.321	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.357	3.479	62	44.938	2.414	67.735	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.490	3.970	63	51.471	2.983	70.718	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.648	4.618	64	58.953	3.562	74.280	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.821	5.440	65	67.523	3.991	78.271	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	1.006	6.446	66	77.339	4.252	82.523	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	1.211	7.657	67	88.583	4.112	86.635	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	1.456	9.113	68	101.460	3.476	90.111	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	1.769	10.882	69	116.210	2.628	92.739				
20	0.150	0.000	0.000	45	4.472	2.175	13.056	70	133.103	1.976	94.715				
21	0.172	0.000	0.000	46	5.122	2.683	15.739	71	152.453	1.512	96.227				
22	0.197	0.114	0.114	47	5.867	3.277	19.016	72	174.616	1.179	97.406				
23	0.226	0.187	0.301	48	6.720	3.896	22.912	73	200.000	0.935	98.341				
24	0.259	0.282	0.583	49	7.697	4.443	27.355	74	229.075	0.744	99.085				
25	0.296	0.351	0.934	50	8.816	4.814	32.169	75	262.376	0.588	99.673				

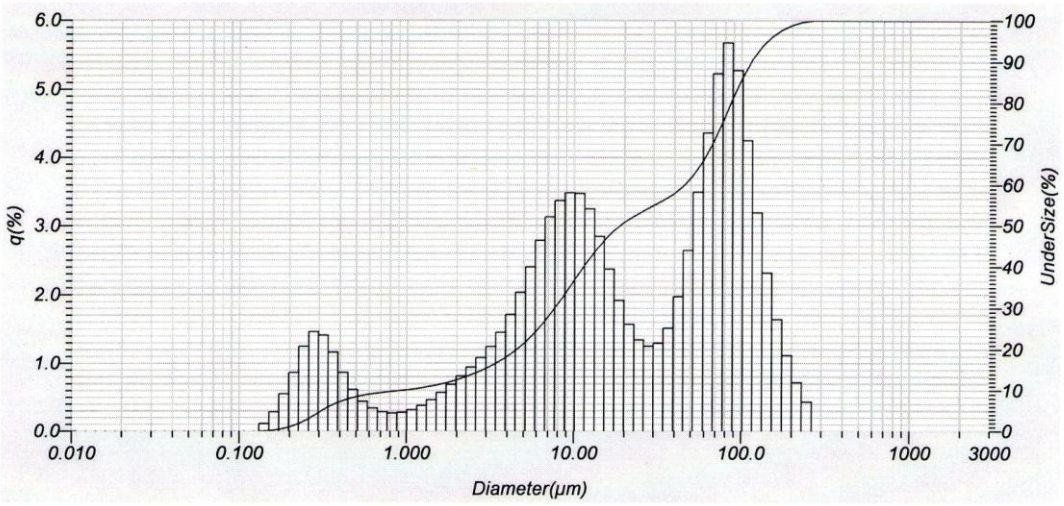
HORIBA Laser Scattering Particle Size Distribution Analyzer Partica LA-950

Horiba LA950 for Windows [Wet] Ver3.50

Sample Name : 500mUpstream3
Material :
Lot Number :
Source :
ID# : 200912151547801
Transmittance(R) : 88.7(%)

Feed liquid level : Middle
Number of times of rinse : 3

Median Size : 18.85178(μm)
Mean Size : 44.88219(μm)
Diameter on Cumulative % : (2)10.00 (%)- 0.9441(μm)
: (9)90.00 (%)- 113.9664(μm)
Cumulative % on Diameter : (6)150.0 (μm)- 95.834(%)
: (7)106.0 (μm)- 87.733(%)
: (8)75.00 (μm)- 74.227(%)
: (9)53.00 (μm)- 63.083(%)
: (10)38.00 (μm)- 57.363(%)



No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)	No.	Diameter(μm)	q(%)	UnderSize(%)
1	0.011	0.000	0.000	26	0.339	1.405	5.903	51	10.097	3.482	36.924	76	300.518	0.000	100.000
2	0.013	0.000	0.000	27	0.389	1.158	7.062	52	11.565	3.471	40.395	77	344.206	0.000	100.000
3	0.015	0.000	0.000	28	0.445	0.859	7.921	53	13.246	3.245	43.639	78	394.244	0.000	100.000
4	0.017	0.000	0.000	29	0.510	0.609	8.530	54	15.172	2.844	46.484	79	451.556	0.000	100.000
5	0.020	0.000	0.000	30	0.584	0.438	8.968	55	17.377	2.367	48.851	80	517.200	0.000	100.000
6	0.022	0.000	0.000	31	0.669	0.335	9.304	56	19.904	1.916	50.766	81	592.387	0.000	100.000
7	0.026	0.000	0.000	32	0.766	0.282	9.586	57	22.797	1.563	52.329	82	678.504	0.000	100.000
8	0.029	0.000	0.000	33	0.877	0.265	9.851	58	26.111	1.339	53.669	83	777.141	0.000	100.000
9	0.034	0.000	0.000	34	1.005	0.275	10.126	59	29.907	1.249	54.917	84	890.116	0.000	100.000
10	0.039	0.000	0.000	35	1.151	0.312	10.438	60	34.255	1.292	56.210	85	1019.515	0.000	100.000
11	0.044	0.000	0.000	36	1.318	0.374	10.812	61	39.234	1.509	57.719	86	1167.725	0.000	100.000
12	0.051	0.000	0.000	37	1.510	0.460	11.272	62	44.938	1.969	59.688	87	1337.481	0.000	100.000
13	0.058	0.000	0.000	38	1.729	0.566	11.839	63	51.471	2.642	62.330	88	1531.914	0.000	100.000
14	0.067	0.000	0.000	39	1.981	0.685	12.524	64	58.953	3.491	65.820	89	1754.613	0.000	100.000
15	0.076	0.000	0.000	40	2.269	0.810	13.333	65	67.523	4.362	70.182	90	2009.687	0.000	100.000
16	0.087	0.000	0.000	41	2.599	0.938	14.271	66	77.339	5.228	75.411	91	2301.841	0.000	100.000
17	0.100	0.000	0.000	42	2.976	1.077	15.348	67	88.583	5.679	81.089	92	2636.467	0.000	100.000
18	0.115	0.000	0.000	43	3.409	1.240	16.588	68	101.460	5.274	86.363	93	3000.000	0.000	100.000
19	0.131	0.000	0.000	44	3.905	1.445	18.033	69	116.210	4.247	90.610				
20	0.150	0.109	0.109	45	4.472	1.705	19.738	70	133.103	3.189	93.799				
21	0.172	0.282	0.391	46	5.122	2.027	21.765	71	152.453	2.311	96.110				
22	0.197	0.547	0.938	47	5.867	2.398	24.163	72	174.616	1.634	97.745				
23	0.226	0.860	1.798	48	6.720	2.783	26.947	73	200.000	1.112	98.857				
24	0.259	1.243	3.041	49	7.697	3.127	30.074	74	229.075	0.715	99.572				
25	0.296	1.456	4.498	50	8.816	3.368	33.442	75	262.376	0.428	100.000				

8.2 Appendix 2

Temperature and relative humidity for three sample storage conditions.

	Room		Freezer		Fridge	
	Temperature (°C)	Humidity (%RH)	Temperature (°C)	Humidity (%RH)	Temperature (°C)	Humidity (%RH)
17/02/2010	21.8	21.9	-18.6	23.4	5.5	19.8
18/02/2010	21.6	29.6	-19.5	32.3	5.4	26.9
19/02/2010	21.4	24.5	-19.4	26.7	5.4	22.2
20/02/2010	20.3	24.3	-20.0	26.5	5.3	21.9
21/02/2010	20.5	30.8	-20.3	33.9	5.3	27.9
22/02/2010	21.7	31.5	-19.1	34.5	5.4	28.1
23/02/2010	22.2	28.4	-18.7	31.1	5.4	25.8
24/02/2010	22.6	35.7	-19.3	39.6	5.9	32.8
25/02/2010	22.8	36.2	-20.5	40.9	5.7	33.5
26/02/2010	22.9	28.9	-20.4	32.1	5.6	26.5
27/02/2010	23.0	32.4	-20.4	36.4	5.7	30.1
28/02/2010	23.2	28.9	-20.4	32.3	5.7	26.6
01/03/2010	22.7	25.2	-20.7	28.0	5.8	23.0
02/03/2010	22.6	24.5	-20.9	27.3	5.8	22.5
03/03/2010	22.7	20.9	-20.8	23.0	5.7	18.9
04/03/2010	23.2	19.8	-20.5	21.6	5.7	17.7
05/03/2010	22.4	18.8	-20.8	20.7	5.8	16.9
06/03/2010	22.9	20.5	-20.8	22.6	5.8	18.6
07/03/2010	20.8	16.1	-21.5	17.5	5.7	14.3
08/03/2010	22.1	17.9	-21.2	19.8	5.7	16.2
09/03/2010	22.7	22.1	-20.9	24.4	5.7	20.4
10/03/2010	22.7	21.4	-20.7	23.7	6.0	19.8
11/03/2010	22.5	20.8	-20.8	22.8	5.7	18.9
12/03/2010	22.4	24.3	-21.0	27.0	5.7	22.8
13/03/2010	22.2	26.9	-21.1	29.7	5.7	25.1
14/03/2010	22.7	24.1	-21.0	26.5	5.7	22.9
15/03/2010	22.9	25.7	-20.7	28.5	5.7	23.9
16/03/2010	23.0	22.5	-20.4	24.7	5.7	20.4
17/03/2010	23.0	30.1	-20.5	33.5	5.7	28.4
18/03/2010	22.8	30.5	-20.5	33.7	5.7	28.0

19/03/2010	23.3	40.2	-20.1	45.2	6.1	37.5
20/03/2010	23.5	40.0	-19.9	45.2	6.2	36.8
21/03/2010	23.1	33.4	-20.0	37.0	6.0	30.4
22/03/2010	23.0	32.5	-20.1	36.1	6.0	30.3
23/03/2010	22.3	34.1	-20.3	37.9	6.0	33.0
24/03/2010	22.4	38.6	-20.3	43.4	5.9	38.1
25/03/2010	22.9	41.9	-20.0	47.3	6.2	40.1
26/03/2010	23.2	33.1	-20.1	37.2	6.3	30.4
27/03/2010	22.9	34.4	-20.3	38.4	6.2	31.6
28/03/2010	22.8	34.6	-20.3	38.7	6.2	31.7
29/03/2010	22.9	39.8	-20.0	44.5	6.2	36.6
30/03/2010	23.0	34.1	-20.0	38.0	6.2	31.1
31/03/2010	23.0	26.3	-20.3	28.9	6.2	23.7
01/04/2010	22.7	24.0	-20.6	26.4	6.2	21.5
02/04/2010	22.6	31.3	-20.5	34.9	6.2	28.8
03/04/2010	22.7	31.2	-20.4	35.3	6.2	29.9
04/04/2010	22.6	27.9	-20.6	30.8	6.2	26.5
05/04/2010	22.6	30.8	-20.7	34.4	6.1	29.7
06/04/2010	22.7	31.1	-20.5	34.4	6.2	28.9
07/04/2010	22.9	34.6	-20.5	38.8	6.2	31.6
08/04/2010	23.4	26.6	-20.3	29.4	6.4	24.1
09/04/2010	23.4	27.9	-20.3	31.1	6.6	25.5
10/04/2010	23.4	28.7	-20.3	31.9	6.5	26.2
11/04/2010	23.4	25.5	-20.3	28.3	6.4	23.2
12/04/2010	23.3	27.3	-20.3	30.4	6.5	25.0
13/04/2010	23.0	26.4	-20.4	29.3	6.2	24.0
14/04/2010	23.0	25.9	-20.6	28.8	5.5	23.7
15/04/2010	23.1	26.6	-20.6	29.6	5.5	24.4
16/04/2010	23.0	26.1	-20.5	28.9	5.5	23.8
17/04/2010	22.7	26.4	-20.6	29.4	5.6	24.1
18/04/2010	22.7	26.3	-20.5	29.2	5.5	24.0
19/04/2010	23.0	28.6	-20.3	31.9	5.5	26.3
20/04/2010	23.2	25.7	-20.0	28.2	5.6	23.2
21/04/2010	22.2	22.9	-20.8	25.2	5.6	20.4
22/04/2010	22.9	23.9	-20.7	26.5	5.5	20.9
23/04/2010	22.9	24.6	-20.7	27.4	5.5	21.5

8.3 Appendix 3

Standard deviation of temperature and humidity data collected.

	Room		Freezer		Fridge	
	Temperature (°C)	Humidity (%RH)	Temperature (°C)	Humidity (%RH)	Temperature (°C)	Humidity (%RH)
17/02/2010	0.3	0.6	0.6	1.1	1.2	1.2
18/02/2010	1.2	3.3	0.5	3.7	0.9	3.4
19/02/2010	1.0	2.4	0.4	2.8	0.9	2.5
20/02/2010	1.1	1.5	0.4	1.9	0.8	1.9
21/02/2010	1.4	3.7	0.6	4.0	0.9	3.5
22/02/2010	1.1	3.7	0.8	4.6	0.9	4.2
23/02/2010	0.8	1.8	1.1	2.3	0.9	2.2
24/02/2010	0.4	1.2	0.7	1.9	1.8	1.9
25/02/2010	0.2	1.1	0.4	1.5	0.9	1.8
26/02/2010	0.2	4.4	0.4	5.1	1.0	4.5
27/02/2010	0.1	0.7	0.3	1.0	0.8	1.5
28/02/2010	0.2	1.8	0.3	2.2	0.8	2.3
01/03/2010	0.5	0.8	0.4	1.0	0.8	1.2
02/03/2010	0.8	1.6	0.4	2.0	0.7	1.8
03/03/2010	0.8	2.8	0.4	3.5	0.8	3.0
04/03/2010	0.5	2.7	0.4	3.2	0.8	2.8
05/03/2010	1.0	1.2	0.5	1.7	0.9	1.6
06/03/2010	0.4	4.1	0.4	4.8	0.7	4.0
07/03/2010	1.7	1.0	0.7	1.3	0.7	1.3
08/03/2010	1.2	2.4	0.5	2.9	0.8	2.5
09/03/2010	0.8	1.2	0.5	1.4	0.9	1.7
10/03/2010	0.7	1.1	0.4	1.4	1.2	1.8
11/03/2010	0.4	1.4	0.3	1.7	0.9	1.4
12/03/2010	0.6	4.0	0.3	4.7	0.8	3.5
13/03/2010	0.6	1.8	0.3	2.2	0.8	1.7
14/03/2010	0.3	1.6	0.2	1.9	0.8	1.8
15/03/2010	0.2	1.7	0.4	2.0	0.8	1.8
16/03/2010	0.2	1.5	0.3	2.0	0.8	1.9
17/03/2010	0.2	3.2	0.3	3.7	0.9	3.2
18/03/2010	0.4	3.6	0.3	4.2	0.8	3.7

19/03/2010	0.3	1.2	0.4	1.8	0.9	2.1
20/03/2010	0.2	1.5	0.3	2.0	0.6	2.1
21/03/2010	0.2	2.0	0.3	2.3	0.8	2.2
22/03/2010	0.1	2.0	0.4	2.6	0.8	2.6
23/03/2010	0.7	4.4	0.4	5.3	0.8	4.9
24/03/2010	0.6	2.9	0.5	3.6	0.8	3.1
25/03/2010	0.3	4.3	0.4	5.0	0.8	4.9
26/03/2010	0.2	0.6	0.4	0.9	0.7	1.3
27/03/2010	0.2	2.4	0.2	2.9	0.7	2.7
28/03/2010	0.2	3.0	0.1	3.6	0.7	3.2
29/03/2010	0.2	1.4	0.4	1.7	0.7	1.8
30/03/2010	0.2	4.3	0.4	5.1	0.7	4.3
31/03/2010	0.2	2.2	0.3	2.6	0.7	2.4
01/04/2010	0.2	1.8	0.4	2.2	0.7	2.0
02/04/2010	0.1	1.8	0.3	2.2	0.7	2.0
03/04/2010	0.1	1.4	0.4	2.4	0.7	1.6
04/04/2010	0.1	4.3	0.4	5.1	0.7	4.4
05/04/2010	0.2	4.9	0.4	5.8	0.7	5.1
06/04/2010	0.2	1.7	0.4	2.2	0.7	2.8
07/04/2010	0.6	1.9	0.4	2.3	0.6	1.9
08/04/2010	0.2	2.5	0.3	3.0	0.6	2.5
09/04/2010	0.1	1.4	0.3	1.6	0.6	1.6
10/04/2010	0.1	1.6	0.2	2.0	0.6	1.9
11/04/2010	0.1	1.3	0.3	1.6	0.6	1.5
12/04/2010	0.2	0.8	0.3	0.9	0.7	1.1
13/04/2010	0.1	1.8	0.3	2.1	0.8	2.0
14/04/2010	0.2	1.4	0.4	1.7	0.9	1.8
15/04/2010	0.1	2.5	0.4	3.1	1.0	2.8
16/04/2010	0.1	1.1	0.3	1.4	0.9	1.6
17/04/2010	0.2	1.5	0.4	1.7	1.0	1.8
18/04/2010	0.1	1.1	0.3	1.3	1.0	1.6
19/04/2010	0.2	3.0	0.3	3.6	0.9	3.3
20/04/2010	0.2	4.3	0.3	5.0	0.9	4.6
21/04/2010	1.0	2.2	0.4	2.6	1.0	2.4
22/04/2010	0.2	2.1	0.4	2.6	1.2	2.9
23/04/2010	0.2	2.0	0.4	2.5	1.0	2.3