UPTEC W 16027
Examensarbete 30 hp
September 2016

UPPSALA UNIVERSITET

SLU

Pharmaceuticals in the Environment – Concentrations Found in the Water, Soil and Crops in Kampala

Läkemedel i naturen – koncentrationer funna i vattnet, marken och grödorna i Kampala

Emma Björnberg
Anna-Klara Elenström

ABSTRACT

Pharmaceuticals in the Environment – Concentrations Found in the Water, Soil and Crops in Kampala

Emma Björnberg and Anna-Klara Elenström

In Kampala, the capital of Uganda, there is an extensive use of water mixed with wastewater for irrigation of crops. The water is taken from Nakivubo channel that flows through the centre of the city, and since the wastewater treatment in the city is insufficient, the channel water might contain pharmaceuticals that are spread to the farmlands and the crops that are grown in Nakivubo wetland. The aim of this Master's thesis was to examine the concentration of some selected pharmaceuticals in water, soil and crop samples collected from Nakivubo channel and the area surrounding it. The water was analysed from five measurement points in the Nakivubo channel and Lake Victoria. The solid samples comprised of soil and crops collected from cocoyam, maize and sugar cane fields in the Nakivubo area. The pharmaceutical analyses were carried out through pharmaceutical extraction (solid phase extraction and QuEChERS) and the use of LC-MS (liquid chromatography combined with mass spectrometry). The capacities of the water and soil to reduce pharmaceuticals were analysed and a risk assessment was made in order to determine if it was harmful to drink water from Lake Victoria, the source of drinking water for Kampala, or to eat the crops that were grown in the wetland.

A majority of the pharmaceuticals studied (42 substances) were detected in the water samples (29 substances). The most common pharmaceuticals detected in the water were atenolol, carbamazepine, sulfamethoxazole and trimethoprim. The antibiotics trimethoprim and sulfamethoxazole showed the highest average concentrations in the various water samples (26 100 ng/l and 3790 ng/l respectively). Fewer pharmaceuticals were detected in the soil compared to the water (11 substances). The pharmaceuticals most frequently found in the soil were carbamazepine and pyrimethamine and they also had the highest average concentrations along with trimethoprim, 4.6-9.4 ng/g, 8.4-14.0 ng/g and 39.6 ng/g, respectively. No pharmaceuticals could be detected in the edible part of maize and sugar cane, but lidocaine, trimethoprim and pyrimethamine were found in detectable concentrations in the yam (on average 1.2-2.2 ng/g). A significant negative correlation could be found between carbamazepine and total suspended solids (TSS) in the water (linear regression: $y = -0.67x + 3.98$, $R^2 = 0.35$, $p < 0.05$, $n = 14$). The risk assessment showed that the concentrations found in the yam and water in Lake Victoria together with the average daily intake of yam and drinking water was not hazardous to the people of Kampala. However, eating more than 0.5 kg of yam daily might pose a risk with regards to pyrimethamine. On the other hand, the concentration in the yam might decrease when it is boiled, and this has not been accounted for.

Keywords: pharmaceuticals, Nakivubo channel, wastewater, soil, crop, wetland, risk assessment, treatment

Department of Energy and Technology, Swedish University of Agricultural Sciences
Lennart Hjelms väg 9
SE-756 51 Uppsala

ISSN 1401-5765

REFERAT

Läkemedel i naturen – koncentrationer funna i vattnet, marken och grödorna i Kampala

Emma Björnberg och Anna-Klara Elenström

I Ugandas huvudstad Kampala är det vanligt att vatten blandat med avloppsvatten från den centrala Nakivubokanalen används för bevattning av grödor. Avloppsreningen i staden är bristfällig och som ett resultat släpps mycket orenat avloppsvatten ut i naturen. Det är dock oklart om det finns läkemedel i Nakivubokanalen som tas upp av jordbruksmark och grödor odlade i Nakivubos våtmark. Syftet med det här examensarbetet var att studera koncentrationen av utvalda läkemedel i vatten-, mark- och grödprover insamlade i och längs Nakivubokanalen. Prover från fem mätplatser studerades i kanalen och Victoriasjön. Mark- och grödprover fanns tillgängliga som samlats in från jams-, sockerrör- och majsfält i och kring Nakivubos våtmark. Läkemedelsanalyserna genomfördes med hjälp av läkemedelsextraktion i form av fastfasextraktion och QuEChERS samt LC-MS (vätskekromatografi kombinerat med masspektrometri). Utöver läkemedelsanalysen studerades markens och vattnets förmåga att rena läkemedel. Det utfördes även en enkel riskbedömning för att se om det var farligt att äta grödor odlade i våtmarken eller dricka vatten från Victoriasjön, som är Kampalas dricksvattenkälla.

De flesta (29 st) av de 42 studerade läkemedlen detekterades i vattenproverna. De vanligast förekommande läkemedlen i vattnet var atenolol, karbamazepin, sulfametoxazol, och trimetoprim. Trimetoprim och sulfametoxazol hade de högsta koncentrationerna i de olika mätpunkterna i vattnet i medeltal, 26 100 ng/l respektive 3790 ng/l. I marken detekterades 11 av de 42 läkemedelsämnena. De vanligast detekterade läkemedlen i marken var karbamazepin samt pyrimethamine och det var också dessa som hade högst koncentrationer i medeltal, tillsammans med trimetoprim. Dessa tre läkemedel hade koncentrationer på 4,6-9,4 ng/g; 8,4-14,0 ng/g respektive 39,6 ng/g. Inga läkemedel kunde detekteras i majsen och sockerrören, men jamsen hade detekterbara koncentrationer av både lidokain, trimetoprim och pyrimethamine (1,2-2,2 ng/g i medeltal). I vattnet erhölls ett signifikant negativt samband mellan karbamazepin och totalt suspenderat material (linjär regression: $y = -0,67x + 3,98$; $R^2 = 0,35$; $p < 0,05$; $n = 14$). Riskanalysen visade att det inte bör vara farligt att äta jamsen eller dricka vattnet från Victoriasjön givet de koncentrationer som uppmättes och de mängder jams och vatten som förtärs dagligen. Det kan dock utgöra en risk att äta mer än 0,5 kg jams om dagen.

Nyckelord: läkemedel, Nakivubokanalen, avloppsvatten, jord, gröda, våtmark, riskanalys, rening

Institutionen för energi och teknik, Sveriges lantbruksuniversitet
Lennart Hjelms väg 9
SE-756 51 Uppsala
ISSN 1401-5765

PREFACE

This Master's thesis concludes our studies in the Master Programme in Environmental and Water Engineering at Uppsala University and Swedish University of Agricultural Sciences. The project comprised 30 ECTS credits and was made on the initiative of the Swedish University of Agricultural Sciences. Supervisor was Sahar Dalahmeh, and Håkan Jönsson was subject reviewer, both working at the Department of Energy and Technology at Swedish University of Agricultural Sciences. This thesis is part of a research project entitled "Pharmaceutical Pollution at Use of Wastewater in Crop Production: Consequences and Mitigation Measures for Soil Ecosystem and Agricultural Productivity in Developing Countries" which was funded by the Swedish research Council (FORMAS) and the Swedish International Development Agency (SIDA).

First of all we would like to thank our supervisor, Sahar Dalahmeh, for all the help, support and patience during our lab work and writing this thesis. Thank you for giving us the opportunity to work with this interesting subject!

We would also like to thank our subject reviewer Håkan Jönsson for the insightful thoughts and help with the thesis. Your advice was much appreciated.

Special thanks to Allan John Komakech and Pablo Gago Ferrerro, Allan for all the help regarding the information about Kampala and Pablo for analysing our samples and helping us understand the procedure and the software used. Thanks to Swedish University of Agricultural Sciences for allowing us to use their laboratories and equipment. Thanks to Lennart Wern at SMHI for help with climate data.

We would like to thank Sana Tirgani, Johanna Krona and Oskar Skoglund for all the practical help during the lab work. We made a good team!

Finally, we would like to thank our families and friends for all the support and encouragement during the whole process. We would not have made it without you!

Emma Björnberg and Anna-Klara Elenström
Uppsala, June 2016

UPTEC W 16027 ISSN 1401-5765
Published digitally at the Department of Earth Sciences, Uppsala University, Uppsala, 2016.

POPULAR SCIENCE SUMMARY

Pharmaceuticals in the Environment – Concentrations Found in the Water, Soil and Crops in Kampala

Emma Björnberg and Anna-Klara Elenström

The use of pharmaceuticals is increasing in the world and with that follows increased emissions of pharmaceuticals to the environment. If only the human consumption is taken into consideration, pharmaceuticals from treated and untreated wastewater is the main source of the pollution. In several countries, water shortage is a serious problem, which has led to that re-use of wastewater in agriculture is both a necessity and a prerequisite for a productive cultivation. With that follow the risks of accumulation and uptake of contaminants, such as pharmaceuticals, in both the soil and in the crops grown. Also proliferation of contaminants to the drinking water is possible. Today, the conventional treatment plants are not constructed to remove pharmaceuticals from the wastewater. Constructed wetlands however are considered to be in some extent effective in treating water from pharmaceuticals and can be used as an adjunct to the conventional treatment plants.

Some pharmaceuticals are persistent in the environment. However, it is not fully determined what impact they have on nature, animals and people when they are released and mixed. There is also a lack of knowledge regarding the presence and concentration of the pharmaceuticals in water, soil and vegetation. The largest knowledge gaps are found in developing countries where only a few studies have examined the prevalence of drugs in nature. The aim of this study was to investigate the presence of pharmaceuticals in water, soil and crops grown in the Nakivubo wetland in the Ugandan capital Kampala, where the crops are irrigated with diluted wastewater. The thesis also aimed to examine whether the content of pharmaceuticals in the crops grown and the raw water in Lake Victoria possess a health risk to the population if consumed. It has also been studied if there exist a natural treatment of pharmaceuticals during the diluted wastewater transport through the environment and if there is a way to reduce the source of pharmaceuticals through irrigated crops with an appropriately chosen method for Kampala.

Thereby, the intent of the thesis was to increase knowledge and awareness, for the residents in and around Nakivubo, about the pharmaceutical presences in Kampala. The intent was also to highlight key areas related to pharmaceutical management and to be a basis for further studies.

In Kampala, only around 7 % of the population is connected to the municipal sewage treatment plant in Bugolobi, and the others rely on pit latrines, plastic bags or open defecation. Many industries in the area do not treat their wastewater either. This means that large volumes of untreated wastewater is released and then transported in the Nakivubo channel, which flows from central Kampala through Nakivubo wetland and then reach its outlet in Lake Victoria. The wetland has received large amounts of untreated wastewater for over 60 years and serves as Kampala's largest water treatment plant with regards to nutrients and metals. Some parts of the wetland are also used for cocoyam, maize and sugar cane cultivation. The crops are for home consumption and irrigated with diluted wastewater.

Water samples from both untreated and treated wastewater were collected at five different locations in the Nakivubo channel and Lake Victoria. Solid samples were collected in the yam, maize and sugar cane fields in the wetland. The samples were collected before this thesis started at two different occasions in late April and early May 2015, and were then

stored frozen. The samples were pre-treated and extracted by two different extraction methods, one for the solid samples, and one for the liquid samples. The pharmaceuticals were separated and identified by liquid chromatography combined with mass spectrometry. In total, the presence of 42 different pharmaceuticals were analysed. The selected pharmaceuticals were based on the most prescribed and sold pharmaceuticals by pharmacies in Kampala 2015.

The results showed that the cultivated crops and the raw water from Lake Victoria should not be harmful for people to consume. The only crop that had a detectable concentrations of pharmaceuticals in the edible parts was yam and the pharmaceuticals lidocaine, pyrimethamine and trimethoprim were detected. The wastewater measured the largest number of pharmaceuticals in varying concentrations between 26 100 ± 30 300 ng/l and 4 ± 2 ng/l in the various sampling places. For some of the pharmaceuticals, like atenolol and carbamazepine, the concentration increased after the water treatment in Bugolobi water treatment facility. The concentration was highest in the untreated wastewater and then declined along the way to the end point in Lake Victoria. It was therefore assumed to have been a natural treatment of the water during the transport through the channel and the wetland. In the soil, 11 different pharmaceuticals were detected in varying concentrations between 2 ng/g and 40 ± 14 ng/g, where pyrimethamine and carbamazepine were the two most frequent detected. The concentrations and pharmaceutical prevalence differed between the soils. Something needs to be done about the poor sanitation situation in Kampala. More toilets with good handling chains for the sewage needs to be installed in slum areas and the treatment of the existing wastewater treatment plant needs to be studied further, in order to reduce the discharge of pharmaceuticals to the environment.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Läkemedel i naturen – koncentrationer funna i vattnet, marken och grödorna i Kampala

Emma Björnberg och Anna-Klara Elenström

Läkemedelsanvändningen ökar i världen och med det följer ökade utsläpp av läkemedel till naturen. Den största utsläppskällan är, endast sett till människors läkemedelsanvändning, från renat och orenat avloppsvatten. På flera platser i världen är vattenbrist ett allvarligt problem, vilket har lett till att återanvändning av avloppsvatten inom jordbruket både är en nödvändighet och en förutsättning för ett produktivt jordbruk. Då följer risker för problem som ackumulering och upptag av föroreningar, exempelvis läkemedel, i både marken och i de odlade grödorna. Även risk för spridning av föroreningar till dricksvattnet finns. I dagsläget är inte de konventionella reningsverken byggda för att rena bort läkemedel från avloppsvatten. Konstruerade våtmarker anses vara effektiva att till en viss grad rena vattnet från läkemedel och kan användas som ett komplement till den konventionella reningen.

Vissa läkemedel är svårnedbrytbara i naturen. Det är dock inte helt fastställt vilken påverkan de har på natur, djur och människor när de släpps ut flera på en gång och blandas till en läkemedelscocktail. Det råder också kunskapsbrist gällande förekomst och koncentration av läkemedel i vatten, mark och vegetation. De största kunskapsluckorna återfinns i utvecklingsländer där endast ett fåtal studier har undersökt förekomsten av läkemedel i naturen. Syftet med denna studie var att studera läkemedelsförekomsten i vatten, mark och grödor odlade i Ugandas huvudstad, Kampalas våtmark Nakivubo, där grödorna bevattnas med avloppsvatten. Studien syftade även till att ta reda på om läkemedlen i de odlade grödorna och vattnet i Victoriasjön utgör en risk för befolkningen vid förtäring, om det sker en naturlig rening av läkemedlen under avloppsvattnets transport genom naturen och om det finns något sätt att minska tillförseln av läkemedel via bevattnade grödor på ett lämpligt valt sätt för Kampala.

Studien avser därigenom ge ökad kunskap och ökad medvetenhet om läkemedelsförekomsten i Kampala för de boende i och omkring Nakivubo. Den hoppas även ha berört och lyft fram nyckelområden som berör läkemedelshanteringen och som kan ligga till grund för vidare studier.

I Kampala är endast ca 7 % av befolkningen anslutna till det kommunala reningsverket, Bugolobi, och resterande förlitar sig på att utföra sina behov i latringropar, plastpåsar eller ute i det fria. Samtidigt har många industrier i området inte heller någon rening av sina utsläpp. Det innebär att stora volymer av orenat avloppsvatten transporteras i Nakivubokanalen som rinner från centrala Kampala ut genom Nakivubo våtmark till utloppet i Victoriasjön. I våtmarken odlas jams, majs och sockerrör för privat bruk på fält som blir bevattnade med avloppsvattnet. Våtmarken har tagit emot stora mängder orenat avloppsvatten i över 60 år och fungerar som Kampalas största vattenrenare med avseende på näringsämnen och metaller. Frågan var om den är lika bra på att ta bort läkemedel ur vattnet.

Vattenprover av både orenat och renat avloppsvatten samlades in på fem olika platser längs med Nakivubokanalen och i Victoriasjön. Jord och grödprover togs från jams, majs och sockerrörs odlingsfält i våtmarken. Proverna samlades in vid två olika tillfällen i slutet av april och i början av maj år 2015, innan denna studie påbörjades och de förvarades nedfrysta. Proverna förbehandlades och extraherades med hjälp av två olika extraktionsmetoder och läkemedlen identifierats med hjälp av masspektrometri. Totalt analyserades förekomsten av

42 olika läkemedel. De utvalda läkemedlen baserades på de mest utskrivna och sålda läkemedlen av apotek i Kampala år 2015.

Studiens resultat visar på att de odlade grödorna och rå vattnet från Victoriasjön inte bör vara farliga för befolkningen att förtära. Den gröda som tagit upp mest läkemedel var jams och i den kunde läkemedlen lidokain, pyrimetamin och trimetoprim detekteras medan inga läkemedel kunde detekteras i de ätbara delarna av de övriga grödorna; majs och sockerrör. I avloppsvattnet uppmättes det största antalet läkemedel i varierande koncentrationer mellan 26 100 ± 30 300 ng/l och 4 ± 2 ng/l i de olika provpunkterna. För några av läkemedlen, till exempel atenolol och karbamazepin, ökade koncentrationen i utflödet från Bugolobis reningsverk. Koncentrationen var högst i det orenade avloppsvattnet och avtog sedan längs vägen till slutpunkten i Victoriasjön. Det antogs därför ha skett en naturlig rening av vattnet under transporten genom kanalen och våtmarken. I marken kunde totalt 11 olika läkemedel detekteras i varierande koncentrationer mellan 2 ng/g och 40 ± 14 ng/g, där pyrimetamin och karbamazepin var de två oftast förekommande. Koncentrationerna och läkemedelsförekomsten skilde mellan jordarna. Det behöver göras något åt den undermåliga avloppssituationen i Kampala, fler toaletter med bra hanteringskedjor för samlat toalettavfall behöver installeras i slumområden och reningen i det befintliga reningsverket behöver studeras vidare med syftet att minska utsläppet av läkemedel till naturen.

DIVISION OF WORK

The two authors have written different parts of this thesis. The distribution of the work between the authors is listed below.

Chapter	Emma	Anna-Klara
Abstract/Referat		X
Preface	X	X
Popular science summary/populärvetenskaplig sammanfattning	X	
1 Introduction	X	
1.1 Aim and objectives, 1.2 Limitations	X	X
2 Background and theory		X
2.1 Pharmaceuticals		X
2.2 Pharmaceutical use in Uganda	X	X
2.3 Characteristics of pharmaceuticals	X	
2.4 Pharmaceutical occurrence and effects on the environment and humans		X
2.4.1 Uptake of pharmaceuticals in crops		X
2.4.2 Human intake of pharmaceuticals via ingestion of crops and drinking water		X
2.4.3 Risk assessment		X
2.5.1 Wetlands	X	
2.5.2 Pharmaceuticals in soil and in farmland	X	
2.6 Review of analytical methods for pharmaceutical determination		X
2.6.1 Extraction of liquid samples	X	X
2.6.2 Extraction of solid samples	X	
2.6.3 Separation and detection, liquid and solid samples		X
3 Description of the study area	X	
3.1 Sewage collection and treatment in Kampala		X
3.1.1 Challenges with the current sewage disposal in Kampala		X
3.2 Nakivubo channel as recipient for wastewater in Kampala	X	
3.3 The geology of the Nakivubo wetland	X	
3.4 The hydrology of the Nakivubo wetland	X	
3.5 The vegetation of the Nakivubo wetland	X	
3.6 Farming in the Nakivubo wetland	X	
3.6.1 Sugar cane	X	
3.6.2 Maize	X	
3.6.3 Cocoyam	X	
4.1.1 Type of samples	X	
4.1.2 Sampling locations and methods	X	
4.1.3 Challenges during the sample collection	X	
4.1.4 Storage and preparation of samples before analyses	X	
4.2 Determination of wastewater quality parameters in the liquid samples	X	X
4.2.1 Phosphate		X
4.2.2 Total phosphorus		X
4.2.3 Nitrate	X	
4.2.4 Total nitrogen	X	
4.2.5 Chemical oxygen demand	X	
4.2.6 Total organic carbon		X
4.2.7 Total solids and total suspended solids	X	
4.3 Determination of soil quality parameters in the soil samples	X	
4.4 Survey of the types of medicines prescribed and sold in Kampala	X	
4.5.1 Types of target compounds analysed in this study		X
4.5.2 Contamination prevention and cleaning of the lab ware	X	
4.5.3 Internal standard		X
4.5.4 Extraction of pharmaceuticals in liquid samples		X
4.5.5 Extraction method for solid samples		X
4.5.6 Instrumental analysis		X
4.5.7 Calculation of the Recovery Samples	X	
4.6 Natural removal of pharmaceuticals	X	

GLOSSARY

Analyte	The compound that is of interest in an analytical study.
Anoxic	Absence of molecular oxygen, the oxygen level is zero (Nationalencyklopedin, 2016b).
Alluvial	Deposition of sediments containing sand, clay and gravel or other substances with help of running water (Nationalencyklopedin, 2016a).
Coextractives	"Additional components of a sample which are extracted along with those of interest, and which may interfere with the analysis" (Middleditch, 1989, p. 164).
Constructed wetlands	Constructed wetlands are built to have similar characteristics as natural wetlands. They work as treatment systems and use the natural processes such as soils, the vegetation of the wetland and their assembly of microbes to improve water quality (US EPA, 2015).
Exhaustiveness	Completeness, all requirements have been meet.
Hypoxic	A system with low oxygen concentrations, it is in the range of 1-30 % saturation.
Lacustrine	Something that has been made in a lake or with impact from the lake, for example sediments (Nationalencyklopedin, 2016h).
Lipid	Substances that can be solved in nonpolar organic solvents, but are usually not soluble in water (Merriam-Webster, 2016).
Matrix	Definition used in this thesis: A medium surrounding a substance that is subject for study (Nationalencyklopedin, 2016k).
Metabolite	Product that has been created through a chemical reaction in the body (Nationalencyklopedin, 2016l).
Seiche	A standing wave upcoming in an enclosed or partly enclosed body of water, for example in a tank, small and/or elongated lake. (Pugh, 1987 cited in Kansiime and Maimuna, 1999).

ACRONYMS

ADI	Acceptable daily intake
COD	Chemical Oxygen Demand
CW	Constructed wetland
DE	Detected
EDI	Estimated daily intake
HCW	Health Care Waste
HPLC	High-performance liquid chromatography
HRT	Hydraulic Retention Time
LC-MS	Liquid chromatography-mass spectrometry
LLE	Liquid/liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
NOAEL	No observed adverse effect level
OM	Organic Matter
PP tubes	Polypropylene Centrifuge tubes
SE	Soxhlet extraction
SLM	Supported liquid membrane extraction
SPE	Solid-phase extraction
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorous
TS	Total Solids
TSS	Total Suspended Solids
UAE	Ultrasound-assisted extraction

WWTP	Wastewater treatment plant
QuEChERS	Quick, easy, cheap, effective, rugged and safe

STATISTICAL DESIGNATIONS

R^2	"The coefficient of determination is a coefficient that indicates how much of the variation in the dependent variable (y) can be explained by variations in the independent variable (x) provided that the relationship between x and y are linearly dependent" (Gunnarsson, 2002). When the value is 1, all measurement points are on the regression line and the y and x variable is linear. The opposite when the value is 0, then no relationship can be seen.
p-value	Determines the significance in the results given from a statistical test. p-value < 0.05 means that the null hypothesis is rejected and hence p-value > 0.05 means that the null hypothesis cannot be rejected.
Residuals	Are used in linear regression and are the deviations of the observed values and the predictions by the model, i.e. the distribution in y-direction. A residual of an observed value is the difference between the observed value and the estimated value with help from the linear regression eruptions or sample mean (Nationalencyklopedin, 2016n).

TABLE OF CONTENTS

1 INTRODUCTION

The use of human and veterinary pharmaceuticals has increased globally in recent years and still does (Mompelat, Le Bot and Thomas, 2009). The pharmaceutical market has grown and between the year 2001 and 2004 their profit had increased with 667 billion U.S. dollars (Statista, 2016).

Pharmaceuticals are constructed to leave the body after their effect has been exerted, hence the pharmaceuticals eventually end up in the wastewater. They are also constructed to be persistent and the degradation in nature is slow for some of them. The main source of pharmaceuticals, used by humans, to the environment is household sewage (Fent, Weston and Caminada, 2006) and some pharmaceuticals have been proven to be hard to get rid of in wastewater treatment plants (Carmona, Andreu and Picó, 2014). As a consequence, pharmaceuticals are constantly released into the environment from treated or untreated wastewater (Daughton and Ternes, 1999). At the same time, the reuse of treated and untreated wastewater in crop cultivation is common in many countries, due to nutritional reversal, when nutrients like phosphorous and nitrogen are reused from sewage, and water scarcity. The reuse can pose risks to the public health, especially if the crops are eaten raw (Drechsel et al., 2010; Fuhrimann et al., 2014). Lots of studies have been done on pharmaceuticals in the developed countries and rather few in the developing countries (Zuccato et al., 2000; Brodin et al., 2013; Prosser and Sibley, 2015). A lot of studies have also been made on constructed wetlands and their ability to be a complement to the conventional wastewater treatment (Verlicchi and Zambello, 2014). Wetlands have played and still play an important role in the livelihoods of people in Africa. This is mainly because of their ability to provide food, fresh water and fuel (Wood, Dixon and McCartney, 2013).

Uganda is one of the countries that reuse wastewater for irrigation of crops cultivated in wetlands. Uganda is an East African country within the equatorial belt, with a population of around 40 million residents in 2015 (Mwakikagile, 2009; Svenska FN-förbundet, 2016). The country has been classified as a low income country by the World Bank with a per capita GDP of US$ 715 year 2014 (The World Bank, 2014a; b). In some areas in Uganda, drained wetlands are used for agriculture and crop farming. In Kampala, the capital of Uganda, almost one sixth, 31 km^2, of the city is covered by wetlands. Nakivubo wetland is the largest wetland in the city and some parts of it is used for cultivation of cocoyam, maize and sugar cane. The wetland is today receiving wastewater from the city representing sewage sludge from half a million people which is approximately five times more than the amount of water that is passing through the municipal wastewater treatment plant in the area. The same wastewater is used for irrigate the crops cultivated in the wetland (Emerton, 2005).

1.1 AIM AND OBJECTIVES

The overall aim of this thesis was to evaluate occurrence and concentrations of selected pharmaceuticals in the wastewater irrigated agriculture, i.e. in irrigation water, the soil and the crops irrigated in Nakivubo wetland in Kampala. Part of the aim was also to assess the safety, with regards to intake of pharmaceuticals, of human consumption of cocoyam, maize and sugar cane crops cultivated in the wetland using wastewater, and drinking water from Lake Victoria. The specific objectives of the study included:

1. Assess the types of the prevalent pharmaceuticals in Kampala based on the most prescribed and sold pharmaceuticals in the city.

2. Evaluate the concentrations of selected pharmaceuticals in wastewater and water running in Nakivubo channel. The concentration should be studied at various measurement points along the channel: at the inflow and outflow of Bugolobi wastewater treatment plant, in the Nakivubo channel and wetland and finally in Lake Victoria.
3. Evaluate the status of the water in Nakivubo channel and Lake Victoria with regards to water quality parameters, such as nutrients and organic matter.
4. Evaluate the concentration of selected pharmaceuticals in soil used for cultivating cocoyam, maize and sugar cane, all irrigated using water running in Nakivubo channel and wetland.
5. Evaluate the occurrence of organic matter, nutrients and some metals in the soil.
6. Evaluate the uptake of selected pharmaceuticals in the edible parts of cocoyam, maize and sugar cane, all irrigated using water running in Nakivubo channel and wetland.
7. Investigate if there has been a natural treatment of the water through its journey in the Nakivubo channel, from the city to Lake Victoria, through the Nakivubo wetland and its soil.
8. Study the distribution of selected pharmaceuticals between the water and the soil.
9. Give suggestion or suggestions for reducing the concentration of pharmaceuticals in the wastewater with a method that is appropriate for Kampala.
10. Assess the human intake of pharmaceuticals through ingestions of crops and drinking water and compare with the acceptable daily intake.
11. Give suggestions for how the intake of pharmaceuticals via irrigated crops could be reduced.

1.2 LIMITATIONS

This thesis was limited to only study the negative effects of pharmaceutical residues on humans, with no regards to environmental effects on soil ecosystem, aquatic and wild life in the study area. The effects of the active ingredients of individual pharmaceuticals were evaluated, and not the effects of mixtures. Neither metabolites nor veterinary medicines were studied. The thesis did not identify point sources of pharmaceutical pollution in the water or fields. Furthermore, no suggestions were presented on how the pharmaceutical load from hospitals and pharmaceutical industries could be reduced. The thesis instead focused on how the pharmaceutical load from humans could be reduced.

2 BACKGROUND AND THEORY

In recent time, the awareness surrounding the effects of pharmaceuticals in the environment has increased. The interest in this subject has grown along with the usage of veterinary and human medicine, and with the improvement of analytical techniques allowing for better detection of pharmaceuticals in samples (Mompelat, Le Bot and Thomas, 2009). When humans consume medicine, usually a large part of it will be excreted and will most likely find its way into water, either directly or by the effluent of wastewater treatment plants (WWTPs) (Fent, Weston and Caminada, 2006). In surface waters, pharmaceuticals can cause harm when they are taken up by fish, or they can make their way into drinking water, affecting humans (Brodin et al., 2013; Mompelat, Le Bot and Thomas, 2009). If farmlands are fertilized with sewage sludge or irrigated with water containing pharmaceuticals, the crops grown on the land can take up pharmaceuticals affecting their growth (Herklotz et al., 2010; Boxall et al., 2006). If the crops contain high enough concentrations of pharmaceuticals, they might pose a health risk to humans.

In Kampala the wastewater treatment is insufficient, resulting in a lot of untreated sewage being released into the environment (African Development Bank, 2016). The Nakivubo channel receives the treated and a lot of the untreated wastewater, while crops grown in the Nakivubo wetland are irrigated with water from the channel. The Nakivubo channel has its outflow in Lake Victoria, and raw water from Lake Victoria is used to make drinking water for the city of Kampala. The Nakivubo wetland is an important part of the treatment of the water before it reaches Lake Victoria (Emerton, 2005). It is not clear how much of the pharmaceuticals consumed in Kampala end up in the channel and subsequently in the food and drinking water of the residents, or how much is removed by the wetland. Most of the studies made on pharmaceuticals in the environment have been made in developed countries that have sufficient wastewater treatment, for example Zuccato et al., (2000); Brodin et al., (2013); Prosser and Sibley, (2015). There are also few studies made on the ability of natural wetlands to remove pharmaceuticals. The studies that have been made in Kampala have primarily focused on wastewater pollution in terms of nutrients and heavy metals, and not pharmaceuticals. This thesis should contribute to increased knowledge surrounding this important subject.

2.1 PHARMACEUTICALS

Pharmaceuticals are mainly large, complex molecules, made of several different components (Brooks and Huggett, 2012, p.64). They are used to treat, cure or prevent diseases, and come in many different shapes. The pharmaceuticals that were studied in this thesis have a wide range of applications (Table 1).

Table 1. The therapeutic groups of the pharmaceuticals studied in this thesis along with their usage and examples of pharmaceuticals from every group

Therapeutic group	Use	Pharmaceutical
Analgesic	Also called painkillers. A type of medicine that serves to reduce pain without affecting consciousness or blocking nerve impulses[a].	Codeine

Therapeutic group	Use	Pharmaceutical
Antibiotic	Chemical substances produced by microorganisms that can be used to treat bacterial infections[b].	Ofloxacin, ciprofloxacin, trimethoprim
Antidepressant	Mood-enhancing drugs that can be used by people that are depressed or suffer from other mental disorders[c].	Citalopram, venlafaxine
Anti-diabetic	Lowers abnormally high glucose levels in the blood that are caused by diabetes[d].	Metformin
Antiepileptic	Prevents or treats epileptic seizures[e].	Carbamazepine, diazepam
Antifungal agent	Treats fungal infections by acting against fungal pathogens[f].	Climbazole, ketoconazole
Antihistamine	Block the effects of histamines, treating allergies and itches[g].	Cetirizine
Antihypertensive	Treats high blood pressure[h].	Irbesartan, losartan
Anti-inflammatory agent	A type of analgesic that reduce inflammation at its source[a].	Ibuprofen, naproxen
Antimalarial	Used to treat malaria[i].	Lumefantrine, pyrimethamine
Antiulcer agent	Treats ulcers in the stomach[j].	Ranitidine
β-blocker	Reduces heart rate, myocardial contractility and blood pressure[k].	Atenolol, metroprolol
Diuretics	Increases the production of urine in order to expel excess liquid from the body. These drugs can be used to treat cirrhosis or oedemas caused by heart failure[l].	Furosemide, hydrochlorothiazide
Lipid regulator	Primarily used to treat high cholesterol[m].	Atorvastatin, bezafibrate, gemfibrozil
Local anaesthetic	Produces a local loss of sensation and pain. Can be used when extracting teeth, for example[n].	Lidocaine

[a](Encyclopædia Britannica, 2016a), [b](Encyclopædia Britannica, 2016c), [c](Nationalencyklopedin, 2016c), [d](Encyclopædia Britannica, 2016d), [e](Nationalencyklopedin, 2016d), [f](Encyclopædia Britannica, 2016e), [g](Nationalencyklopedin, 2016e), [h](Nationalencyklopedin, 2016f), [i](Nationalencyklopedin, 2016j), [j](Medical Dictionary, 2008), [k](Frishman, Cheng-Lai and Chen, 2000), [l](Nationalencyklopedin, 2016g), [m](Borton and Tidy, 2014), [n](Encyclopædia Britannica, 2016b).

The shape of the medicine, the way it is administrated and the chemical and physical properties of the active ingredients, all affect the amount of medication taken up by the body.

Some of the medication will simply pass through the body without being absorbed. The part that does get absorbed will either leave the body unchanged or transform into more water-soluble forms (Apoteket, 2005). Pharmaceuticals used by humans will mostly end up in excreta (Brooks and Huggett, 2012, p.172). Urine is a major source of pharmaceuticals in the environment, and if the compound has been metabolized then sweat can also significantly contribute to the pharmaceutical load (Brooks and Huggett, 2012, p.173). Where there is a wastewater network, substances travel to wastewater treatment plants and then partly into water bodies.

2.2 PHARMACEUTICAL USE IN UGANDA

The most common diseases that cause many deaths annually in Uganda are malaria, HIV/AIDS, tuberculosis, meningitis and lower respiratory infections, which are all communicable diseases. Non-communicable diseases (NCDs) (Chronic diseases none-inherit) are also becoming more common. Self-harm, diabetes and road injuries have at least doubled since 1990 (Ministry of Health, Uganda, 2015). A problem seen in the Ugandan healthcare is that only 35 % of the public health providers could perform correct diagnosis of at least four out of the five most common diseases including diarrhoea with dehydration. Only 15 % of them were able to treat these diseases correctly (Martin and Wane, 2013). There are also some shortcomings in the way the country is handling pharmaceuticals. There are currently no systems that control the availability and the use of antibiotics, neither in the public nor in the private sector. Antibiotics are widely available, even without any prescription. Also traditional or indigenous medicines are widely spread in the country without adequate regulation. Some improvements have been done, for example the number of pharmacists in the public sector have grown from 77 in 2011/12 to 376 in 2013/14 (Ministry of Health, Uganda, 2015).

In a study by Hasunira (2008) for WHO, 1051 households in Uganda (some of them in Kampala) were interviewed about their access to and use of pharmaceuticals. Results showed that 72 % of the households were close to a public health care facility. However, 64 % of the households reported that they usually could not afford pharmaceuticals and the majority (77 %) of the households lacked medication with proper packaging and labelling (Hasunira, 2008). Nearly 80 % of the private pharmacies were situated in three of the larger cities in Uganda, one of them being Kampala (WHO, 2006). There exists two pharmaceutical manufacturers which are operating in Kampala. One of them, Cipla Quality Chemical Industries Limited, was located in the drainage area of the Nakivubo wetland (Figure 1). They are focusing on producing antiretroviral, antimalarial and hepatitis-B medicines. Their antimalarial medicine, lumartem contains the active ingredients artemether and lumefantrine (CIPLA Quality Chemicals Industries Limited, 2014). Despite the national production, essential medicine demand far exceeded supply in Kampala. In 87 % of the cases, the residents were unable to acquire medicine due to lack of money, or the pharmacies being out of stock (WHO, 2006).

There are seven hospitals in Kampala according to Mugambe et al. (2012). The International hospital Kampala was located 500 m from the Nakivubo channel and within the drainage area of the Nakivubo wetland. Every hospital create healthcare waste (HCW) where 20 % of it is waste that needs special attention, for example hazardous chemicals, scalpels and pharmaceuticals. Low income countries have generally bad or non-existent enforcement of management systems, which cause troubles in methods of taking care of the waste i.e. in the collection, treatment and disposal process. In many low income countries the waste is burned in pits and on the ground, meanwhile the liquid is dispensed without appropriate treatment

(Verlicchi, Galletti, Petrovic and Barceló, 2010; Manyele and Anicetus, 2006). The HCW management in Uganda was unfortunately similar to other low income countries in 2009 (Healthcare Waste Management Technical Working Group, 2009). Mugambe et al. (2012) studied the HCW from three of the hospitals in Kampala and found that pharmaceuticals were collected together with infectious waste in separate labelled bins. Thereafter, the waste was burned in incinerators either by onsite or offsite incineration. On average the hospitals emitted 0.24 kg infectious waste/patient/day (Mugambe et al., 2012). They also found that hospitals treating diseases that need more medication, like tuberculosis, released more pharmaceuticals to the environment than for example hospital focusing on short time medication use for diseases like malaria (Mugambe et al., 2012).

2.3 CHARACTERISTICS OF PHARMACEUTICALS

The distribution of pharmaceuticals between the liquid and solid phases depends on the physicochemical properties of the pharmaceuticals and their partitioning coefficients. Most of the pharmaceuticals are highly ionic in nature and have a low solubility. The most important coefficients that determine the distribution are the linear solid-water distribution coefficient (K_d), the organic carbon-water partition coefficient (K_{oc}), the octanol-water distribution coefficient (K_{ow}) and the logarithmic acid dissociation constant (pKa). Values on these coefficients for selected pharmaceuticals have been compiled (Table 2).

K_d is defined according to Tolls (2001, p.3397) "as the ratio of the concentrations in a sorbent phase and in a water phase at equilibrium", where in this case the sorbent is a solid. High K_d-values indicate that the compound prefers the solid phase and low K_d-values indicate that the compound is more prone to be distributed in the liquid phase. The value depends on the acidity in the solid phase, i.e. the pH of the solid (Franco, Fu and Trapp, 2009). A high K_d-value indicates that the pharmaceutical tend to absorb to the soil and hence might accumulate (Kibbey et al., 2007). K_d can be estimated with help from an equation described in Tolls (2001):

$$K_d(L/kg) = \frac{C_{Solid}\left(\frac{ng}{g}\right)}{C_{Liquid}\left(\frac{ng}{l}\right)} \times 1000 \qquad (1)$$

where C_{Solid} is the concentration of the compound in the solid phase (ng/g) and C_{Liquid} is the concentration in the liquid phase (ng/l).

Another way of measuring the distribution is the K_{oc} coefficient, which also take the carbon content of the sorbent into account. K_{oc} is a more correct estimate for those pharmaceuticals that are neutral hydrophobic chemicals. They depend on the carbon content in the sorbent for their ability to adsorb to the soil (Tolls, 2001). The K_{oc}-value is proportional to the K_d-value and can be estimated according to the equation:

$$K_{oc}(l/kg\ TOC) = K_d/f_{oc}\text{, and } f_{oc} = \frac{TOC\ (\%\ TS)}{Tot{-}C\ (\%\ TS)} \qquad (2)$$

Where f_{oc} is the fraction of organic carbon in soil, TOC is the total organic carbon (% TS) and Tot-C is the total carbon (% TS). The K_{oc}-coefficient is a good indicator for determining the mobility of organic compounds, especially in soil. Higher values of K_{oc} indicate that the compound will be stronger attached to the organic carbon in the soil, hence the compound will be less mobile (Nollet and Rathore, 2015). But the K_{oc}-coefficient could be overrated as a

mobility indicator since it only takes the carbon sorption into account and ignores sorption to other components (U.S. Environmental Protection Agency, 1996).

K_d can also be adjusted to the carbon content in the solid phase, e.g. in soil, with help from equation (2) circumscribed. Then the equation according to Chen et al. (2013) takes the following form:

$$K_d(l/kg) = K_{oc} \times f_{oc} \quad (3)$$

The definition of the dimensionless K_{ow} given from Pontolillo and Eganhouse (2001, p.2) state that K_{ow} is "the ratio of the concentration of a chemical in n-octanol and water at equilibrium at a specified temperature" (Pontolillo and Eganhouse, 2001). I.e. in this case the sorbent is n-octanol instead of a solid. The distribution coefficient shows if the compound tends to be hydrophobic or hydrophilic, if it prefers water or non-aqueous conditions. K_{ow} has an important role in describing how chemicals act in the environment e.g. their ability to bioaccumulate in organisms (Sangster, 1997). High values of K_{ow} indicate that the compound is more hydrophobic and therefore more likely to be found in the solid phase (Levén et al., in press).

Most pharmaceuticals are present as ions with different charges e.g. positive or negative, and some are present without any charge, as neutral. Actually only few pharmaceuticals are neutral or hydrophobic, around 5 – 10 % and one example is carbamazepine (Table 2). The more neutral a compound is, the more it will be divided into lipids and will therefore be found in the sludge of wastewater treatment plants and in living organisms. Temperature and pH of the surrounding environment affect the pharmaceuticals ionizing extent, as well as the type of the functional groups present (Brooks and Huggett, 2012, pp. 64-65). The logarithmic proteolysis constant, pKa, describes the acidic characteristics of the pharmaceuticals, i.e. how strong the compound acts as an acid. Pharmaceuticals that are strong acids have low pKa-values (Nationalencyklopedin, 2016m). Pharmaceuticals with a positive charge, compounds with basic characteristics, are more likely to be found in suspended particles, sediment and soil while negatively charged pharmaceuticals usually dissolves in surface waters (Brooks and Huggett, 2012; da Silva et al., 2011).

Table 2. Selected pharmaceutical and their chemical and physical properties including, molecular weight (MW), chemical formula, half-life time in soil and water, water solubility at 25 °C, the organic carbon-water partition coefficient (K_{oc}), logarithmic octanol-water distribution coefficient (log K_{ow}) and logarithmic dissociation constant (pKa). All values without any reference mark are modelled and taken from ChemSpider, 2016. The bold log K_{ow}-values are estimated and the others are database matched

Pharmaceutical	MW (g/mol)	Chemical formula	Half-life		Water solubility at 25 °C (mg/l)	K_{oc} (l/kg)	log K_{ow}	pKa
			Soils (days)	Water (days)				
Acetaminophen	151.163	$C_8H_9NO_2$	30	n.a.	14 000	61.72	0.46	9.38[a]
Amitriptyline	277.403	$C_{20}H_{23}N$	120	60	9.71	504 700	4.92	9.40[a]
Amoxicillin	365.404	$C_{16}H_{19}N_3O_5S$	75	37.5	3433	865.5	0.87	n.a.
Atenolol	266.336	$C_{14}H_{22}N_2O_3$	75	37.5	685.2	148.1	0.16	9.60[c]
Carbamazepine	236.269	$C_{15}H_{12}N_2O$	462–533[d]	37.5	17.66	3 871	2.45	7.00[c]
Cetirizine	388.888	$C_{21}H_{25}ClN_2O_3$	120	60	101.3	6 993	**-0.61**	2.70/3.57/7.56[b]
Ciprofloxacin	331.341	$C_{17}H_{18}FN_3O_3$	1 155–3 466	n.a.	11 480	35.51	0.28	6.16/8.63[a]
Clarithromycin	747.953	$C_{38}H_{69}NO_{13}$	n.a.	n.a.	n.a.	n.a	3.16[a]	8.90[a]
Climbazole	292.761	$C_{15}H_{17}ClN_2O_2$	120	60	8.281	566.3	**3.76**	n.a.
Codeine	299.364	$C_{18}H_{21}NO_3$	120	60	12 200	1 305	1.19	8,21[a]
Diazepam	284.740	$C_{16}H_{13}ClN_2O$	75	37.5	50	11 200	2.82	3.40[a]
Diclofenac	296.149	$C_{14}H_{11}Cl_2NO_2$	3-20[e]	37.5	4.518	200-630[g]	4.51	4.15[a]
Furosemide	330.744	$C_{12}H_{11}ClN_2O_5S$	120	60	149.3	188.3	2.03	3.80/7.50[b]
Hydrochlorothiazide	297.739	$C_7H_8ClN_3O_4S_2$	9-11[f]	60	1 292	79.59	-0.07	7.90[a]
Irbesartan	428.529	$C_{25}H_{28}N_6O$	75	37.5	0.05991	$8.15x10^7$	**5.31**	4.08/4.29[b]
Ketoconazole	531.431	$C_{26}H_{28}Cl_2N_4O_4$	n.a.	n.a.	0.087[a]	n.a.	4.35[a]	3.96/6.75[b]
Lidocaine	234.337	$C_{14}H_{22}N_2O$	120	60	237.7	908.6	2.44	8.01[a]
Losartan	422.911	$C_{22}H_{23}ClN_6O$	75	37.5	0.938	910 000	**4.01**	5.50[a]
Metformin	129.164	$C_4H_{11}N_5$	30	15	$1.00x10^6$	140.9	**-1.4**	12.4[b]
Metroprolol	267.364	$C_{15}H_{25}NO_3$	75	37.5	4 777	62.24	1.88	9.6[b]
Metronidazole	171.154	$C_6H_9N_3O_3$	75	37.5	25 700	10	-0.02	2.38[b]
Omeprazole	345.416	$C_{17}H_{19}N_3O_3S$	120	60	82.28	4 000	2.23	1.2[b]

Pharmaceutical	MW (g/mol)	Chemical formula	Half-life		Water solubility at 25 °C (mg/l)	K_{oc} (l/kg)	log K_{ow}	pKa
			Soils (days)	Water (days)				
Pyrimethamine	248.711	$C_{12}H_{13}ClN_4$	120	60	121.3	1 569	2.69	7.34[a]
Salbutamol	239.311	$C_{13}H_{21}NO_3$	30	15	300 000	31.67	**0.64**	10.3[b]
Sulfamethoxazole	253.278	$C_{10}H_{11}N_3O_3S$	2-7[h]	37.5	3 942	1 531	0.89	5.70[c]
Trimethoprim	290.318	$C_{14}H_{18}N_4O_3$	17.3-179[i]	60	2 334	905	0.91	7.12[c]
Venlafaxine	277.402	$C_{17}H_{27}NO$	120	60	266.7	1 464	**3.28**	3.28[a]

[a](ChemIDplus Advanced, 2016), [b](PubChem, 2016) , [c](Bonnet et al., 2010), [d](Walters, McClellan and Halden, 2010) , [e](Al-Rajab, Sabourin, Lapen and Topp, 2010) , [f](Lin and Gan, 2011), [g](Xu, Wu and Chang, 2009), [h](Liu et al., 2010), [i](Kodešová et al., 2016), n.a. not available, bold estimated log K_{ow}

2.4 PHARMACEUTICAL OCCURRENCE AND EFFECTS ON THE ENVIRONMENT AND HUMANS

Pharmaceuticals are created with the intent that they will have a biological effect (Sundstøl Eriksen et al., 2009, p.142). It is therefore clear that they will affect the environment if they are released there. Pharmaceuticals are made of several different components and those that are of importance when studying effects on the environment are the active ingredients and the metabolites created from the pharmaceuticals (Kümmerer, 2008, p.4). Pharmaceuticals can also contain adjuvants or pigments and dyes, but these usually do not affect the environment in a significant way (Kümmerer, 2008, p.4).

The German Federal Agency evaluates environmental risk assessments (ERAs) of pharmaceuticals before they are marketed. Out of 120 complete ERAs, 10 % of the pharmaceuticals were shown to have a potential environmental risk (Küster and Adler, 2014). These substances comprised of analgesics, hormones, antibiotics and antidepressants (Küster and Adler, 2014). The risk associated with pharmaceuticals in the environment lies in the tendency of the compounds to bioaccumulate, their degradability and their ecotoxicity (Apoteket, 2005). It is important to note that it is not only the concentration of a particular pharmaceutical that comprises the risk in the environment (Apoteket, 2005). A compound has bioaccumulated in an organism if the concentration is higher inside the organism than in the surrounding nature and in the food of the organism (Apoteket, 2005). When micro-pollutants accumulate in the environment it leads to food chains becoming toxic, thereby distorting the ecological balance and causing environmental pollution (Katukiza et al., 2012).

Antibiotics are bioavailable and some of them are not easily degradable, which means that they can affect the environment for a long period of time (Apoteket, 2005). In the environment the antibiotics may either inhibit functions among the microorganisms or the microorganisms will build up a resistance to the antibiotics. Both of these effects can lead to a change in ecosystem functioning and threaten the biodiversity (Apoteket, 2005). The consequence of resistant bacteria spreading is, of course, the risk of humans getting infected with resistant bacteria resulting in sicknesses that cannot be cured by antibiotics.

Pharmaceuticals that end up in water bodies can also cause problems. For example: in a study done by Brodin et al. (2013), the effects of the antidepressant drug oxazepam were studied on wild European perch. According to the study, the drug altered the feeding rate and social behaviour of the fishes, even at a low, diluted concentration of 1.8 μg/l. This had ecological and evolutionary consequences on the fishes (Brodin et al., 2013). Endocrine disruptors in the environment can lead to developmental disorders and they can affect the reproductive capacity of wild animals. A high content of the female sex hormone oestrogen in waters can lead to a higher number of females in some fish populations (Apoteket, 2005).

Sulfamethoxazole and naproxen are known to be persistent for more than a year in all natural waters (Zuccato et al., 2000). It is primarily anti-inflammatory agents and antiepileptic drugs that are found in drinking water in Europe. Anti-inflammatory agents are found because of their high consumption worldwide, and antiepileptic drugs such as carbamazepine can be found because of their high persistence (Mompelat, Le Bot and Thomas, 2009). Pharmaceuticals in drinking water usually have a lower concentration than those found in surface waters. If the concentrations in the surface water are around 100 ng/l, the concentrations in the drinking water are generally below 50 ng/l (WHO, n.d.a).

2.4.1 Uptake of Pharmaceuticals in Crops

It has been established that crops that are grown on contaminated soils can take up pharmaceuticals. Boxall et al. (2006) for example, showed that lettuces and carrots that are grown on soil contaminated with veterinary medicines could take up those pharmaceuticals at detectable levels. Dolliver, Kumar and Gupta (2007) studied the uptake of the antibiotic sulfamethazine in maize, lettuce and potato and found that all crops had absorbed it. The accumulation in plant tissue was 0.1-1.2 mg/kg dry weight, with a higher concentration in maize and lettuce than in potato.

Plants can take up pharmaceuticals from contaminated soils through their roots, and this is the main pathway of contaminants into crops. The amount of medication (contaminant) absorbed by the crop depends on the physical and chemical properties of the soil (Sundstøl Eriksen et al., 2009, p.40). Contaminants may also be taken up by direct contact between the crop tissue and soil, and shoots may absorb gaseous and particulate contaminants above ground. Contaminants can also be degraded and removed from the crops, by metabolism and transpiration (Sundstøl Eriksen et al., 2009, p.41). Contaminants are transported in the crop from the roots to the leaves and grain. Cabbage that has been irrigated with water that contains pharmaceuticals has a higher concentration of pharmaceuticals in the root structure than in the leaf and stem, which has a much lower concentration (Herklotz et al., 2010). This suggests that the part of the crop that is further away from the ground usually accumulates less pharmaceuticals. Carter et al. (2014) concluded in their study that plant uptake of pharmaceuticals is affected by the physicochemical properties of the compounds (e.g. water solubility and log K_{ow}), plant species (i.e. lipid content) and the distribution of the plant above and below ground. The concentration of pharmaceuticals in the soil also seem to affect uptake. Olliver, Kumar and Gupta (2007) found in their study that a high concentration of antibiotic in the manure resulted in a higher uptake in the crop, compared to low concentrations of the antibiotic in the manure. There are a large number of factors that affect the uptake of pharmaceuticals. It is a complicated process that cannot be explained by just one factor.

For neutrally charged compounds, the most important factor for plant uptake of pharmaceuticals is the log K_{ow}-value (Carter et al., 2014). Maximum translocation occurs at a log K_{ow} value around 1.78 (Biggs et al., 1982 cited in Ryan, Bell, Davidson and O'Connor, 1988; Carter et al., 2014). At values < 0.5 the pharmaceuticals are too hydrophilic to enter the plant, they are more prone to stay in the pore water. At values > 3 the pharmaceuticals are too hydrophobic to be taken up by the crop; they bind harder to the organic matter in the soil (Duarte-Davidson and Jones, 1996). Ionized compounds are less likely to be taken up by plants than neutral compounds (Trapp, 2000; Carter et al., 2014). Carter et al. (2014) for instance, found that there was up to 600 times larger uptake of unionized pharmaceutical carbamazepine in ryegrass compared with the ionized pharmaceuticals diclofenac, fluoxetine and propranolol.

2.4.2 Human Intake of Pharmaceuticals via Ingestion of Crops and Drinking Water

Unintentional human exposure to pharmaceuticals can happen through eating crops if the crops have taken up pharmaceuticals. If the edible part of the crop is above ground, the pharmaceuticals must translocate from the roots to the upper parts of the crop (Sundstøl Eriksen et al., 2009, p.43). Toxicity is one of the issues related to human intake of pharmaceuticals. Most pharmaceuticals have side effects, and most are only supposed to be consumed for a limited period of time. It is true however, that the concentrations of pharmaceuticals found in crops and drinking water are far lower than the therapeutic doses.

There is no certain evidence on what the long-term effects on humans are from low exposure of pharmaceuticals through food and drinking water (Colaneri, 2014). There are however, documented effects on aquatic life (Brodin et al., 2013; Apoteket 2005).

2.4.3 Risk Assessment

In order to determine the risks with eating crops grown on contaminated soil and drinking water contaminated with pharmaceuticals, a risk assessment can be made. Everything is toxic if consumed in high enough doses, even water (Trautmann and National Science Teachers Association, 2001). For this reason, a value called the acceptable daily intake (ADI) can be calculated in order to see how much of a given substance can be consumed without becoming toxic for those consuming it. The ADI shows how much of a given pharmaceutical that can be consumed each day of a person's lifespan without evocating adverse effects, as reported by Prosser and Sibley (2015). The ADI for a pharmaceutical is calculated with the help of the no observed adverse effect level (NOAEL) (mg/kg/day) or the lowest therapeutic dose for adults (mg/day) divided by the weight of the average adult and a safety factor (Prosser and Sibley, 2015). The safety factor can account for different responses between humans, the sensitivity of e.g. children and – if the lowest therapeutic dose was used to calculate the ADI instead of NOAEL – the fact that the lowest therapeutic dose not represent a no-effect level. The safety factor can also account for pharmaceuticals that have a higher toxic effect or unwanted side effects, such as cytotoxic drugs or hormonally active steroids (Prosser and Sibley, 2015; Watts et al., 2007).

In the risk assessment, the estimated daily intake (EDI) is compared with the ADI. The EDI is calculated by multiplying the concentration of pharmaceuticals in the crops with the daily consumption of the crops and the dry matter, divided by the weight of the average adult. After both the ADI and the EDI have been determined, the hazard quotient can be calculated. The hazard quotient is given by dividing the EDI with the ADI. If the hazard quotient is ≥ 0.1, the consumption of the food is a potential hazard to humans (Prosser and Sibley, 2015).

2.5 THE ABILITY OF WETLANDS AND SOILS TO REMOVE PHARMACEUTICALS

2.5.1 Wetlands

According to Aber et al. (2012) one of the most cited definition of a wetland is utilized by the U.S. Fish and Wildlife Service made by Cowardin, et al. (1979):

"Wetlands are lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water… wetlands must have one or more of the following attributes: 1) at least periodically, the land supports predominantly hydrophytes, 2) the substrate is predominantly undrained hydric soil, and 3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of each year."

Hydraulic mode, soil, vegetation and microbes are important components in a wetland (Li et al., 2014). Nakivubo wetland is in places a submerged (covered) and in other places a subsurface wetland with horizontal water flow, and therefore only those types of wetland will be discussed further.

Wetlands are one of the most productive ecosystems in the world and the amount of carbon accumulated depends on the balance between productivity and decay (Aber, Pavri and Aber, 2012). The most specific for the soil of a natural wetland is that it is anaerobic and a so called hydric soil with a high content of carbon. Organic matter has a great cation exchange ability, which in reality means that hydric soils with high organic content behave like sponges, they absorb additional nutrients, cations like ammonium and potassium and other pollutants (Welsch et al., 1995, p.80). The microbial activity and their functions in wetlands are relatively unexplored and much more research needs to be done (Bodelier and Dedysh, 2013).

There has been little research on natural wetlands and their ability to reduce pharmaceutical content in wastewater. Most authors have focused on constructed wetlands (CW) and their ability to replace conventional treatment steps, i.e. secondary or tertiary treatments, in wastewater treatment plants and the parameters which influence their treatment capacity (Verlicchi and Zambello, 2014). The transition between the natural and constructed wetland is complex since a natural ecosystem is more complex than a constructed ecosystem. Hence, in this thesis the parameters that affect the treatment capacity of CWs are believed to have similar effects on the removal capacity of natural wetlands. There are many researchers that believe that CWs would be a good alternative as a secondary or tertiary wastewater treatment step for removing pharmaceuticals. However knowledge about the pharmaceutical removing capacity of the wetlands is still low due to lack of reported studies and unanimous results (Li et al., 2014).

The parameters that have most influence on the removing capacity of constructed wetlands are vegetation, evaporation rates, hydraulic retention time (HRT), redox potential, photodegradation and temperature as seasonal variability (Verlicchi and Zambello, 2014). The connection between the physicochemical properties of the pharmaceuticals and their behaviour in a CW have been studied (Verlicchi and Zambello, 2014). Lee et al. (2011) and Park et al. (2009) have tried to find a correlation between the hydrophobic characteristics (log K_{ow}) and removal but did not find any. Lee et al. (2011) however, did see that more hydrophilic compounds like naproxen and atenolol were more efficiently removed than compounds that are more hydrophobic like carbamazepine. Whereas no relationship between the chemical structure of the pharmaceuticals, their functional groups, and the wetlands removing capacity have been found (Verlicchi and Zambello, 2014).

It is still unclear how the presence of vegetation and particular species can improve the removal capacity of CWs. It depends on a number of factors like the plants interaction with microorganism communities, the nature of the wastewater and environmental conditions (Verlicchi and Zambello, 2014). It has however been established that planted constructed wetlands have a higher removal capacity than unplanted wetlands, especially during the summer period for pharmaceuticals like ibuprofen, naproxen and diclofenac (Hijosa-Valsero et al., 2011). On average, the removal effects decreases during winter, with less vegetation and with higher water levels in constructed horizontal subsurfaces wetlands (Li et al., 2014). The presence of plants increases the evaporation due to their transpiration, greater plant size and more activity and thus during the summer the evapotranspiration rates increases even more (Verlicchi and Zambello, 2014). The evaporation decreases the outflow volume and increases the retention time of the water in the wetland, which means longer time for the pharmaceuticals and the environment of the wetland to interact with each other. This increases the removal for some pharmaceuticals (Kadlec and Wallace, 2008). Matamoros, García and Bayona (2008) found that higher hydraulic retention time (HRT) leads to higher

removal capacity for most of the selected compounds due to promotion of biodegradation and photodegradation. Also, mainly hydrophobic compounds like hormones are removed (Kadlec and Wallace, 2008).

Photodegradation, photochemical transformation of a molecule into lower molecular weight fragments, usually in an oxidation process, is widely used in the degradation of pollutants like pharmaceuticals by UV-based processes (IUPAC, 1997). Since pharmaceuticals generally contain aromatic rings or other functional groups that can either react with photogenerated transient compounds in water or absorb solar radiation directly, they can be removed by photodegradation processes. However, today there is no general rule for how the pharmaceuticals react to photodegradation (Verlicchi and Zambello, 2014). According to Matamoros et al. (2012) photodegradation and biodegradation are the most important removal pathways for pharmaceuticals in water. They also found that the effect on a pilot plant was higher than on a full-scale polishing pond, indicating that a scaling effect is of importance (Matamoros, Sala and Salvadó, 2012).

Biodegradation, decomposition of organic materials by the influence of living organisms, is one important removal pathway (Matamoros, Sala and Salvadó, 2012). Ciprofloxacin can decrease the bacterial activity and thereby reduce the overall removal capacity of the constructed wetland (Li et al., 2014). High temperatures generally improve the biodegradation of pharmaceuticals, since microorganism are more active when it is warm (Verlicchi and Zambello, 2014; Sylvia, 2005).

Redox potential, a condition at a specific location that affects the possibility of a chemical compound to acquire electrons and hence be reduced, affects the removal efficiency (Atkins and Jones, 2007). A low redox potential is related to anaerobic conditions and a high redox potential are linked to aerobic conditions (Hijosa-Valsero et al., 2010). Varying conditions, combinations of anaerobic and aerobic, tend to help biodegradation of different organic micropollutants (Matamoros, García and Bayona, 2008; Hijosa-Valsero et al., 2010).

2.5.2 Soil and Farmland

Pharmaceuticals have been found in soil due to irrigation with insufficiently treated wastewater. It has been seen that some compounds would accumulate in the soil when the soil constantly is receiving pharmaceuticals through water and hence possess environmental risks such as contamination of groundwater and uptake in plants (Kinney, Furlong, Werner and Cahill, 2006). Different pharmaceuticals behave different in the soil regarding the physiochemical characteristics of both the compound and the soil. According to Kibbey et al. (2007) the pharmaceuticals adsorption ability will vary and is hard to determine since it depends on complicated pH-interactions. The compounds partitioning coefficient K_d could for some pharmaceuticals be a good indicator of their absorption ability. Soil properties such as high organic material (OM) and clay content will also increase the amount pharmaceuticals adsorbed.

The major transport process for antibiotics that adsorb strong to the soil seems to be thorough big macropores (Thiele-Bruhn, 2003). The pharmaceuticals that easily adsorb will, in fields with surface irrigation, be found in the upper layer of the soil and decrease drastic when the soil depths increases. For example, it has been seen that the high adsorbed 4-n-nonylphenol was not detected beyond 10 cm depth in loamy sand soil and that the concentrations of all the selected pharmaceuticals decreased with the depth (Chen et al., 2013). Soil properties such as big pore sizes and less OM and clay will increase the leaching from the soil to the groundwater. The pharmaceuticals that are most prone on leaching are those with low K_d-

values (Chen et al., 2013). Chen at al. (2013) also studied the mass balance and found that degradation and adsorption were the two major pathways for the pharmaceuticals. Evaporation and leaching did together only account for approximately 2-3% in the mass balance for the studied pharmaceuticals.

The soils microbial activity is important to consider in the degradation procedure. Xu et al. (2009) showed that the decomposition rate increases when microbes are present in the soil. OM and clay content will decrease the decomposition rate of the microbes as well as high initial concentrations of the pharmaceuticals also tend to do (Xu, Wu and Chang, 2009). Photodegradation has been seen to decompose antibiotics, but in the soil at the surface, this process will be neglected since only a small amount will be decomposed (Thiele-Bruhn, 2003).

2.6 REVIEW OF ANALYTICAL METHODS FOR PHARMACEUTICAL DETERMINATION

The procedure of analysing pharmaceuticals is usually divided into five steps: sampling, sample preparation (or extraction), separation, detection and data analysis (Pavlović et al., 2007). During the sample preparation the pharmaceuticals are extracted from the sample, and during the separation the different pharmaceuticals are separated from each other with the use of e.g. chromatography. The pharmaceuticals are then detected with the help of analysis methods such as mass spectrometry.

When analysing pharmaceuticals in different matrices (e.g. soil, crop or water) the samples need to be prepared before they can be analysed. The goal with the preparation is to convert the sample into a more suitable format for analysis. There are a number of ways in which to do this but all methods have the same basic goals according to Smith (2003):

- Remove potential interferences.
- Increase the concentration of the analyte.
- Convert the analyte to a more suitable format for detection or separation.
- Provide a method that is robust and can be reproduced without the sample matrix affecting the result.

The extraction can be difficult if matrix effects influence the analyte. Matrix effects are defined as properties in the matrix that influence the ability to recover the analyte. Pharmaceuticals for example, can adsorb to organic matter, which makes it harder to extract them and therefore harder to detect (Pavlović et al., 2007).

2.6.1 Extraction of Liquid Samples

Different methods for extracting and analysing pharmaceuticals from aqueous environmental samples have been developed during recent years. The biggest challenge with analysing pharmaceuticals are the complexity of different compounds and their ability of either being more acidic or more basic. The polarities of the pharmaceuticals, along with their ability to either be lipophilic or hydrophilic and hence bind to different phases, is one more challenge (Gros, Petrović and Barceló, 2006). The presence of coextractives makes it harder to identify and quantify the target compounds (Hao et al., 2005). Examples of extracting methods for aqueous samples are: liquid/liquid extraction, supported liquid membrane extraction and solid-phase extraction.

In liquid/liquid extraction (LLE) an immiscible solvent is added to the sample and afterwards the sample is mixed or vortexed. The intention is that after the sample and the extracting solvent have separated, the analyte molecules will have moved to the extracting solvent (organic phase), and the interferences will stay in the sample (aqueous phase) (Nationalencyklopedin, 2016o). When the two phases are completely separated, the solvent containing the analyte will be removed. After the organic phase has been collected, it is evaporated with an inert gas and then reconstituted with an appropriate HPLC-solvent. The advantages with the LLE are that it is simple and has reasonable selectivity. The drawbacks with the LLE are that sometimes interferences are coextracted, or emulsions are formed which make the separation of the phases incomplete and therefore it is harder to collect only one of the phases (CHROMacademy, n.d.).

Membrane extraction was developed as an attempt to automate LLE (Pavlović et al., 2007). The most important membrane extraction method is the supported liquid membrane (SLM) extraction. The SLM has three phases, two aqueous (one donor and one acceptor) and one organic phase. The organic phase is immobilised inside a porous hydrophobic membrane and then put between the two aqueous phases. The analytes will then travel from the donor to the acceptor through the membrane (Pavlović et al., 2007). The main driving force for the transportation of the analytes is mostly the concentration gradient. By removing the analyte from the acceptor the gradient can be increased (Smith, 2003). This technique has several advantages compared to classical LLE: cleaner extracts, less consumption of organic solvents and easier automation, to name a few (Pavlović et al., 2007).

Solid-phase extraction (SPE) has largely replaced classic LLE and is now the most common extracting technique in environmental areas (Pavlović et al., 2007). In SPE, the analytes are divided between a solid phase and a liquid phase. The analytes have a higher affinity for the solid phase than the liquid phase and are therefore retained in the solid phase. The analytes are later extracted from the solid phase with an elution solvent (Smith, 2003). One advantage with SPE, is that different compounds simultaneously is extracted in only one extracting step. Important factors influencing the SPE method occur during the pretreatment of the samples e.g. different pH-levels, the temperature of the samples and the elution solvent. One other widely used way is to combine two different SPE materials or categorising two SPE groups depending on their physicochemical properties and the pharmaceutical classification. In that way, two or more extracting step will be performed and the method will be more specific in targeting pharmaceuticals with different characteristics. On the other hand, it leads to a more complicated and time-consuming performance (Gros, Petrović and Barceló, 2006).

According to Gros et al (2006), C_{18} as an SPE sorbent is most commonly used followed by Oasis HLB (hydrophilic-lipophilic balanced) cartridges (Waters, USA) using pretreatment of sample conditions. The biggest advantages with Oasis HLB are their combination of the lipophilic and the hydrophilic polymers that make it possible to extract acidic, neutral and basic compounds from a wide pH-range. The extractions are generally performed simultaneously and at neutral pH, but the preferable pH-level vary between studies, e.g. it depends on the target pharmaceutical group. This generalisation and analysis of several group of compounds makes the analytical range of the method wide but in need of compromising, which leads to not the most optimal condition for each of the compounds (Gros, Petrović and Barceló, 2006). In this thesis SPE has been used with Oasis HLB cartridges to extract pharmaceuticals from the liquid samples.

2.6.2 Extraction of Solid Samples

It is hard to analyse solid samples since most analytical instruments only can analyse liquid samples. Therefore, the first step in the sample preparation is to transfer the targeted compounds from the solid phase into a liquid phase (Luque de Castro and Priego-Capote, 2010). The sample preparation is considered by Azzouz and Ballesteros (2012) to be the most polluting step during the whole analyse process because of the use of organic solvents.

It is difficult to extract pharmaceuticals from environmental solid samples due to their low concentration in the environment (µg/l-ng/l) and also because of the variable physicochemical characteristics of the pharmaceuticals and the solid materials. There is often a strong interaction between the pharmaceuticals and the solid material, which makes them hard to extract (Sanchez-Prado, Garcia-Jares and Llompart, 2010). Analysing pharmaceuticals in plant tissues possess even additional challenges, since the presence of waxy materials, fats and pigments endanger matrices inferences. The sample preparation therefore needs to have high accuracy and analytical precision (Wu, Dodgen, Conkle and Gan, 2015). If the extraction includes a lot of different pharmaceuticals with different physicochemical and sorption characteristics, it is almost impossible to find a method that meets all the requirements of the pharmaceuticals, therefore compromising is necessary. This creates non-ideally conditions for all pharmaceuticals (Runnqvist et al., 2010).

Different methods that have been used by researchers for extracting pharmaceuticals from environmental solids are methods such as Soxhlet extraction (SE), ultrasound-assisted extraction (UAE), pressurized-liquid extraction (PLE), combined microwave-assisted extraction (MAE) with solid-phase extraction and the quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Peng et al., 2006; Pérez-Carrera et al., 2010; Tadeo, Sánchez-Brunete, Albero and García-Valcárcel, 2010; Aznar et al., 2014; Azzouz and Ballesteros, 2012; Peysson and Vulliet, 2013).

Soxhlet extraction (SE) is one of the oldest techniques of solid sample preparation and it was developed in 1879 by von Soxhlet. Since then, it has been the most widely used extraction technique for almost a century. The conventional SE is a simple method and little training is needed before use. Another advantage is that it can extract a bigger sample mass than recent methods like MAE (Luque de Castro and Priego-Capote, 2010). The main shortcomings of the method are long extraction time and use of large amount of solvents compared to recent developed methods (Luque de Castro and Priego-Capote, 2010; Tadeo et al., 2010). The conventional method has laid the ground for development of a variety of modifications that focus on maintaining the advantages and to minimise the shortcomings e.g. high-pressure SE, automated SE, ultrasound-assisted SE and microwave-assisted SE (Luque de Castro and Priego-Capote, 2010). According to Luque de Castro and Priego-Capote (2010), the Soxhlet extraction has been and practically still is, a panacea in its field.

One of the recently developed methods is the method called ultrasound-assisted extraction (UAE) and it uses ultrasound radiation for extracting compounds from solid samples. Commonly used devices for the sonication are waterbath, probes and sonoreactors. The theory behind UAE is to create cavitation, which occurs when ultrasonic waves crosses a liquid media. Cavitation is a physical process where lots of tiny bubbles are generated, growing, oscillating, splitting and then collapsing. In the way the bubbles are acting, they can be seen as micro-reactors, with a pressure near 1000 atm and temperatures near 5000 ºC. This reaction creates pitting and mechanical erosion of the solids and later particle breakage, due to the physical properties of the cavitation (Santos and Capelo, 2007). Parameters that influence the

extraction efficiency are sample amount, sonication time, the ultrasound device and sample particle size. To optimise the extraction, different factors such as solvent type, amplitude of sonication and temperature are important to reflect on. But the main thing to consider regarding the efficiency of the method is the targeted compounds different characteristics, which will determine the most efficient procedure (Tadeo et al., 2010). Advantages of this method are that it is rapid, have low solvent consumption and is reliable (Aznar et al., 2014).

The method used in this thesis is the so called quick, easy, cheap, effective, rugged and safe (QuEChERS) method, which according to Peysson and Vulliet (2013) has exactly those properties. The method was first developed in 2003, for extracting pesticides from fruit and vegetables (Anastassiades, Maštovská and Lehotay, 2003). It has then been used to determine pharmaceuticals in soil (Salvia et al., 2012) and in sewage sludge (Peysson and Vulliet, 2013). The benefits of this method are in particular that the extraction is simple, has low solvent consumption and at the same time is rapid (Salvia et al., 2012). QuEChERS is based on "a salting-out extraction" with acetonitrile as the solvent used most (Peysson and Vulliet, 2013). The exact method procedure used in this thesis has been developed and validated by the Swedish University of Agricultural Sciences. This method has been proved to be good in analysing some of the selected pharmaceuticals analysed in this thesis[1].

2.6.3 Separation and Detection, Liquid and Solid Samples

In this thesis the separation and detection step (or instrumental analysis) comprised of high-performance liquid chromatography (HPLC) for separating the samples and mass spectrometry (MS) for detecting the samples. This method is called LC-MS and utilizes the strengths of both HPLC and MS (Simonsen and Lindegren, 2005, pp.268-269). In HPLC a sample is eluted through a column with a mobile phase and a stationary phase. The components of the sample will reach the end of the column at different times, since components will adsorb to the stationary phase for a varying amount of time. The components are therefore separated and can be analysed by the mass spectrometer (Simonsen and Lindegren, 2005, p.272). In MS the molecules are ionized and decomposed into small fragments. If the molar weight and the frequency between the fragments are measured, the original molecule can be identified (Simonsen and Lindegren, 2005, p.258).

[1] Sahar Dalahmeh, Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala. 05.05.2016

3 DESCRIPTION OF THE STUDY AREA

Wetlands cover 10-13 % of Uganda's total land surface and they are home to a lot of different and unique species. Kampala, the capital of Uganda, is located on the southern side of the country, near Lake Victoria (Kansiime and Maimuna, 1999). Nakivubo wetland, a submerged and subsurface flow wetland[2], 3.8 km southeast from Kampala (00°18'N, 32°38'E) with an altitude of 1 135 m above sea level, is the largest wetland area in the capital city (Figure 1). Its surface area is 5.3 km^2 and the catchment area range over 40 km^2 (Kyambadde et al., 2004; COW/VKI, 1998 cited in Emerton, 2005). The wetland provides Kampala with lots of important ecosystem services. It takes care of most of Kampala's industrial and domestic wastewater and acts as a buffer zone before the water reaches Lake Victoria and has done so for more than 60 years (Emerton, 2005; Kansiime and Maimuna, 1999).

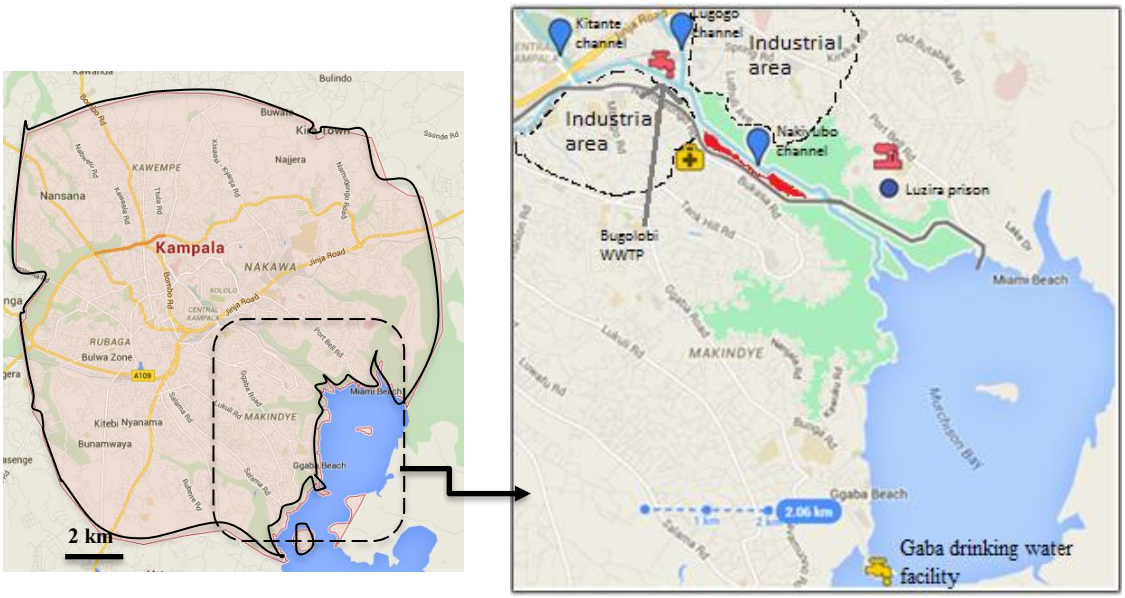

Figure 1. *Illustrating Kampala city to the left and the studied area to the right. Nakivubo wetland is shown in green and the Kasanvu Slum in red. A railway line (grey line) and the Nakivubo channel are running through the area and ends in Murchison Bay in Lake Victoria. In the north is the industrial area. The International Hospital Kampala and Cipla Quality Chemical Industries Limited facilities are marked with representing symbols. Bugolobi WWTP (wastewater treatment plant) and Gaba drinking water facility are both marked on the map, just as the Luzira prison, Kitante and Lugogo channel. Source: Map data ©2016 Google.*

A railway line running from Kampala city to Port Bell, is dividing the area into two sectors, north and south. The northern side is more dominated by papyrus vegetation, cocoyam and sugar cane cultivation than the southern side. It is also more influenced by humans, some of the area has been changed into industrial zones while the southern sector is relatively more intact (Emerton, 2005, p.77). That is mainly due to that the southern side is directly connected to the Lake Victoria while the north side is not[3]. The south side however consists mostly of wetlands, with floating vegetational islands, with miscanthidium grass and papyrus

[2] Sahar Dalahmeh, Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala. Email 10.02.2016

[3] Allan John Komakech, Department of Agriculutural & Bio systems Engineering, Makerere University, Kampala. Email 25.04.2016

vegetation, and some cocoyam and maize cultivation (Mbabazi et al., 2010a; Mbabazi et al., 2010b).

More than 100 000 people live in the outskirts of the Nakivubo wetland, in both high cost and low cost housings. Kampala's main industrial area is located north of the wetland (Figure 1) and has more than 200 different industries. The facilities produce everything from soft drinks, batteries, shoes, paper and soaps to pharmaceuticals, as described earlier (2.2). The wetland is under a major threat from development and expansion of the urban area in Kampala. The area near the Nakivubo wetland and the wetland itself are regarded as prime sites for urban development. One of the reasons is the low price on the land compared to other available land areas in the Kampala vicinity (Emerton, 2005).

3.1 SEWAGE COLLECTION AND TREATMENT IN KAMPALA

The sewered area of Kampala serves 7.3 % of the population while the majority of the population (92.7 %) in Kampala relies on on-site treatment of their waste, for example pit latrines or septic tanks (African Development Bank, 2016). The pit latrine is a common on-site sanitation system among the urban poor (Nakagiri et al., 2015). A simple pit latrine consists of a large pit dug into the ground, with a slab on top of it that has a hole in the middle where excreta can fall through (Figure 2). Around the slab there is a shed with a door, and the hole usually has a lid over it to prevent problems with odour and flies (WHO, n.d.b). The size of the pit varies with the number of people using the latrine and for how many years the users anticipate it will be needed. WHO (n.d.b) recommends a pit volume of at least 0.06 m^3/person/year.

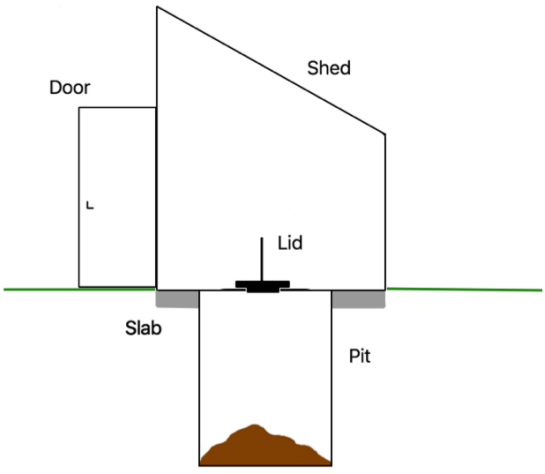

Figure 2. *Basic sketch of a simple pit latrine. The figure is not drawn to scale.*

If the pit latrine is built on a site with a high water table it can be elevated as not to pollute the groundwater. An elevated pit latrine is built with the pit above ground. The pit can be built with e.g. stones, concrete slabs or earth-covered bamboo and the shed is reached via stairs or a ladder (UNHCR, 2009). When the pit latrines are full they can either be emptied or covered with soil. The users must themselves take the initiative to empty the pits. The pit can be emptied either by tankers with suction pumps or they can be emptied manually with buckets and shovels (Pickford and Shaw, 2005). If the pit is covered a new pit will have to be dug. In those cases where there is limited space emptying may be the only option (Jenkins, Cumming and Cairncross, 2015). If the pit latrines are well-constructed and correctly operated and maintained, they can isolate, store and even treat the excreta to some extent, which lowers the health hazards surrounding untreated excreta (Nakagiri et al., 2015).

The National Water and Sewerage Corporation, a government owned public utility company in Uganda, operates two sewage treatment plants in Kampala: Bugolobi, which is a conventional treatment plant and Lubigi, which relies on waste stabilisation ponds (National Water and Sewerage Corporation, 2016). The Lubigi sewage treatment plant has the capacity to treat 5,400 m^3 wastewater/day and lies in the outskirts of Kampala. Lubigi treats piped wastewater as well as faecal sludge brought in from private cesspools, i.e. pit latrines and septic tanks (National Water and Sewerage Corporation, 2016). The Bugolobi sewage treatment plant has the capacity to treat 33,000 m^3 wastewater/day and lies near the Nakivubo channel. The wastewater consists mostly of piped sewage from the business district in Kampala (National Water and Sewerage Corporation, 2016). The effluent from both of the sewage treatment plants is checked so that the quality is in compliance with national environmental standards (National Water and Sewerage Corporation, 2016).

3.1.1 Challenges with the Current Sewage Disposal in Kampala

Due to technical shortcomings in the piping system, the Bugolobi sewage treatment plant receives only 55 % of the sewage produced in the Nakivubo catchment that is supposed to be treated. The remaining sewage is released into wetlands in the area (African Development Bank, 2016). Part of the piping system is old and often breaks down or get blocked which results in untreated sewage flowing out onto the streets and down into the channel. Some sewer channels from buildings are connected directly to tributaries of the Nakivubo channel (Kayima et al., 2008).

Pit latrines are cheap and also easily constructed and maintained. Therefore they are often used in urban poor areas in developing countries (Nakagiri et al., 2015). These areas, called slums, are inhabited by 60 % of the urban residents in Kampala (Kulabako, Nalubega and Thunvik, 2007). Among the urban poor in Kampala, 95 % of the residents have access to an on-site sanitation facility, and 70 % of the residents use shared latrines. The shared latrines are used by 30 persons, or 7 families, on average. Only 47 % of the latrines are clean enough to be used and almost half (45 %) of the latrines are abandoned after five years due to being full or broken (Günther et al., 2011). Since the latrines are often shared by a lot of people, they fill up fast and have a low hygienic standard.

Factors that prevent the use of pit latrines among the slum dwellers include, but are not limited to: latrines being filthy, high pit filling rates, long waiting times since the latrines are shared by a lot of people, impacts of flooding on the cleanliness of the latrines and latrines being inaccessible. The latrines are inaccessible if they have steep steps leading up to them, making them difficult to access for children, pregnant women and the disabled. They are also inaccessible if they lie far away from the homes of the residents making them feel unsafe for women and children, especially after nightfall (Kwiringira et al., 2014).

If the latrines are in bad shape, people will resort to open defecation instead of using the existing latrines. The use of polythene bags for disposing of excreta is also common in these situations (Kwiringira et al., 2014). In Uganda, 2 % of the urban population resorts to open defecation (WHO/UNICEF, 2015). Even latrines of the improved kind can fail in its purpose to provide sanitary protection if misused and not cleaned properly (Kwiringira et al., 2014). This means that it is not only the number of latrines that is important, but also the hygiene and cleanliness of the existing latrines.

The challenges surrounding dirty latrines are many. According to the study made by Kwiringira et al. (2014) some people will not clean after themselves in the pit latrine, even if cleaning supplies are available. This does not encourage a continuous cleaning of the latrines. Latrine cleaners also have a low status, which makes matters worse. Even if some users manage to keep their latrines clean, others that do not use latrines will litter the surroundings of the pit latrine with excreta and polythene bags (Kwiringira et al., 2014). This also discourages the use of the latrines.

Emptying pit latrines in urban slums also poses a challenge. According to Kwiringira et al. (2014), unlined pit latrines are not emptied for fear of them collapsing. Because of the dense housing in the slums, cesspool emptier services have difficulties reaching the latrines with their vehicles, and subsequently emptying the latrines. The high cost of emptying them was also a hindrance (Kwiringira et al., 2014; Katukiza et al., 2012). In those cases where the residents could not afford the emptying of the latrine, they would hire local contractors that manually empty the above ground latrines by making a hole in the side of the faecal sludge chamber (Katukiza et al., 2012). This poses a health risk for the people emptying the latrine, and it is not possible to control where the sludge ends up. According to African Development Bank (2016) and Kayima et al. (2008), untreated sewage from septic tanks and pit latrines are often discharged into the environment or the Nakivubo channel. During the rainy seasons the dirt is washed away from the slums and into the Nakivubo channel (Kayima et al., 2008).

3.2 NAKIVUBO CHANNEL AS RECIPIENT FOR WASTEWATER IN KAMPALA

The 12.3 km long constructed Nakivubo channel act as Kampala's main drainage channel and carries water from the inner city, industrialised area and from all the residents. It runs through the city and 4.5 km runs through the wetland before it reaches its outflow in Inner Murchison Bay within Lake Victoria (Fuhrimann et al., 2014; Figure 1). It was constructed in year 1958 and was designed for leading storm water from the city to the Lake Victoria as fast as possible. Wastewater from the residents flows largely in open ditches directly into the channel and into the wetland, since most people are not connected to any pipeline (3.1). The wastewater volume represent raw sewage produced by more than half a million people or 40 % of all residents in Kampala (Emerton, 2005). The water is flowing freely from the Nakivubo channel into the wetland along the path of the channel, as well as through two separate outlets into the wetland (Emerton, 2005).

Kampala's sewage treatment plant, Bugolobi, also has its outlet in the wetland. Two sewage outflows from Luzira Prison are other point sources that enter the southern parts of the wetland directly. Industrial effluents also flow into the wetland and up to a third of the companies do not treat their wastewater. Both the treated and the untreated wastewater contain environmental and health hazardous such as oils, lubricants, nitrates, phosphates, acids and heavy metals (zinc and mercury). The majority of the wastewater and between 75-85 % of all nutrients enter the wetland through the two outflows of the Nakivubo channel. Nevertheless, the intake of all Kampala's piped drinking water is taken from Gaba around 3 km south west from the wetlands outflow to Murchison Bay (Emerton, 2005). The wastewater flow in the wetland is not evenly distributed over the whole wetland area. Some zones do not have any interaction with the wastewater at all (Kansiime and Maimuna, 1999).

The wetland is effective in removing microbes and bacteria and it has a high capacity for nutrient retention. According to Emerton (2005) it chemically, physically and biologically removes pollutants and sediments that enter from the wastewater. It does so by sedimentation

and by mineralisation processes. The retention time of the wetland is today only 0.5 – 2 days (Emerton, 2005). Currently, the water treatment and water purification done by the wetland are approximately worth between US$ 1 and 1.75 million a year, depending on which calculation method that has been used (Emerton, 2005).

3.3 THE GEOMORPHOLOGY OF THE NAKIVUBO WETLAND

The outermost strata in the Nakivubo wetland lie on a foundation consisting of granite gneisses. The soil consists mostly of alluvial and lacustrine sand, silt and clay layers. The upper layers of the vegetation zone in the alluvial soil consist of semi-liquid organic material followed by dissolved iron reddish ferruginous in loams and further down in clays. Previous studies have found that the underlying soils consist of 30 m thick impervious layer of clays (9-36 m). Peat formation is very low in the area, mostly due to the flood regime. The high annual temperatures and the almost neutral pH in the area are favouring microbes and their activity. Therefore, the decomposition rates and oxygen inputs, due to turbulence, are high. But overall the oxygen levels observed in the wetland were very low because of the high oxygen consumption during the organic matter decomposing. Either hypoxic or anoxic conditions were observed in most of the compartments of the wetland (Kansiime and Maimuna, 1999).

3.4 THE HYDROLOGY OF THE NAKIVUBO WETLAND

The hydraulic and hydrological flow characteristics in the Nakivubo wetland together with a description of the water balance, will give some basic information for understanding the purification capacity of the wetland and later on its ability to treat pharmaceuticals.

Kampala's climate is important to understand because it affects the factors that normally control a wetland. The climate together with the geomorphology in the area are controlling the water level and the nutrient status of the water, which in turn controls the distribution and type of the wetland (Kansiime and Maimuna, 1999). According to Kansiime and Maimuna (1999) the climate is a moist sub-humid climate with two rainy seasons, March to May and October to November, and annual average temperatures around 21 ºC (Figure 3). Both Kampala and Lake Victoria are exposed to lots of tropical thunderstorms, Kampala is even thought to be the capital city in the world that receives most storms (Thompson and Hamilton, 1983 cited in Kansiime and Maimuna, 1999). Kampala is prone to seasonal flooding in periods of intense rainfall (The Republic of Uganda, 2010).

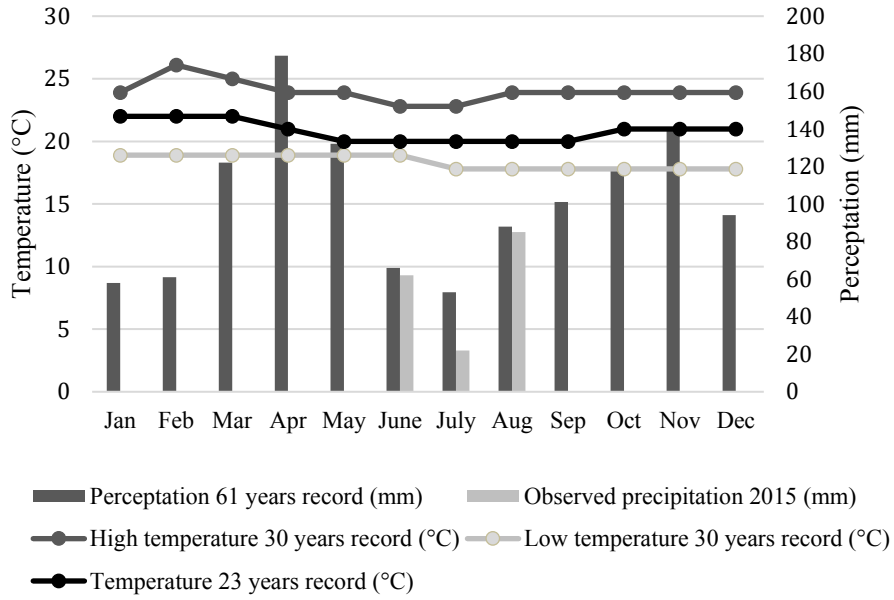

***Figure* 3.** *Monthly average precipitation and temperature data for Kampala city, for a varying number of years, compared with observed monthly average precipitation data year 2015 in June, July and August. Data source:* (Weatherbase, 2016; Uganda National Meteorological Authority, 2016).

Kyambadde et al. (2004) found that the storm, municipal and industrial wastewater loading to the wetland varied together with storm periods. The volume varied approximately with a factor three, with a range from 35 000 to 103 200 m^3/day. Around half of the total volume was from the central parts of the city around the Bugolobi sewage treatment plant and the second largest inflow was from the outflow of the Kitante channel to the Nakivubo channel (Kyambadde et al., 2004);(Figure 1). With high water flows it takes less than a day for the water to travel from the city to the upper parts of the wetland and in dry weather conditions, four days. The rock underground in the area is of the kind that precludes the possibility of a sub-artesian water flow and therefore almost no groundwater enters the wetland (Kansiime and Maimuna, 1999).

Another phenomena that contributes to the water inflow is seiches from Lake Victoria. In average they have 11 cycles per day where one period of the cycles consist of 135 minutes. Kansiime and Maimuna (1999) did some calculations and came to the conslusion that the wetland receives about 837 300 m^3 of water per day from the lake. It is 8 times more than the wastewater inflow from the channel. The daily water inflow from the seiches corresponds to 30 % of the total amount of the water in the wetland, that is approximate 2 875 000 m^3. Water will also flow out from the wetland and the netto inflow from the seiches is estimated to be 108 800 m^3/d. Although a large volume of water is taking part in the exchange process, not all depths and parts are included. It is mostly the same water that is being recycled between the wetland and the lake. It is primarily the parts of the wetland that are close to Lake Victoria that are taking part in this procedure. This water exchange process tend to affect the water purification capacity of the wetland because of varying retention times and because of resuspension and transport of sediments (Kansiime and Maimuna, 1999).

All these in- and outflows can be illustrated in one water mass balance equation specified for the wetland:

$$\frac{dV}{dt} = Q_{Into} + R - ET + Q_S + Q_I \quad (4)$$

where dV/dt is the rate of change of water volume in the wetland, Q_{Into} is the inflow from the Nakivubo channel and nearby water streams, R the precipitation multiplied with the surface area, ET is the evapotranspiration multiplied with the surface area, Q_S is the basin sub-surface water inflow and Q_I is the net value of the water inflow and outflow from and to Lake Victoria. All parameters are in m^3/d. In average the largest flow paths are from the Nakivubo channel and the exchanges with Lake Victoria, around 100 000 - 110 000 m^3/d. Evapotranspiration, perciptation and subsurface flows are in average around 2 000 - 10 000 m^3/d each and no ground water infiltrates the wetland (Kansiime and Maimuna, 1999; Figure 4).

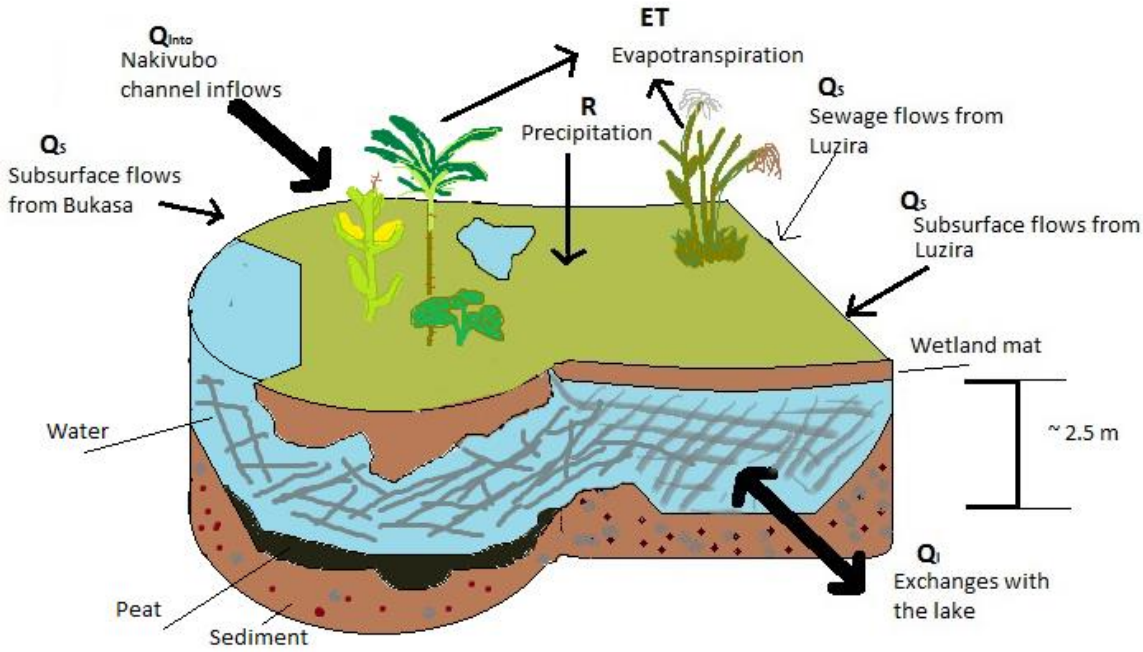

Figure 4. *A schematic picture illustrating the Nakivubo wetland, the crops growing and the mass balance of the water, the in- and outflows. In reality the crops are growing on different sides of the wetland. The thinnest arrow represent water flow in the range of 1 500 - 500 m^3/day, the arrows in middle size are representing water flow in the range of 2 000 - 10 000 m^3/d and the biggest arrows range are 100 000 - 110 000 m^3/d according to Kansiime and Maimuna (1999).*

3.5 THE VEGETATION OF THE NAKIVUBO WETLAND

The vegetation in the wetland is co-dominated by the indigenous aquatic macrophytes Cyperus papyrus and Miscanthidium violaceum Robyns grass. The papyrus is found in most parts of the wetland and can both be rooted directly to the sediments and on floating mats with a thickness round 1.5-2 m. The miscanthidium grass is found in the middle of the wetland on floating mats around 1-1.6 m thick. They are both good as nutrient remover from the wetland since both take up high amounts of nitrogen and phosphorous. Papyrus plants are harvested by the locals and they are thereafter sold and used for making fences, thatching houses and craft making as fuels. Whereas miscanthidium grass is not taken out from the wetland except for rare occasions like building temporary houses for soldiers (Kansiime and Maimuna, 1999). The original vegetation has however decreased. Between the years 1999 and 2004, 50 % of the original vegetation has been replaced by sugar canes and cocoyam cultivation (Kyambadde et al., 2004).

3.6 FARMING IN THE NAKIVUBO WETLAND

Wetlands in Africa are important for the locals because they produce lots of ecosystem services. A lot of people depend on their productivity regarding food and fuel (Aber, Pavri and Aber, 2012). In Nakivubo wetland, most of the farming is done by locals and the cultivated crops are cocoyam, maize, cassava and sugar canes. Cocoyam is the most cultivated crop, with around 70-80 % of all cultivated crop area (Kansiime and Maimuna, 1999). The crops are farmed by small holding farmers and they are mainly for home consumption and the rest are sold to bypassing city dwellers and in markets (Mbabazi et al., 2010a; Mbabazi et al., 2010b).

Wastewater is used in farming for irrigation and as a source of nutrients (Fuhrimann et al., 2014), but the farmers do not irrigate their crops with wastewater intentionally. It just happens to be that this suitable free land is located in a wastewater logged wetland. For crops like maize and sugar cane to be grown, farmers have made channels and furrows to carry away the excess water and grow the crops on ridges, which are surrounded by channels. Cocoyams are more capable of tolerating water logged soils and they are therefore cultivated directly in the wetland which is filled with water contaminated with wastewater from the channel. No channels are made to carry away any excess water. As a consequence, all the crops will be in contact, in some way, with the diluted wastewater throughout the year[4].

3.6.1 Sugar cane

Sugar cane is a tall perennial tropical grass with unbranched stems, on average 4-5 m high and 5 cm in diameter. Sugar canes prefer sunny and warm weather conditions, with mean temperatures around 28-30 ºC during growing season and access to at least 1 500 mm of rain annually. For them to grow, the soil needs to be well drained, fertile and moist. The planting material is obtained from the harvested cane or from a seed nursery. The canes are mature and ready for harvest after 15-24 months, much depending on species and the season (Mukiibi, 2001). The sugar content in the canes varies from area to area, but in Uganda the average is about 9 % (Fortune of Africa Uganda, 2016). The sugar canes grown in the Nakivubo wetland are today only chewed on raw by the locals and are not processed for raw sugar production[5].

3.6.2 Maize

Maize belongs to the grass family and is an annual cereal (Nationalencyklopedin, 2016i). During the growing season the maize species in Uganda prefers about 635 mm of rain and mean temperatures around 20-22 ºC. The seeds are mostly obtained from the previous harvest (Balirwa, 1992). Maize requires nutritious soils and is in most need of nitrogen, nevertheless no extra fertilizer is added to the soil by the farmers in the area. Most of the farmers plant maize by hand using a chop and plant method (Mukiibi, 2001, p.56). The fruit cobs are ready to harvest after approximately three to five months, depending on species and environmental conditions (Ashley, 2016). Maize can be stored for a long time and is among other crops a staple food in Uganda. Their good storage makes them excellent to store for future need in case of food shortage or drought (Balirwa, 1992).

[4] Allan John Komakech, Department of Agriculutural & Bio systems Engineering, Makerere University, Kampala. Email 17.05.2016

[5] Allan John Komakech, Department of Agriculutural & Bio systems Engineering, Makerere University, Kampala. Email 12.04.2016.

3.6.3 Cocoyam

Cocoyam (Colocasia esculenta) is a root tuber crop in the plant family dioscorea and the eatable parts are grown under the ground. Ideal conditions for yam growing are rain, at least 1000 mm spread over five to six months, deep and friable and well drained soils. One yam crop only give yields once a year and it takes around eight months before the yam can be harvested. The yields per plant is approximately 15-20 kg, but damages during harvest is common. Yam cultivation still relays on traditional farming methods and little has been done to improve them (Mukiibi, 2001). Only few farmers in Uganda buy mother seed yams, instead most of the farmers obtain them from their own fields (Ocitti p'Obwoya, 1991 cited in Mukiibi, 2001, p.230). In a study made by Talawana (2009), 90 cocoyam farmers in the Lake Victoria area in Uganda were interviewed. They found that the cocoyam were generally grown without any adequate landscape control, such as water supply and the fertilizer inputs to the fields were very low, despite some sporadic manure inputs. Nearly 50 % of the farmers reported that the yield were declining due to decrease in arable land, soil fertility and changing environmental conditions (H A L Talwana, 2009).

Farmers do not have methods for conserving the yams when they have been harvested so the majority of the produced cocoyam were eaten directly by the families. All farmers said that they cooked the yam in some way before eating them. Common methods were steaming peeled or unpeeled tubers, mixed together with beans, groundnut or beef stew and some reported that they make chips and/or porridge on the tubers (H A L Talwana, 2009). Normally, cocoyam is also consumed at breakfast once a week. Some problems with the cocoyam consumption are the long cooking time and that they tend to irritate the skin during peeling and when not properly cooked tubers are eaten. The leaves of the cocoyams are also consumed as vegetables (H A L Talwana, 2009).

4 MATERIAL AND METHOD

4.1 SAMPLE COLLECTION AND PREPARATION

4.1.1 Type of Samples

Water, soil and crop samples were collected from different points within the wastewater-irrigated fields in Kampala along Nakivubo channel. Water samples included wastewater inflow and outflow of the Bugolobi wastewater treatment plant, in the Nakivubo channel divide, in the yam field in the wetland as well as in Lake Victoria (Table 3). Sahar Dalahmeh collected the samples in the spring of 2015. Samples were collected at two occasions and in total 20 wastewater samples from 6 different sampling spots were collected (Table 3). The soil and the crop samples were collected in yam, maize and sugar cane fields in the wetland. In total 9 samples were collected from random selected spots in each field and each sampling occasion (Table 4).

Table 3. Description of wastewater samples; their locations, collection dates, number of samples, GPS coordinates and altitude. Source: Sahar Dalahmeh

Sample ID	Location	Collection date	Number of samples	GPS coordinates	Altitude
Inflow	Inflow of Bugolobi wastewater treatment plant	2015-04-29 2015-05-05	2 2	0.31935°N, 32.6062°E	1 166 m
Outflow	Outflow of Bugolobi wastewater treatment plant, at the entrance of Nakivubo channel	2015-04-29 2015-05-05	2 2	0.31620°N, 32.60812°E	1 158 m
Channel divide	Nakivubo channel, where it divides into two	2015-04-29 2015-05-05	2 2	0.30263°N, 32.62279°E	1 153 m
Wetland YF	Wastewater collected in the yam field where the crops are cultivated	2015-04-29 2015-05-05	2 2	0.29755°N, 32.63215°E	1 142 m
Lake Victoria	Raw water from lake Victoria	2015-05-05	2	0.25493°N, 32.6381°E	1 141 m
Gaba	Lake Victoria at Gaba	2015-04-30	2	0.243650°N, 32.641504°E	1 141 m
Total			***20***		

Table 4. Description of crop and soil samples; their location, collection date, number of samples, GPS coordinates and altitude. Source: Sahar Dalahmeh

Sample ID	Location	Collection date	Number of samples	GPS coordinates	Altitude
Yam	Yam plant on yam field	2015-04-29 2015-05-05	9 9	0.29755°N, 32.63215°E	1 142 m
Maize	Maize plant on maize field	2015-04-29 2015-05-05	9 9	0.29798°N, 32.63176°E	1 145 m
Sugar cane	Sugar cane plant on sugar cane field	2015-04-29 2015-05-05	9 9	0.29863°N, 32.6302°E	1 144 m
Soil yam field	Soil yam field	2015-04-29 2015-05-05	9 9	0.29755°N, 32.63215°E	1 142 m
Soil maize field	Soil maize field	2015-04-29 2015-05-05	9 9	0.29798°N, 32.63176°E	1 145 m
Soil sugar cane field	Soil sugar cane field	2015-04-29 2015-05-05	9 9	0.29863°N, 32.6302°E	1 144 m
Total			***108***		

4.1.2 Sampling Locations and Methods

The water was collected in the upper 50 cm water surface by hand using a bucket and a rope. The water was mixed to obtain a sample that was as representative as possible. Then around one and a half litres of water was taken from the bucket and stored in plastic bottles (Figure 5). Farmers collected the water samples from the wetland because of the tough terrain and dangerous animals like snakes in the area. All the water replicates were taken at approximately the same spot. Water from all sampling spots were also collected on the first sampling day, 29 April 2015, directly into 50 ml polypropylene centrifuge tubes (PP tubes) and pre-treated with sulphuric acid for conservation. The samples were later used for conventional wastewater analyses.

Figure 5. *Pictures from Uganda during the water sample collection. To the left Allan Komaketch collecting water in the channel. In the middle Sahar Dalahmeh is collecting wastewater from inflow to Bugolobi wastewater treatment plant. To the right Sahar Dalahmeh and Allan Komaketch pour wastewater from the Nakivubo channel into a plastic bottle. © Sahar Dalahmeh & Allan Komaketch with permission.*

Edible parts of the crops were collected by hand, i.e. yam root, maize fruit-grain and the sugar cane stem. They were collected by randomly walking in the field. The crops were rinsed and sliced into smaller pieces and then stored in 50 ml PP tubes. The yam tubes were washed with water. The soil samples were randomly collected from the three cultivated fields at the same random spots as the crops. They were collected with help from a hand soil sampling auger from the top horizon at 0-25 cm deep. The sample was taken from the middle of the core and stored in both plastic sample bags sealed with a steel wire and in 50 ml PP tubes. All samples were frozen right after they had been collected.

The random selected sampling spots in the fields were not documented. The fields in the wetland are also randomly cultivated and no documented boundaries between them exist. The map is therefore only showing the locations of the three fields approximately (Figure 6).

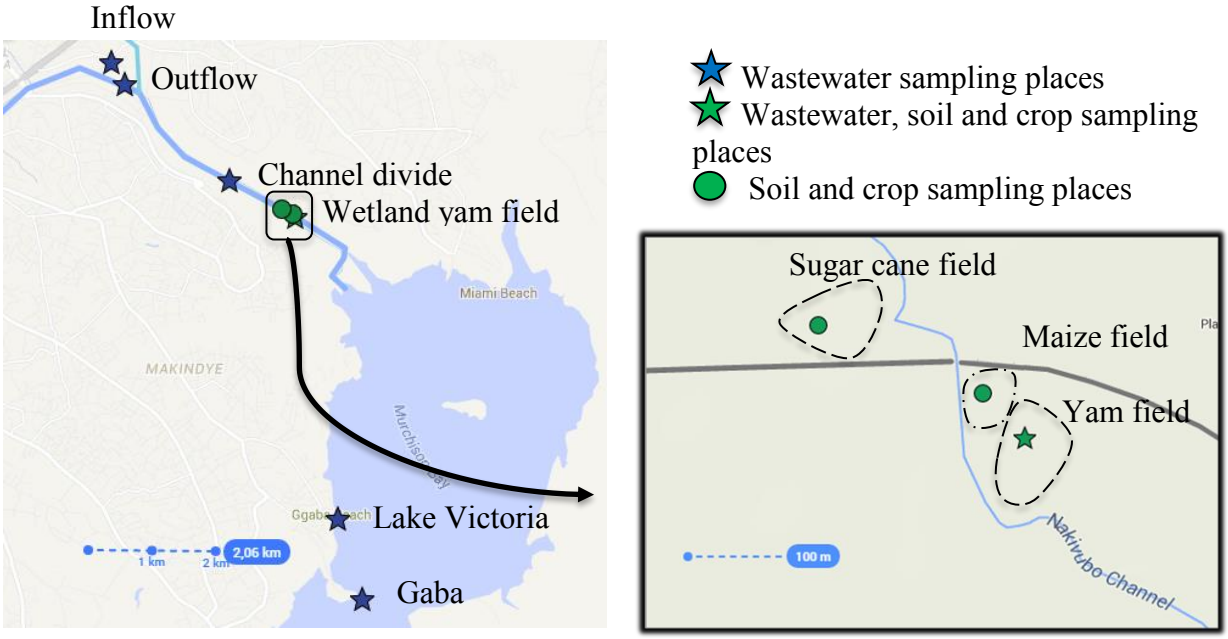

Figure 6. *The sampling spots for wastewater (stars in blue or green), soil and crop (circles or star in green) along the Nakivubo channel and in Lake Victoria. To the left: The scale bar is 2.06 km. To the right: Enlarged picture of the sampling spots in the wetland. The scale bar is 100 m. The three different fields are divided by the Nakivubo channel and a railway line (in grey). The boundaries of the fields are only illustrated approximately. Source: Map data ©2016 Google.*

4.1.3 Challenges During the Sample Collection

The biggest problems that may affect the results occurred during the second day of sampling collection, 5 May 2015. During the morning it was raining at medium intensity, which might have impacted the results mainly for the liquid samples, due to resuspension of solids and dilution of water. On the same day, the wastewater treatment plant Bugolobi was not working. It had a break-down a couple of days earlier and was not able to treat any of the incoming water. All wastewater came out from the plant without proper treatment although it might have been sedimentation of the bigger particles. That means that the outflow samples from the fifth of May is untreated wastewater.

4.1.4 Storage and Preparation of Samples before Analyses

All samples were kept frozen at -18 °C until transported to Sweden. Thereafter, all the solid samples were freeze dried in a freeze dryer at -40 °C (Edwards) and then stored in a freezer with a temperature around -20 ºC. The wastewater samples were frozen and stored in the same freezer at around -20 ºC.

4.2 DETERMINATION OF WATER QUALITY PARAMETERS IN THE LIQUID SAMPLES

In order to draw conclusions about the effectiveness of the treatment of wastewater in Kampala in general, the concentrations of phosphorus as phosphate (PO_4-P), total phosphorous (TP), nitrogen as nitrate (NO_3-N), total nitrogen (TN), chemical oxygen demand (COD), total organic carbon (TOC), pH, total solids (TS) and total suspended solids (TSS) were measured in the liquid samples. The pH was measured with indicator paper before filtration of the water samples. The biochemical oxygen demand during five days (BOD_5) in the water samples from the 29 April 2015 was determined in Kampala by Sahar Dalahmeh. It was done according to standard methods. Further analyses on the soil samples such as pH, nutrients, carbon content and selected metals were performed in an external lab at the Swedish University of Agricultural Sciences.

The number of replicates for each water analysis and each sample varied (Table 5). The different analyses required different amounts of water and there was only a limited amount of water available that needed to be shared between the analyses. Therefore, some of the samples were diluted. The dilution factor (DF) accounts for the dilution of the different samples, and is calculated by dividing the final volume with the initial volume. A DF of one corresponds to an undiluted sample, while a DF of two corresponds to a sample with an equal amount of sample water and Milli-Q water. The analyses carried out on the Lake Victoria samples were performed at a different day than the rest of the analysis. Therefore, two or more blanks were created on all the analysis performed.

Table 5. Number of replicates per sample for each water analysis; phosphorus as phosphate (PO_4-P), total phosphorous (TP), nitrogen as nitrate (NO_3-N), total nitrogen (TN), chemical oxygen demand (COD), total organic carbon (TOC), biochemical oxygen demand during five days (BOD_5), total solids (TS) and total suspended solids (TSS). The dilution factor for each analysis is also presented. The COD and the TOC tests were conducted using standard kits with both low and high detection limits. LV stands for Lake Victoria

	Number of replicates per sample						
Analysis	Inflow	Outflow	Channel	Wetland	Lake Victoria	Blank	Dilution factor (DF)
PO_4-P	3	3	3	3	3	2	1
TP	1	1	2	2	3	3	2 (LV = 1)
NO_3-N	3	3	3	3	3	2	1
TN	1	2	2	2	3	2	2 (LV = 1)
COD (high)	3	3	3	3	0	2	1 (Inflow = 2)
COD (low)	0	0	0	0	3	1	1
TOC (high)	3	3	3	3	0	1	10
TOC (low)	0	0	0	0	5	1	10
BOD_5	2	2	1	2	2	2	1
TS	3	3	4	3	2	0	1
TSS	4	4	4	4	3	0	1

4.2.1 Phosphate

The phosphorus as phosphate concentration was determined using the Spectroquant® Phosphate Test by Merck, Germany. In the test, orthophosphate ions react with molybdate ions and form molybdophosphoric acid. This is reduced to phosphomolybdenum blue by ascorbic acid, and can be determined photometrically. Detailed description of the analysis can be found in Appendix A.

4.2.2 Total Phosphorus

The total phosphorus concentration was determined using the Spectroquant® Crack Set 10 by Merck, Germany. In the test, compounds containing phosphorus are digested in sulphuric acid and peroxodisulfate, and transformed into orthophosphate. Detailed description of the analysis can be found in Appendix A.

4.2.3 Nitrate

The nitrate as nitrogen concentration was determined using the Spectroquant® Nitrate test by Merck, Germany. In the test, sulfuric and phosphoric solution nitrate ions react with 2.6-dimethylphenol (DMP) and then form 4-nitro-2.6-dimethylphenol. The solute will be pink and the strength of the colour reveals the nitrate content. The solute can be determined photometrically. Detailed description of the analysis can be found in Appendix A.

4.2.4 Total Nitrogen

The total nitrogen concentration was determined using Spectroquant® Crack Set 20 by Merck, Germany. The test is based on Koroleff's method where organic and inorganic nitrogen compounds are transformed into nitrate. This is done by an oxidizing agent treatment in a thermoreactor. Detailed description of the analysis can be found in Appendix A.

4.2.5 Chemical Oxygen Demand

The chemical oxygen demand (COD) was determined using a Spectroquant® COD cell test by Merck, Germany. In the test, silver sulfate acts as the catalyst while the water sample is oxidized with a hot sulfuric solution of potassium dichromate. The solute transforms then from yellow into different shades of green depending on the COD content and can be determined photometrically. Detailed description of the analysis can be found in Appendix A.

4.2.6 Total Organic Carbon

The concentration of TOC was determined using a Spectroquant® TOC cell test by Merck, Germany. In the test, carbon-containing compounds are digested in sulphuric acid and peroxodisulfate, and are transformed into carbon dioxide. The carbon dioxide reacts with an indicator solution and the colour is determined photometrically. Detailed description of the analysis can be found in Appendix A.

4.2.7 Total Solids and Total Suspended Solids

The total solids (TS) and total suspended solids (TSS) were determined using a water filtration unit, an oven and a scale. The tests are similar to each other and were performed in a similar way. A detailed description of the analysis can be found in Appendix A.

4.3 DETERMINATION OF SOIL QUALITY PARAMETERS IN THE SOIL SAMPLES

The three soils were sent away for laboratory analysis to the external laboratory soil and plant at the Swedish University of Agricultural Sciences. The parameters that were examined were pH, total solids (TS), total nitrogen (Tot N), total carbon (Tot C), total organic carbon (TOC), phosphorus (P), iron (Fe), aluminium (Al), manganese (Mn) and zinc (Zn). The methods used were according to Swedish standards SS-ISO 13878 and SS 02 83 11 for tot-C and TN, and for macro and micro nutrients in soil, respectively. Approximately 6 gram from each of all 9

collected soil samples were taken and put together. In total 47.7 g, 54.4 g and 55.8 g of yam, sugar cane and maize soil respectively, were prepared and sent to the external laboratory.

4.4 SURVEY OF THE TYPES OF MEDICINES PRESCRIBED AND SOLD IN KAMPALA

A questionnaire was developed to collect data about the types and amounts of medicines mostly sold in Kampala by Sahar Dalahmeh. They were used as support to identify the pharmaceuticals which were most adequate to study. The questionnaires were distributed among 15 anonymous pharmacies in Kampala. The survey included questions which enabled identification of the types of the mostly prescribed and sold medicines, and the disposal methods of old and unused medicines. Some of the questions were:

1) Which two medicines are most prescribed for common diseases like diarrheal, malaria, food poisoning, stomach pain, heart problem and so on?
2) At which quantity are they sold per year?
3) Is there any information distributed to the customer on how to dispose of unused medication?
4) Where is the expired/unused medicine disposed?
5) Have the concerned authority given any instructions/restrictions of how to handle unused or expired medication?

The questionnaires were compiled by using Excel (Microsoft, 2013). Only pharmaceuticals prescribed were taken into account. Other preventive medications such as charcoal for food poisoning and magnesium for stomach pain were neglected.

4.5 ANALYSIS OF PHARMACEUTICALS IN THE SAMPLES

4.5.1 Types of Target Compounds Analysed in this Study

The target compounds that were analysed in this study are listed in Appendix B. The compounds were selected taking into consideration the compounds that were most sold in Kampala. The selected pharmaceuticals included pharmaceuticals that were used for various therapeutic uses.

4.5.2 Prevention of Contamination and Cleaning of the Lab Ware

Pharmaceuticals found in wastewater, soils and crops are expected to occur in very low concentrations (µg/l-ng/l) (2.6.2). This puts high demands on the purity of the equipment for avoiding unnecessary contaminations. Therefore the laboratory work first of all started with preparation and cleaning of all equipment needed for the extraction. The cleaning was done according to standard methods depending on size and material of the equipment to avoid contaminants as much as possible. For example, all plastic material were rinsed with 99.9 % pure methanol (Appendix D) and all glass ware were burned in an oven at 400 °C over the night. For a more detailed description of the cleaning process for all lab ware used, Appendix C. All automatic pipettes used were calibrated beforehand.

4.5.3 Internal Standard

Internal standards containing the pharmaceuticals to be studied (Appendix B) were prepared by Sahar Dalahmeh. The internal standards are used to calculate the concentration of the compounds in the samples in the separation and detection step. During the analysis, the

concentration of the samples is not given as an absolute value, but is rather given in comparison of the internal standard. If an internal standard representative of the sample is added to each sample before the analysis, the concentration of the compound can be calculated in comparison to the internal standard, since the concentration of the internal standard is known.

4.5.4 Extraction of Pharmaceuticals in Liquid Samples

The extraction of the liquid samples was performed using SPE. 20 liquid samples were analysed (Table 3) along with one blank made of Milli-Q water. Oasis HLB cartridges were used to extract the pharmaceuticals (Appendix D). The cartridges were eluted with methanol, which was collected in glass tubes and later evaporated in an N-EVAP™112 nitrogen evaporator (Appendix D) in order to increase the concentration of the analyte. After the evaporation the samples were reconstituted with a methanol/Milli-Q water solvent and put in the freezer until the instrumental analysis. For detailed description of the lab procedure, preparation and extraction, see Appendix E.

4.5.5 Extraction Method for Solid Samples

The extraction of the solid samples was performed using the method QuEChERS. 18 soil samples and 18 crop samples were analysed. The solid samples were crushed and then put three by three in 50 ml PP tubes together with Na_2-EDTA, acetonitrile with acetic acid and QuEChERS extract pouches (Appendix D). When the pharmaceuticals had been extracted from the samples to the liquid, the liquid was put in glass tubes and was evaporated in an N-EVAP™112 nitrogen evaporator (Appendix D) in order to increase the concentration of the analyte. After the evaporation the samples were reconstituted with a methanol/Milli-Q water solvent and put in the freezer until the instrumental analysis. For detailed description of the lab procedure, preparation and extraction, see Appendix F.

4.5.6 Instrumental Analysis

A detailed description of the pharmaceutical analysis with LC-MS can be found in Appendix G.

Lumefantrine, ibuprofen and naproxen proved to be troublesome during the analysis for different reasons, and these were therefore not included. Lumefantrine had solids in the stock solution while naproxen and ibuprofen were not ionized in the mass spectrometer for unknown reasons. There were also some troubles with getting the concentrations of the different compounds. Sometimes, the internal standards were not detected, or the wrong internal standard was detected in the analysis. Since the software (UNIFI Scientific Information System) only could calculate the concentration of a certain compound with the help of the corresponding internal standard, the concentration of those compounds were not shown, or the wrong concentration was shown. The concentrations of some of those compounds were therefore calculated manually with the help of linear regression. The regression line was created with values from the calibration curve of the compound. The response of the compound in the calibration curve was on the y-axis and concentration of the compound in the calibration curve was on the x-axis. The equation from the regression line (Table 6) together with the response of the compound from the chromatogram gave the requested concentration. The response of the compound was given by measuring the height of the intensity in the chromatogram.

Table 6. Characteristic factors for all linear regressions made for the compounds with inaccurate or missing concentrations. Two calibration methods have been used, one with help of the compounds' corresponding internal standards (IS) and one without IS, when the IS was not detected. Number of measurement points (n) used in the curves and the curves equations with R^2-value are presented. X is the sample's concentration and Y is the sample's response. A mathematical correction factor was calculated for some of the pharmaceuticals to correct the calculated results and make the results comparable with the concentrations given directly by the software (UNIFI Scientific Information System)

Sample	Pharmaceutical	Calibration method	n	Equation	R^2	Correction factor
Water	Diclofenac	Without IS	9	$X = \frac{Y - 488.3}{17.93}$	0.997	4.4 ± 1.1 (n=4)
	Metformin	Without IS	8	$X = \frac{Y + 173.7}{38.75}$	0.8657	-
	Sulfamethoxazole	Without IS	8	$X = \frac{Y - 3335.6}{47.2}$	0.9087	5.1 ± 2.1 (n=3)
	Omeprazole	Without IS	7	$X = \frac{Y + 584.8}{244.8}$	0.9427	-
Water, soil and crops	Trimethoprim	With IS	9	$X = \frac{Y - 6 \times 10^{-7}}{0.0063}$	1	-
	Pyrimethamine	With IS	9	$X = \frac{Y - 1 \times 10^{-6}}{0.0069}$	1	-

The limits of detection (LODs) and the limits of quantification (LOQs) for the compounds were calculated with the help of the signal-to-noise ratio given in the software UNIFI Scientific Information System. The concentration of the compound of interest in a representative sample was divided by the signal-to-noise ratio which gave the LOD for that compound in the sample. The LOD was then multiplied by 10/3 which gave the LOQ for that compound in the sample. Representative LODs and LOQs were calculated for the water, the soil and the crops.

4.5.7 Calculation of the Recovery Samples

A recovery test was used to see how much of the targeted compounds were recovered from the tested matrices using the previously described methods of extractions. For this purpose, two liquid samples (100 mL each) of the water sample Gaba, 30 April 2015 and two solid samples at each field for the 29 April 2015 samples, for both soil and crop (1g each) were tested. The recovery test was done by spiking the samples with 200 µl of a standard pharmaceutical solution containing a mixture of all pharmaceutical compounds tested in this thesis at concentration of 1 ng/µL. The recovery samples were prepared, extracted and analysed following the same procedure as the original samples. The percentage of the compound recovered was calculated using the concentration detected in the recovery sample, the concentration detected in the original sample and the actual concentration of standard solution. The actual concentration was calculated using:

$$Actual\ concentration\ (ng/g\ or\ ng/l) = \frac{Conc_{PS}\ (ng/\mu l) \times V_{PS}\ (\mu l)}{W_{Sample}\ (g\ or\ l)} \quad (5)$$

where the $Conc_{PS}$ is the concentration of the pharmaceutical solution of the tested compounds, V_{PS} is the volume of pharmaceutical solution added and W_{Sample} is the weight or the volume of the sample. Weight of the sample for the water samples is the volume in litre.

The percentage of the recovery was calculated according to:

$$Recovery\ (\%) = \frac{Conc_{Recovery\ sample} - Conc_{Original\ sample}}{Actual\ concentration} \quad (6)$$

where $Conc_{Recovery\ sample}$ is the concentration detected in the recovery sample and $Conc_{Original\ sample}$ the concentration detected in the original sample. In the cases were no concentration of the original sample existed, the LOD value was used and when that was not possible a value of zero was used as the concentration.

4.6 NATURAL REMOVAL OF PHARMACEUTICALS

The pharmaceuticals that were detected in majority of the soil samples were investigated to see if they were significantly differed in their concentrations between the measurement points, i.e. in the yam, sugar cane and maize soil. To examine the data distribution, if the data was normal distributed or not, Shapiro-Wilk test was used in the program R version 3.1.2. The three soils were assumed to be independent of each other, and therefore t-test was used. The data was adjusted to fit the requirements in the program R version 3.1.2, i.e. all fields had the same number of measurement points and where no concentration was available the limit of detection or limit of quantification was used. Unfortunately, due to low number of measurement points of the wastewater samples the difference between the points could not be tested statistically.

Before the pharmaceutical extraction, the total suspended solids were filtered off and was not taking part of the extraction, hence its pharmaceutical content is unknown. The correlation of selected pharmaceuticals in the water and the total suspended solids in the water were determined using linear regression in Excel (Microsoft, 2013). The p-value was determined in the program R version 3.1.2. The data was transformed logarithmically to become normally distributed and the residuals of the regression lines were analysed by plotting the new calculated y-values subtracted by the old y-values and by creating a residual versus order plot in Excel. To examine if the residuals were normal distributed a normal probability plot was created in Excel.

4.7 RISK ASSESSMENT

In order to calculate the EDI, information was needed about the food habits of the people in Kampala. In a survey conducted in 2008, the food consumption of women in Kampala was studied (Harvey, Rambeloson and Dary, 2008). 314 women between the ages of 15 and 49 in Kampala were interviewed about their food habits. The mean daily consumption of roots and tubers (yam) was about 120 g/day, maize grain was 35 g/day and the mean daily consumption of sugar was about 40 g/day (Harvey, Rambeloson and Dary, 2008). No information could be found about the daily drinking water consumption in Uganda, but it is assumed to be one litre in this thesis, based on recommendations (Livsmedelsverket, 2016). The average weight of adults in Africa is 60.7 kg (Walpole et al., 2012).

The risk analysis was conducted under the assumption that the food consumed by women ages 15-49 in Kampala was representative for the whole adult population. The safety factor used in this thesis was 1000. The safety factor represents three factors of 10 to account for different responses between humans, the sensitivity among certain groups of the population, e.g. children and the lowest therapeutic dose not representing a no-effect level.

Since no no-effect level could be found for the given pharmaceuticals, the ADI was calculated by

$$ADI\ (\mu g/kg/day) = \frac{LTD\ (mg/day)\ \times\ 1000\ \mu g}{Weight\ (kg)\ \times\ SF} = \frac{LTD\ (\mu g/day)}{60\ kg} \quad (7)$$

where LTD is the lowest therapeutic dose and SF is a safety factor of 1000. The weight is the average weight of adults in Africa. The EDI was given by

$$EDI\ (\mu g/kg/day) = \frac{C\left(\frac{ng}{g}\ or\ \frac{ng}{l}\right)\times\ 0.001\ \mu g\ \times\ FC\left(\frac{g}{day}\ or\ \frac{l}{day}\right)\times\ DM\ (\%)}{Weight\ (kg)} \quad (8)$$

where C is the concentration of the pharmaceutical in the crop dry matter or water, FC stands for food consumed, being crops or water and DM is the dry matter. The DM was only used when calculating the EDI for the crops. This was because the concentrations of the pharmaceuticals only were given in the dry matter, and the EDI was supposed to represent the whole crop. The dry matter in the yam was assumed to be 26.2 %, based on average findings in Agbor Egbe and Treche (1984).

5 RESULTS AND DISCUSSION

5.1 LABORATORY WATER QUALITY PARAMETERS ANALYSIS RESULTS

5.1.1 Phosphate and Total Phosphorous

Phosphorus analysis showed that the average TP and the PO_4-P concentrations at the inflow of the Bugolobi WWTP were 9.9 mg/l and 4.4 ± 0.3 mg/l respectively and both of those were higher than what was measured at the effluent wastewater, 6.3 mg/l and 4.0 ± 0.1 mg/l (Figure 7). The concentrations of TP and PO_4-P in the channel (10.5 ± 0.1 and 5.4 ± 0.1 mg/l) were higher than the effluent water, however. The TP and PO_4-P concentrations in the wetland were somewhat lower than in the channel. Lake Victoria had the lowest concentrations of TP and PO_4-P giving 0.16 ± 0.03 and 0.05 ± 0.00 mg/l respectively. All the TP concentrations are higher than the PO_4-P concentrations. The blanks are low, but the TP blank is higher than the TP concentration in Lake Victoria (0.3 ± 0.1 mg/l).

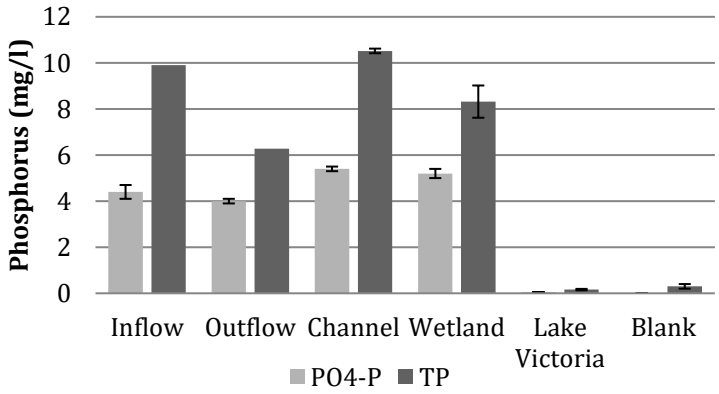

Figure 7. *Total phosphorus (TP) and phosphate (PO_4-P) concentrations expressed in mg/l in water samples from inflow and outflow of the Bugolobi WWTP, Nakivubo channel, Nakivubo wetland and Lake Victoria the 5 May 2015. The blanks were made of Milli-Q water. All results are mean values with standard deviations showing as black lines except for TP inflow, TP outflow and PO_4-P blank since only one replicate was analysed for those samples (Table 5).*

The standard deviations are mostly low, showing that there was not much variation among the replicates, the TP concentration in the wetland being the sole exception.

5.1.2 Nitrate and Total Nitrogen

The TN was 56 mg/l in the influent wastewater and 53.1 ± 4.1 mg/l in the effluent wastewater. Channel water showed 18.2 ± 1.4 mg/l of TN, which was lower than 29.7 ± 9.2 mg/l, measured in the wetland water. Lake Victoria showed 2.4 ± 0.1 mg/l TN (Figure 8). The variations were lower for the NO_3-N. The inflow and outflow of Bugolobi WWTP showed concentrations of 3.9 ± 0.5 and 1.9 ± 0.1 mg/l, respectively. The channel and the wetland had the same average concentration of 2.4 mg/l, with standard deviations of 0.1 and 0.2 mg/l. Lake Victoria had the lowest NO_3-N concentration of 1.3 ± 0.2 mg/l. NO_3-N concentrations were a very small percentage of TN concentration in inflow, outflow and wetland (4-8 %) while the NO_3-N concentration was a somewhat larger part of TN in the channel and Lake Victoria (13 and 54 % respectively).

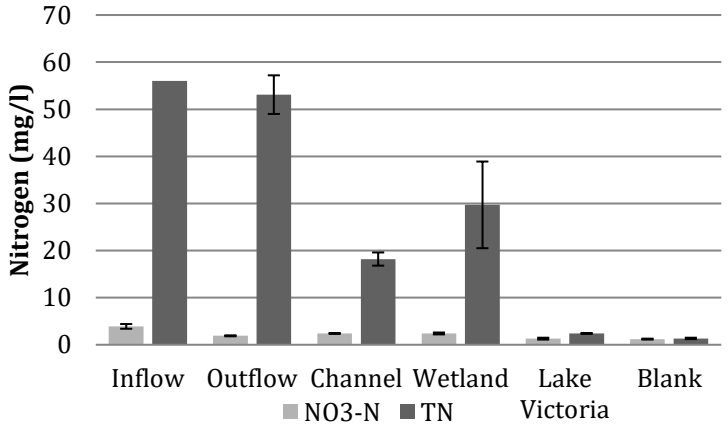

Figure 8. *Total nitrogen (TN) and nitrate (NO_3-N) concentrations expressed in mg/l in water samples from inflow and outflow of Bugolobi WWTP, Nakivubo channel, Nakivubo wetland and Lake Victoria the 5 May 2015. The blanks were made of Milli-Q water. All results are mean values with standard deviations showing as black lines except for TN inflow since only one replicate was analysed (Table 5).*

The standard deviations for the NO_3-N samples are relatively low, while the TN concentrations in the outflow, channel and wetland vary more.

5.1.3 Chemical Oxygen Demand and Total Organic Carbon

The COD and the TOC concentrations show the same patterns between the measurement points: the concentration is at its highest at the inflow of the WWTP (975 ± 149 and 3773 ± 36 mg/l respectively), it decreases at the outflow (393 ± 40 and 3187 ± 240 mg/l) and in the channel (153 ± 12 and 2867 ± 196 mg/l) and increases again in the wetland (548 ± 317 and 3280 ± 624) (Figure 9). The COD and TOC concentrations in Lake Victoria are very low compared to the other measurement points, with concentrations of 14 ± 7 and 273 ± 46 mg/l respectively. The concentrations in the blanks for the COD and TOC tests are much lower than the COD and TOC concentrations in the measurement points.

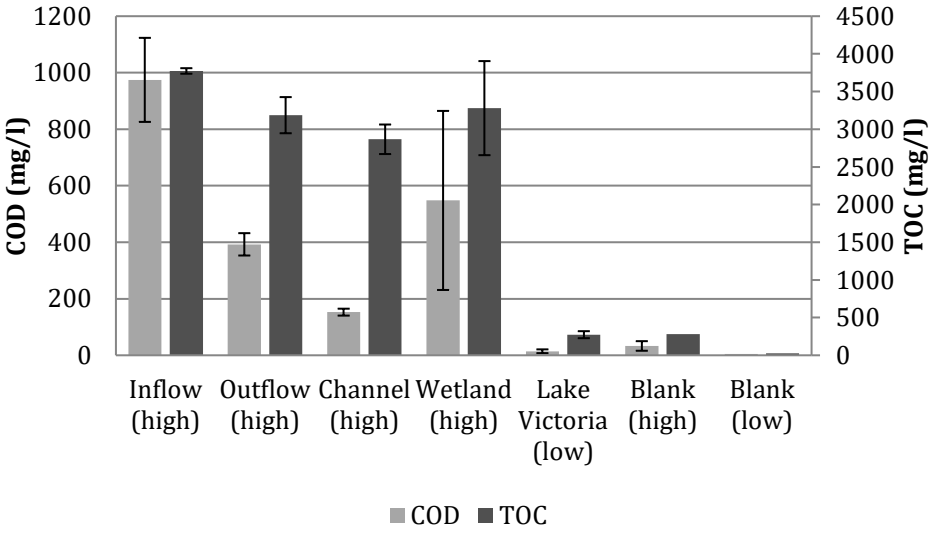

Figure 9. *Concentrations (mg/l) of chemical oxygen demand (COD) and total organic carbon (TOC) along different parts of the Nakivubo channel and Lake Victoria the 5 May 2015. Note that the COD concentration is presented on the left axis and the TOC concentration on the right axis. Different measuring kits were used to determine the TOC and the DOC, one with a high detection range and one with a low. The inflow, outflow, channel and wetland concentrations were determined using the kits with the high detection ranges while Lake Victoria was determined using the kits with low detection ranges. The concentrations are averaged with the black lines showing the standard deviation for all the values except for the blanks. The blanks were made of Milli-Q water had only one replicate each except for the blank with the high detection range for COD (Table 5).*

The standard deviation for the COD concentration in the wetland was quite high. The COD concentration in the other measurement points didn't vary as much, except for the blank made by the kit with the high detection range. The TOC concentration in the wetland also varied the most. The TOC concentration that varied the least was at the inflow to the Bugolobi WWTP.

5.1.4 Biochemical Oxygen Demand

The BOD_5 concentrations decline steadily along the Nakivubo channel, showing 535 ± 37 mg/l in the inflow wastewater and 21 ± 10 mg/l in Lake Victoria (Figure 10). The blank's concentration is similar to Lake Victoria's (22 ± 0.6 mg/). The standard deviations were relatively low for the different measurement points except for Lake Victoria's.

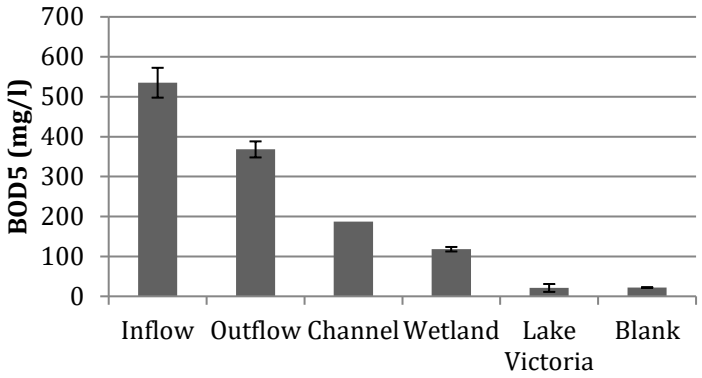

Figure 10. *Concentrations of BOD_5 (mg/l) the 29 April 2015 at the inflow and outflow of the Bugolobi WWTP, at the Nakivubo channel, in the wetland irrigated with water from Nakivubo channel, in Lake Victoria, and in a blank made of Milli-Q water. The values are all averaged with standard deviations shown as black lines, except for the channel sample that only had one replicate (Table 5).*

5.1.5 Total Solids and Total Suspended Solids

The inflow concentrations of TS and TSS were medium high (476 ± 222 and 206 ± 81 mg/l) but at the outflow the TS concentration had increased (683 ± 70 mg/l) while the TSS concentration has decreased (101 ± 28 mg/l) (Figure 11). In the channel, the TS concentration had decreased (574 ± 83 mg/l) while the TSS concentration has increased again (478 ± 434 mg/l). The TS and TSS concentrations were both much higher in the wetland (1190 ± 1218 and 251 ± 394 mg/l) than in Lake Victoria (126 ± 58 and 10 ± 5).

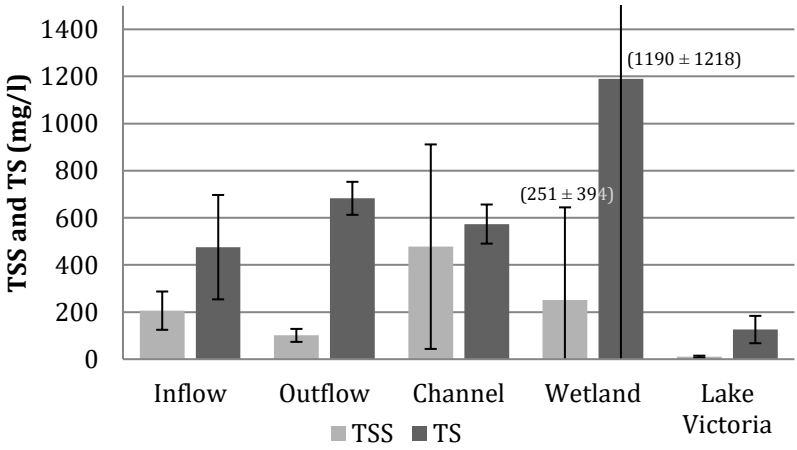

Figure 11. *Concentrations (mg/l) of total suspended solids (TSS) and total solids (TS) along different parts of the Nakivubo channel and Lake Victoria the 29 April and the 5 May 2015. The concentrations are shown as mean values with standard deviations showing as black lines. The standard deviations for the wetland samples didn't fit in the figure and their respective spans have been disclosed in the figure. The number of replicates analysed for each sample were two to four (Table 5).*

The standard deviations in this test were the highest among the conventional analyses. Especially the standard deviations for the wetland samples are extremely high and couldn't fit in the figure (Figure 11). The TSS concentration in the wetland also has a high variation. Those measurement points that had the smallest variation were Lake Victoria and the outflow of Bugolobi WWTP.

5.1.6 pH

All water samples had the same pH value of 8, except for one of the replicates at the outflow of Bugolobi WWTP, 29 April 2015, and one of the replicates in the Nakivubo channel, 29 April 2015. Those had a pH value of 7 before the extraction started. The pH value was obtained with indicator paper.

5.2 LABORATORY WATER QUALITY PARAMETERS ANALYSIS DISCUSSION

5.2.1 PO_4-P, TP, NO_3-N and TN

The nutrients studied (PO_4-P, TP, NO_3-N and TN) showed different patterns between the measurement points (Figure 7; Figure 8). All of the nutrients had decreased to some extent between the inflow and the outflow of the Bugolobi WWTP, showing that the water received a partial treatment by the WWTP, in spite of the WWTP not working properly on the day when the samples were taken. Both of the nutrients increased downstream of the outflow however; the phosphorus concentration increased in the wetland and the nitrogen concentration increased in the channel. This seems to indicate that there were additions of nutrients in the channel and the wetland. The concentration of the nutrients was very low in Lake Victoria, presumably because the water was diluted. Seen from an eutrophication perspective however, the TP concentration was very high, suggesting that the Lake – and by extension, the Nakivubo channel – is eutrophic.

The TP concentrations increased and decreased more between the measurement points than the PO_4-P, showing that there are primarily other forms of phosphorus that affect the TP variations in the concentration, for example organic phosphorus or particulate phosphorus (Figure 7). If the PO_4-P values are compared to those obtained by Fuhrimann et al. (2014), they are in the same range. The NO_3-N concentrations were much lower than the TN concentrations at all the measurement points except Lake Victoria (Figure 8). The majority of the TN is presumably made up of ammonium. This conclusion is supported by the results obtained by Fuhrimann et al. (2014), who also found low NO_3-N concentrations, but much higher concentrations of ammonium. The ammonium concentrations were of the same magnitude as the TN concentrations in this thesis. The lack of nitrification suggests that the water is anaerobic.

The blanks for the different tests were quite low, showing that the tests seem to be reliable. It should be noted however that the TP blank had higher concentrations than the Lake Victoria sample. This indicates that the method might not be very sensitive, or that the concentration in Lake Victoria is very low. The standard deviations were low for the PO_4-P and NO_3-N tests, which show that the results ought to be quite certain. It should be noted however, that the TP test had the lowest number of replicates making the results more uncertain (Table 5). The TP and the TN tests had larger standard deviations which can be explained by the fact that the samples were diluted during these tests. Another possible explanation is that in the tests organic matter and particles are degraded. This can lead to uncertainties in the results since the particles can settle if the liquid is not homogenized, and it is also difficult to extract all of

the phosphorus and nitrogen from the particles. Furthermore, the degradation also contributes to the uncertainty of the result since it is an additional step in the analysis.

The tests were made on wastewater samples collected on the 5 May 2015, the day when the Bugolobi WWTP wasn't working properly, which means that the concentrations showed here might not represent the true average concentrations at the outflow of the WWTP and in the Nakivubo channel.

5.2.2 COD and TOC

The COD and the TOC tests showed the same patterns: their concentrations declined between the inflow of the Bugolobi WWTP and the channel divide, but the concentrations increased again in the wetland. This indicates that more organic matter is added to the water in the wetland. The wetland is used to grow yam, which might explain the increase of organic matter at that point. Other plants such as grass and macrophytes also grow in the wetland. Still it is interesting that the COD and TOC increase in the wetland, as planted wetlands are constructed to treat wastewater. Yet total concentrations are not the full story and it should be noted that both COD and TOC change to more inert forms, as is discussed in the next section. Both the COD and TOC concentrations were low in Lake Victoria, but this is expected since the water was diluted.

The blanks made for the COD and TOC tests were much lower than the COD and TOC concentrations in the measurement points, showing that the tests seem reliable even though the blank made for the TOC test with the high detection range had noticeable pollution in it. The standard deviations for the wetland samples were high in both of the tests, showing that the highest variation in the concentrations was at that point of measurement. This is probably because of the high number of plants grown in the wetland, as the number of particles varied in the samples. The standard deviation for the inflow COD sample (Figure 9) was also high, showing that there is a higher degree of variation and uncertainty in that result. This can be explained by the fact that the inflow COD samples had a dilution factor of two while all the other samples had a dilution factor of one (Table 5). When the sample is diluted the variations are magnified when the true concentrations are calculated. Furthermore, inflow water is by nature more heterogeneous than treated water. Fuhrimann et al. (2014) also measured the COD concentrations along the Nakivubo channel and Lake Victoria. Their COD concentrations' 95 % confidence interval ranged between 211.3 and 303.5 mg/l. Those concentrations are comparable to the averages found here, even if the concentrations in this thesis varied more (Figure 9).

The tests were made on wastewater samples collected 5 May 2015, the day when the Bugolobi WWTP wasn't working properly, which means that the concentrations showed here might not represent the true average concentrations at the outflow of the WWTP and in the Nakivubo channel.

5.2.3 BOD_5

The BOD_5 concentration decreased steadily between the measurement points, unlike the COD or TOC concentrations (Figure 10). The COD and TOC concentrations both increased in the wetland, unlike the BOD_5. This indicates that the organic matter that is polluting the wetland water was not easily biodegradable, just as can be expected if it is humic substance. The standard deviations are quite low, showing that there is little variation in the concentrations. This can also be due to the fact that the samples only had two replicates each (Table 5).

5.2.4 TS and TSS

The TS and TSS concentrations showed different patterns, they increased and decreased alternately (Figure 11). The standard deviations were among the highest seen in this thesis, showing a very large variation in both TS and TSS concentrations. A possible explanation for this could be inconsistencies in the lab work. The TS concentrations were supposed to be measured before the sample filtration started, however due to unfortunate circumstances in some cases, the TS concentrations were measured after the filtration had started, which resulted in a disproportionate amount of solids left in the sample. Before the measuring of TSS the bottle containing the sample was supposed to be shaken well, but it is possible that the bottle was not always shaken enough. The filters and bowls used to measure the TS and TSS were weighed on different scales with different accuracies, which also may have affected the results. Some of the samples had negative results because the filters or bowls weighed less after the test, but these were not included in the results. The high variation in concentrations can also be explained by the fact that the means are comprised of measurements from both the 29 April and the 5 May, and the variations between the two sampling days show in the results. The outflow samples and TS concentration in the channel are those that have the lowest variation. If these tests were to be repeated, lab procedures would be followed more carefully, and all samples would be weighed on the same scale.

Fuhrimann et al. (2014) presented TSS concentrations with a 95 % confidence interval of 140.8-256.7 mg/l. Those concentrations are of the same magnitude as the ones found in this thesis, even if the concentrations in this thesis had a larger variation.

5.3 WASTEWATER TREATMENT AND QUALITY

Uganda has regulations about how much contaminants are allowed in the wastewater that is discharged onto land or water bodies set by the National Environment Management Authority (NEMA) (NEMA, 1999). The wastewater analysis results were compared to the maximum permissible limits of PO_4, NO_3-N, TN, COD, BOD_5 and TSS concentrations in wastewater discharged in Uganda, and it was apparent that almost all of the limits were exceeded (Table 7). It is clear that the water is polluted, and this could also explain the high number of pharmaceuticals found in the water samples.

Table 7. The maximum permissible limits for some wastewater parameters as reported by NEMA (1999), compared with the concentrations found in the outflow of Bugolobi WWTP and Nakivubo channel. The values are averaged, and based on different number of replicates (Table 5). The PO_4 concentrations were calculated by multiplying the PO_4-P concentrations by 3.066. Concentrations exceeding the limits are marked with bold text

Parameter	Maximum permissible limits (mg/l)	Outflow (mg/l)	Channel (mg/l)
PO_4	10	**12.3**	**16.6**
NO_3-N	10	1.9	2.4
TN	20	**53.1**	18.2
COD	100	**393**	**153**
BOD_5	50	**368**	**187**
TSS	100	**101**	**478**

The pollution from pit latrines and the malfunctioning WWTP can be two possible explanations for the exceeding concentrations. Pit latrines have been shown to significantly pollute shallow aquifers in slum areas of Kampala with nutrients (nitrogen and phosphorus) (Nyenje et al., 2013; Nyenje et al., 2014). Nyenje et al. (2013) studied the leaching at two

sites: one waste dump and a site with two pit latrines. Results showed that the two pit latrines increased the nutrient load of NO_3, NH_4 and PO_4 downgradient of the pit latrine-site. The pit latrines discharged their contents into the groundwater. 2-20 % of N input and 0-1 % of P input leached to the groundwater from the pit latrines. Apparently, a lot of the nutrients were retained in the soil or in the pit latrine. If the nutrients are leaking, there is a risk that pharmaceuticals are leaking from the latrines as well. Another explanation could be Kasanvu slum which lies very close to Nakivubo channel (Figure 1). As of late 2011, only two public toilets were available for the 1000 people living there, resulting in a lot of open defecation (Kasanvu slum's appalling hygiene, 2011), meaning that a lot of heavily polluted storm water will reach the channel when it rains. Sewage from Kasanvu slum is most likely polluting the Nakivubo channel.

5.4 SOIL ANALYSIS

5.4.1 Results

The TOC content showed as percent of TS, was highest in the yam soil, 35.7 % of TS and lowest in the maize soil, 4.9 % TS (Table 8). The Tot-C is almost the same as TOC. The total nitrogen content was highest in the yam soil, 2.8 % of TS while the phosphorous concentration was highest in the sugar cane soil, 2 350 mg/kg TS. All the three soils contained large amounts of iron (Fe) and aluminium (Al). All soils contained zinc and manganese. The pH was almost 7 for all soils.

Table 8. Measured soil parameters such as pH, total solids (TS), total nitrogen (Tot N), total carbon (Tot C), total organic carbon (TOC), phosphorus (P), iron (Fe), aluminium (Al), manganese (Mn) and zinc (Zn)

Parameters	Yam soil	Maize soil	Sugar cane soil
pH	7.0	7.1	7.0
TS %	58.3	98.0	99.5
Tot N (% TS)	2.8	0.4	0.5
TOC (% TS)	35.7	4.9	7.0
Tot C (% TS)	35.8	5.0	6.9
P (mg/kg TS)	1 070	1 560	2 350
Fe (mg/kg TS)	23 200	40 300	58 500
Al (mg/kg TS)	33 700	41 600	59 300
Mn (mg/kg TS)	776	837	1 040
Zn (mg/kg TS)	116	246	538

5.4.2 Discussion

All of the three soil samples had a neutral pH-level, around 7. The total nitrogen content was highest in the yam soil, 2.8 % of ts (Table 8), although almost no external fertiliser, such as nitrogen or phosphorus was added to the fields (3.6). This could be due to that the yam soil was saturated in wastewater and then more nitrogen was adsorbed in the soil as the nutrients enter via the wastewater irrigation. As seen in the water samples in the channel and the wetland, the total nitrogen, total phosphorous and phosphate content were high (Figure 7; Figure 8). The nitrogen concentration is also due to the high TOC of the soil, 35.7%, as soil organic matter contains nitrogen. The phosphorous content was highest in the sugar cane soil, almost twice as high as the content in the other two soils (Table 8). Different sugar cane species have been seen to take up 0.34-0.56 kg phosphorus per tonne of cane. The species with the highest uptake were expected to be effective in removal of phosphorous (Rakkiyappan et al., 2007). The average optimal yield in Uganda is 89 ton sugar cane/ha, and

therefore the average phosphorus removal is 30.26-49.84 kg P/ha (Isabirye et al., 2013). But not all phosphorous is available for the crops, since it can attach really strong to the soil and be hard for the crops to take up (Eriksson et al., 2005). Phosphorous adsorbs to both aluminium and iron and makes strong connections (Eriksson et al., 2005). The aluminium and iron content were really high in all soils and highest in the sugar cane soil (Table 8). That could also explain why the phosphorous content was higher in the sugar cane soil. However, more studies need to be done to clarify the reason. In a study made by Gao et al. (2010) total phosphorous was found in concentrations around 600 mg/kg in unrestored and restored wetlands in China. This is much lower than the concentrations found in this thesis (Table 8).

The soil contained the metals manganese (Mn) and zinc (Zn). That indicates that the wastewater loading into the fields is high, since most of the contaminants are expected to enter via the wastewater. It has earlier been seen that the soil could be Mn and Zn contaminated since high values have been found in different crops (Mbabazi et al., 2010b). The organic content in the soil was lower in the sugar cane and maize fields compared with the yam field (Table 8). The TOC content in the soils depend on the balance between the litter production and the degradation (Eriksson et al., 2005). Since the yam field is saturated with water, it is assumed to be anaerobic and therefore microbiological degradation is lower than in the more aerobic sugar cane and maize fields (Eriksson et al., 2005).

5.5 SURVEY OF THE TYPES OF MEDICINES PRESCRIBED AND SOLD IN KAMPALA

5.5.1 Results

The active ingredients in the pharmaceuticals which had been prescribed and sold in largest quantities by pharmacies in Kampala for different types of diseases were summarised (Appendix H). The bars show the number of pharmacies which responded that these compounds were prescribed or sold in large quantities. In total 47 different pharmaceuticals had been prescribed (Appendix H), and 12 of them were antibiotics and 12 were added as internal standard in this analysis. The most frequently prescribed and sold pharmaceuticals reported by the pharmacies were ciprofloxacin, cetirizine and metformin, and they were all analysed in this thesis (Table 9).

Table 9. All pharmaceuticals for all diseases mentioned more than three times by the 15 pharmacies which responded. The bold text are pharmaceuticals matching pharmaceuticals analysed in this thesis. Lumefantrine and ibuprofen could not be analysed for different reasons, but are still included in the list since they were intended to be analysed

Pharmaceutical	Pharmacies mentioning	Pharmaceutical	Pharmacies mentioning
Ciprofloxacin	**17**	Valproic acid	7
Cetirizine	**15**	**Amoxicillin**	**7**
Metformin	**15**	Duo-Cotexine	6
Metronidazole	**14**	Levofloxacin	6
Omeprazole	**14**	Microgynon	6
Amlodipine	**12**	Nifedipine	6
Salbutamol	**12**	Cold cap[1]	6
Doxycycline	11	Amoxyl	5
Rifampicin	11	Clotrimazole	5
Coartem **(**artemether/ **lumefantrine)**	**13**	Digoxin	5

Pharmaceutical	Pharmacies mentioning	Pharmaceutical	Pharmacies mentioning
Glibenclamide	10	**Losartan**	**5**
Cefixime	9	Prednisolone	5
Levonorgestrel	9	Cloxacillin and Ampicillin	4
Loperamide	9	**Ibuprofen**	**4**
Phenylpropanolamine	8	Loratadine	4
Ketoconazole	**7**		

[1] Contains the active ingredients decongestants, acetaminophen (APAP), and antihistamines

The two pharmaceuticals diclofenac and lamotrigine, were also mention as the most prescribed, two respective one time by the pharmacies. They have both been analysed in this thesis.

The quantity of the most sold pharmaceuticals was not compiled. Ten of fourteen pharmacies answered that they gave information to the consumer on how to dispose unused or expired medicines. Only three pharmacies told their customers to return the unused/expired medications to the pharmacy for destruction. Two pharmacies said that they told the customer to get rid of unused/expired medicines by throwing them in pit latrines or in dustbins and one advised the customers to throw them in a safe place.

Eight of the twelve pharmacies said that the expired/unused medicines were collected in a separate bin and then were taken for destruction. Four pharmacies were giving the medicines to the NDA (national drug authority) for destruction and three pharmacies took them to the city Nakasongola 140 kilometres north of Kampala for destruction. All thirteen pharmacies said that the concerned authority have given them instructions/restrictions of how to handle unused or expired medications.

5.5.2 Discussion

The aim of the survey was to obtain knowledge about the pharmaceuticals that were prescribed in Kampala, since this information was hard to find. About 24 %, 10 of the 42 analysed pharmaceuticals in this thesis, were among the most prescribed pharmaceuticals by the pharmacies questioned in Kampala and two more (diclofenac and lamotrigine) were sold by three pharmacies (Table 9). The questionnaires these results relay on, did not cover all pharmaceuticals prescribed, used and disposed of in Kampala. Other sources, like the hospitals and the pharmaceutical manufactories in the area also contributed to pharmaceuticals to the environment (2.2), and not all pharmacies in Kampala were asked to participate in the survey as well. Some of the filled in forms were also hard to read since the pharmacies filled them in by hand and not all pharmaceuticals written were readable. Of the pharmacies contributing, the majority answered that they were taking care of expired and unused pharmaceuticals in an adequate way. Only a few were advising their costumers to get rid of the pharmaceuticals by disposing of them in an environmentally harmful way, e.g. dispose of them in a pit latrine (5.5).

In this thesis other prevention medications such as charcoal and zinc were neglected since they are not conventional pharmaceuticals. Ten of the most prescribed pharmaceuticals are covered in the analysis (Table 9). Unfortunately, the quantity of the pharmaceuticals prescribed could not be compiled due to different units and misunderstanding of the question

by the pharmacies. Therefore the survey only gives a qualitative measure on the most pharmaceuticals prescribed and sold in large quantities in Kampala.

It is hard to determine the pharmaceutical use in Kampala. Lack of regulations on antibiotic medications enables antibiotic pharmaceuticals to be prescribed in great extent. In the survey twelve antibiotics were commonly mentioned of all 47 different pharmaceuticals prescribed (Appendix H). On the other hand, only a minority of the people could afford or had the opportunity to buy pharmaceuticals when they are needed them (2.2).

Antiretroviral diseases like, HIV, is an illness that 1.6 million people in Uganda live with (AVERT, 2015). It is also a sensitive subject and many people does not want to talk about it. The antiretroviral (ARV) medications are provided to the people free of charge by the governmental health centres. That means that they are rarely stocked at pharmacies and only really advanced ARV medicines are stocked at selected pharmacies. To be able to freely access the ARV medications the public only need to be tested positive at a health care centre[6]. The use of these medications is also expected to increase in the future due to better preventive methods (Alcorn, 2011). The ARV medications and their physicochemical properties have not been studied in this thesis. However due to the use and manufacturing, ARV pharmaceutical flows in the area ought to be significant. They need to be determined in further studies.

5.6 PHARMACEUTICALS IN THE WATER SAMPLES

5.6.1 Results

A total of 29 out of 42 pharmaceutical compounds were detected in the water samples. Notable examples are atenolol, carbamazepine and trimethoprim for being found in detectable concentrations in almost all of the replicates with ranges of 40-1636, 106-751 and 4625-26055 ng/l respectively (Table 10). Some of the compounds were detected but could not be quantified since they were below the LOQ, for example ranitidine and sotalol.

Table 10. The concentrations (means ± standard deviations) of the compounds detected in the water samples from the inflow and outflow of Bugolobi WWTP, Nakivubo channel divide, Nakivubo wetland and in Lake Victoria. The n value shows in how many replicates the compound or internal standard was detected, four being the highest. LOD stands for limit of detection, LOQ stands for limit of quantification and DE stands for detected. A value of DE means that the pharmaceutical was detected in the sample but had an indeterminable concentration. Values marked in italics have been calculated. In the cases where neither the compound nor the internal standard was found in the sample, the cell has been marked with a stroke

Pharmaceutical	Inflow (ng/l)	Outflow (ng/l)	Channel (ng/l)	Wetland (ng/l)	Lake Victoria (ng/l)
Acetaminophen	DE (n = 2)	DE (n = 2)	DE (n = 2)	DE (n = 3)	-
Amoxicillin	DE (n = 4)	DE (n = 3)	DE (n = 1)	-	-
Atenolol	949 ± 464 (n = 4)	1640 ± 444 (n = 4)	151 ± 134 (n = 4)	40 ± 31 (n = 4)	< LOQ (n = 3), < LOD (n = 1)

[6] Allan John Komakech, Department of Agriculutural & Bio systems Engineering, Makerere University, Kampala. Email 17.05.2016

Pharmaceutical	Inflow (ng/l)	Outflow (ng/l)	Channel (ng/l)	Wetland (ng/l)	Lake Victoria (ng/l)
Bezafibrate	< LOQ (n = 1), < LOD (n = 3)	< LOQ (n = 2), < LOD (n = 2)	< LOD (n = 3)	< LOD (n = 4)	< LOD (n = 4)
Carbamazepine	394 ± 210 (n = 4)	751 ± 471 (n = 3), DE (n = 1)	155 ± 94 (n = 4)	106 ± 35 (n = 4)	< LOD (n = 4)
Cetirizine	14.3 ± 13.2 (n = 3), < LOD (n = 1)	DE (n = 3), < LOD (n = 1)	DE (n = 3), < LOD (n = 1)	16.2 ± 12.6 (n = 3), DE (n = 1)	< LOD (n = 2)
Ciprofloxacin	DE (n = 1), < LOD (n = 1)	DE (n = 4)	DE (n = 3), < LOD (n = 1)	DE (n = 1)	< LOD (n = 2)
Clarithromycin	< LOQ (n = 1), DE (n = 1)	DE (n = 3)	DE (n = 1), < LOD (n = 2)	< LOD (n = 4)	< LOD (n = 4)
Climbazole	DE (n = 1)	DE (n = 1)	-	< LOQ (n = 1), DE (n = 1)	< LOD (n = 4)
Codeine	164 ± 70 (n = 3), < LOD (n = 1)	272 ± 26 (n = 2), DE (n = 1)	31 (n = 1), < LOD (n = 3)	< LOD (n = 4)	< LOD (n = 4)
Diclofenac	*397 ± 212* (n = 4)	*243 ± 236* (n = 3)	*153 ± 88* (n = 3)	123 ± 31 (n = 4)	< LOD (n = 4)
Furosemide	712 ± 635 (n = 4)	DE (n = 2)	137 (n = 1), < LOD (n = 2)	< LOD (n = 3)	337 (n = 1), < LOD (n = 3)
Hydrochlorothiazide	352 ± 136 (n = 4)	1030 ± 280 (n = 3), DE (n = 1)	139 ± 0.2 (n = 2), < LOD (n = 2)	< LOD (n = 4)	< LOD (n = 4)
Irbesartan	102 ± 19 (n = 2), < LOD (n = 2)	129 (n = 1), DE (n = 3)	< LOD (n = 3)	< LOQ (n = 3), < LOD (n = 1)	< LOD (n = 4)
Lidocaine	53 ± 19 (n = 3), < LOD (n = 1)	135 (n = 1), DE (n = 1)	48 (n = 1), < LOD (n = 3)	15 ± 10 (n = 2), < LOD (n = 2)	< LOD (n = 4)

Pharmaceutical	Inflow (ng/l)	Outflow (ng/l)	Channel (ng/l)	Wetland (ng/l)	Lake Victoria (ng/l)
Losartan	206 ± 257 (n = 2), DE (n = 1), < LOD (n = 1)	935 (n = 1), DE (n = 2)	111 ± 89 (n = 3), < LOD (n = 1)	95 ± 38 (n = 3), DE (n = 1)	< LOD (n = 4)
Metformin	-	*141* (n = 1)	*18* (n = 1)	*30* (n = 1)	< LOD (n = 1)
Metronidazole	DE (n = 4)	DE (n = 1)	DE (n = 1)	< LOD (n = 1)	< LOD (n = 4)
Metoprolol	5.4 (n = 1), < LOD (n = 3)	< LOD (n = 4)	< LOD (n = 4)	< LOD (n = 4)	< LOD (n = 4)
Omeprazole	< LOQ (n = 3)	< LOQ (n = 4)	< LOD (n = 2)	< LOD (n = 2)	< LOD (n = 3)
Pyrimethamine	*45 ± 52* (n = 2), < LOD (n = 2)	*513* (n = 1), DE (n = 1), < LOD (n = 2)	*22* (n = 1), < LOD (n = 3)	*4 ± 2* (n = 3), < LOD (n = 1)	< LOD (n = 4)
Ranitidine	< LOQ (n = 1), < LOD (n = 2)	< LOQ (n = 1), < LOD (n = 1)	< LOD (n = 3)	< LOD (n = 4)	< LOD (n = 4)
Roxithromycin	96.1 (n = 1), DE (n = 1)	-	< LOD (n = 2)	< LOD (n = 4)	< LOD (n = 4)
Salbutamol	40.3 ± 6.6 (n = 3), < LOD (n = 1)	57.8 ± 3.6 (n = 2), < LOD (n = 1)	20.9 (n = 1), < LOD (n = 3)	8.1 ± 1.6 (n = 3), < LOD (n = 1)	< LOD (n = 4)
Sotalol	< LOD (n = 4)	< LOQ (n = 3), < LOD (n = 1)	< LOD (n = 4)	< LOD (n = 4)	< LOD (n = 4)
Sparfloxacin	DE (n = 1), < LOD (n = 2)	< LOD (n = 2)	< LOD (n = 4)	< LOD (n = 4)	< LOD (n = 4)
Sulfamethoxazole	*3790 ± 968* (n = 4)	*3590 ± 538* (n = 4)	*2500 ± 2390* (n = 4)	2460 ± 2200 (n = 4)	< LOD (n = 3)

Pharmaceutical	Inflow (ng/l)	Outflow (ng/l)	Channel (ng/l)	Wetland (ng/l)	Lake Victoria (ng/l)
Trimethoprim	*8430 ± 12 300* (n = 3), < LOD (n = 1)	*26 100 ± 30 300* (n = 3), DE (n = 1)	*4630 ± 2290* (n = 4)	*4630 ± 30* (n = 3), < LOD (n = 1)	< LOQ (n = 1), < LOD (n = 3)
Venlafaxine	< LOD (n = 2)	DE (n = 2)	< LOD (n = 3)	41.5 ± 34.2 (n = 2), < LOD (n = 2)	< LOD (n = 4)

Only two of the pharmaceuticals that had detectable concentrations in both the inflow and outflow of Bugolobi WWTP showed a decrease in concentration at the outflow. These were sulfamethoxazole and diclofenac. The removal of those pharmaceuticals was 5 % and 39 %, respectively.

The standard deviations for the compounds in the water samples were generally very high. The value of the standard deviation for losartan at the inflow of Bugolobi WWTP is even higher than the mean. Atenolol, carbamazepine, codeine, hydrochlorothiazide, irbesartan, lidocaine, losartan, pyrimethamine, salbutamol and trimethoprim all had a higher concentration at the outflow of Bugolobi WWTP than at the inflow, showing average increases between 18 ng/l (salbutamol) and 17 600 ng/l (trimethoprim). Most pharmaceuticals show a decrease in concentration in the channel, wetland and Lake Victoria, compared to the inflow and outflow of Bugolobi WWTP. The exceptions were furosemide and metformin that showed an increase in concentrations in Lake Victoria and the Nakivubo wetland, respectively. The decrease in concentration between the outflow and the channel varied between 30 % (sulfamethoxazole) and 96 % (pyrimethamine).

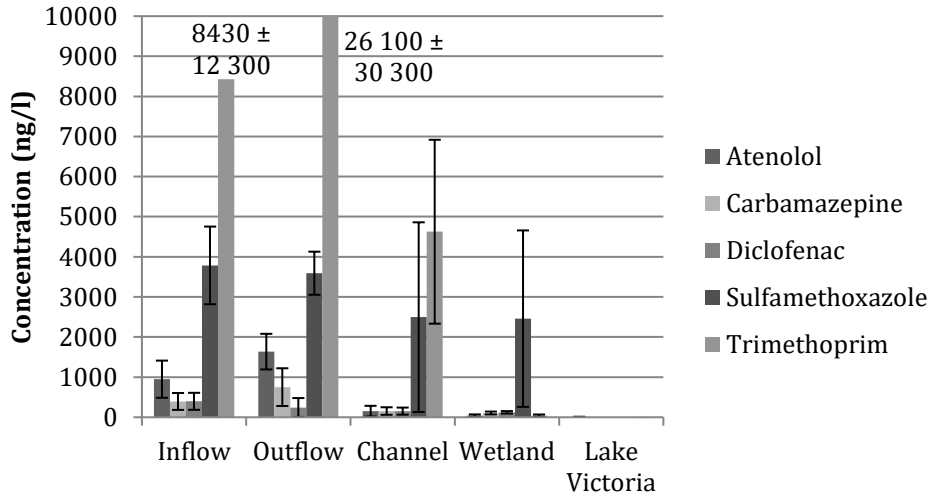

Figure 12. *The average concentration of selected pharmaceuticals at the inflow and outflow of Bugolobi WWTP, Nakivubo channel, Nakivubo wetland and Lake Victoria. The standard deviations are marked with black lines. The concentration and standard deviations of trimethoprim at the inflow and outflow of Bugolobi WWTP could not fit in the figure, and are instead given in numbers in the figure.*

Trimethoprim showed the largest average concentrations found in the samples (39-26 100 ng/l) and sulfamethoxazole showed the second highest (2460-3790 ng/l) (Figure 12). Sulfamethoxazole and trimethoprim also showed the largest standard deviations. Thirteen of the compounds studied did not have a detectable concentration in any of the replicates. The LODs for the channel, wetland and Lake Victoria samples were generally lower than the LODs for the inflow and outflow (Table 11). The pharmaceuticals with the largest LODs were amoxicillin and gemfibrozil, while cetirizine and pyrimethamine had the lowest. The LODs for acetaminophen and metformin could not be calculated due to incomplete data.

Table 11. The limits of detection (LODs) and limits of quantification (LOQs) of the compounds found in the water samples. Two different LODs and LOQs were calculated: one was representative for the compounds found in the inflow and outflow of the Bugolobi WWTP while the other was representative of the compounds found in Nakivubo channel, Nakivubo wetland and Lake Victoria. Some limits were indeterminable for different reasons, and these have been marked with a stroke

	Inflow, outflow		Wetland, channel, Lake Victoria	
Pharmaceutical	LOD (ng/l)	LOQ (ng/l)	LOD (ng/l)	LOQ (ng/l)
Acetaminophen	-	-	-	-
Amoxicillin	-	-	269	898
Atenolol	41	137	6	19
Bezafibrate	-	-	10.9	36.4
Carbamazepine	12.3	41.0	5.3	17.6
Cetirizine	1.0	3.3	-	-
Ciprofloxacin	294	979	87	290
Clarithromycin	-	-	68	228
Climbazole	-	-	2.9	9.7
Codeine	43	144	8	26
Diclofenac	-	-	12.2	40.6
Furosemide	169	562	68	227
Gemfibrozil	-	-	395	1320
Hydrochlorothiazide	27.9	93.0	22.9	76.3
Irbesartan	-	-	67	222
Lidocaine	12.1	40.4	2.4	8.0
Losartan	9.2	30.6	9.2	30.6
Metformin	-	-	-	-
Metronidazole	-	-	18.6	62.0
Metroprolol	2.3	7.7	7.7	25.7
Omeprazole	-	-	18.3	61.1
Pyrimethamine	-	-	2.0	6.7
Ranitidine	-	-	38	127
Roxithromycin	-	-	73	244
Salbutamol	4.8	16.0	-	-
Sotalol	-	-	33	111
Sparfloxacin	42	140	37	122
Sulfamethoxazole	-	-	15.6	52.0
Trimethoprim	57	190	4	14
Venlafaxine	-	-	14.1	46.9

The recovery for the liquid samples ranged from 0.3 % (ketoconazole) to 155 % (metroprolol) (Table 12). Acetaminophen, amlodipine, cetirizine, clotrimazole, fluoxetine, ofloxacin and sertraline all had a recovery of 0 %. Apart from those, the liquid samples generally had a high recovery with 17 pharmaceuticals having a recovery above 90 %.

Table 12. The average recovery in % for the liquid samples based on values from water collected at Gaba WTP the 30 April 2015. The values were calculated using equation (5). The recovery could not be calculated for acetaminophen, cetirizine, pyrimethamine and trimethoprim since the data was inadequate; these values have therefore been marked with a stroke

Pharmaceutical	Recovery (%)	Pharmaceutical	Recovery (%)
Acetaminophen	-	Irbesartan	98.2
Amitriptyline	4.9	Ketoconazole	0.3
Amlodipine	0	Lamotrigine	98.4
Amoxicillin	17.4	Lidocaine	92.6
Atenolol	92.5	Losartan	93.7
Atorvastatin	113	Metformin	1.3
Bezafibrate	95.4	Metronidazole	75.4
Carbamazepine	120	Metroprolol	155
Cetirizine	-	Ofloxacin	0
Citalopram	101	Omeprazole	7.7
Ciprofloxacin	98.8	Oxazepam	61.3
Clarithromycin	14.1	Pyrimethamine	-
Climbazole	3.0	Ranitidine	84.2
Clotrimazole	0	Roxithromycin	125
Codeine	80.7	Salbutamol	92.5
Diazepam	102	Sertraline	0
Diclofenac	86.2	Sotalol	86.4
Fluoxetine	0	Sparfloxacin	135
Furosemide	75.7	Sulfamethoxazole	65.1
Gemfibrozil	68.3	Trimethoprim	-
Hydrochlorothiazide	99.4	Venlafaxine	92.9

5.6.2 Discussion

Atenolol, carbamazepine, diclofenac, lidocaine, losartan, pyrimethamine salbutamol, sulfamethoxazole and trimethoprim were all found in detectable concentrations at the inflow and outflow of Bugolobi WWTP, Nakivubo channel and Nakivubo wetland (Table 10). Trimethoprim and sulfamethoxazole had the highest mean concentration and these were also two of the most common found drugs in a study by K'oreje et al. (2016) of pharmaceuticals found in surface waters in Kenya. K'oreje et al. (2016) also had a high detection frequency of diclofenac in river water, along with carbamazepine. Aus der Beek et al. (2016) has concluded that sulfamethoxazole, trimethoprim, diclofenac, naproxen and ibuprofen are the five most commonly found pharmaceuticals in aquatic environments globally. This suggests that if naproxen and ibuprofen could have been analysed in this thesis those would have been found in detectable concentrations in many of the samples as well. Atenolol has low lipophilicity, and a low affinity for sorption to sediment, which could explain its persistence through the water (Küster et al., 2010). Thus, the pharmaceuticals that were found in the most samples in this thesis also seem to be the most common and persistent drugs found in other studies. The lack of pharmaceuticals at detectable concentrations in Lake Victoria is believed to be caused by the dilution in the lake. A similar result was obtained for the wastewater parameters (5.1).

Atenolol, carbamazepine, codeine, hydrochlorothiazide, irbesartan, lidocaine, losartan, pyrimethamine, salbutamol and trimethoprim all had a higher mean concentration at the outflow of Bugolobi WWTP than at the inflow. This seems to suggest that many

pharmaceuticals in the WWTP are transformed back to the mother substances analysed in this thesis. WWTPs are designed to remove suspended solids and degradable dissolved organic matter, but are lacking when it comes to pharmaceuticals and other similar pollutants (IVL, 2015). It is not unusual that some compounds' concentrations increase in the effluent of a WWTP compared to the influent. β-blockers (such as atenolol) are generally hard to remove from wastewaters (IVL, 2015). Carmona, Andreu and Picó (2014) found an increase in concentration in the effluent for a couple of different pharmaceuticals, e.g. diclofenac and gemfibrozil. Poor or variable removal has been shown for carbamazepine and hydrochlorothiazide in Spain (Gros et al, 2010). Göbel et al. (2007) found in their study an increase of trimethoprim concentration in a WWTP in Switzerland. The increase of these medicines can be explained by transformation of the compounds or desorption from particulate matter during the treatment, and could be an explanation for the increased concentrations in this thesis as well (Carmona, Andreu and Picó, 2014). The increasing concentrations of the above mentioned pharmaceuticals could be explained by the poor removal rates of those compounds in the WWTPs. However, since a lot of the sewage that should be treated in Bugolobi is released into the environment due to inadequate piping, the increase could also be explained by the untreated sewage that flows into the channel. Since only two pharmaceuticals showed removal in the WWTP, it is assumed that the treatment in regards to pharmaceuticals is not very good in Bugolobi, which was not surprising considering that the WWTP did not function on one of the sampling days.

The concentration of pharmaceuticals at the Nakivubo channel measurement point was generally much lower than at the outflow of Bugolobi WWTP. There seem to be no large point sources to the channel, but the measurement point lies close to the Kasanvu slum, which could be a diffuse source of pollution. TP and TSS results seem to indicate this as well (Figure 7; Figure 11).

Trimethoprim and sulfamethoxazole showed the highest mean concentrations of all the compounds in the water samples (Figure 12). The concentrations for trimethoprim were especially high. It should be noted however, that the concentrations have been calculated manually and are therefore not as certain as the results that were obtained directly from the UNIFI software (version 1.7, Waters, 2013). The high mean concentrations could be due to the persistence of these substances in the environment as has been discussed above, but a considerable part of trimethoprim's concentration is believed to be overestimated, due to the manual procedure. The concentration should not be seen as absolutely correct, but rather as an indication of the relationship between the measurement points.

Cetirizine, metformin and ciprofloxacin were the three most commonly sold pharmaceuticals studied in this thesis (Table 9). Ciprofloxacin and cetirizine were detected in a lot of the water samples (inflow, outflow, channel and wetland) but their concentrations couldn't always be determined. A relationship between the most sold pharmaceuticals and frequency of detection can still be observed.

There were generally high standard deviations among the water samples. This was due to the high variability in concentrations between the measurement days. In some cases, the concentrations in the water were twice as high (or even higher!) on the first day of measurement compared to the second day. This suggests that there is a high variability of concentrations on a day-to-day basis. It is also possible that the concentrations on the first day were unusually high, or the concentrations could have been unusually low on the second day. More measurements might have revealed a pattern. Metformin and furosemide had an

increase of concentration downstream, at Nakviubo wetland and Lake Victoria respectively (Table 10). Since there are so few replicates that had detectable concentrations of those pharmaceuticals it is hard to say if there was additional pollution of those compounds in the wetland and Lake Victoria or if it was just variability in the results that was seen.

Only five of the pharmaceuticals studied were not recovered in the water samples: amlodipine, clotrimazole, fluoxetine, ofloxacin and sertraline (Table 12). Some other compounds had a very low recovery, between 0.3 and 17.4 %, these were amitriptyline, amoxicillin, clarithromycin, climbazole, ketoconazole, metformin and omeprazole. These results indicate that another method would have been more suitable to extract those pharmaceuticals. With regards to the rest of the compounds, the method could be deemed successful, especially since a lot of the compounds had a recovery above 90 %.

5.7 HOW TO ACHIEVE A SAFE SEWAGE DISPOSAL IN KAMPALA

Since it has been proven that the sewage disposal in Kampala is inadequate at some places, and since pharmaceuticals consumed by humans mostly end up in excreta, it is logical to assume that the pharmaceutical load from humans can be diminished if the sewage is collected and treated in a sufficient way. The slum areas in Kampala seem to be in most need of proper sanitation and therefore this thesis will give suggestions on how to improve the sewage disposal in those areas.

In order to correct the situation surrounding the unhygienic pit latrines, there is need of a plan focused on the cleaning of the latrines. It is important that everyone who uses the latrines knows how to properly use and clean them. People also need to take responsibility for the cleanliness of the latrines. In order to incentive cleaning on a regular basis, Kwiringira et al. (2014) suggest that the users either hire a cleaner or take turns cleaning themselves. If these solutions are implemented, it is important that they are coordinated and supervised, with penalty for those that do not follow the rules.

While it is important that the existing latrines are working satisfactory, there is also need of additional sanitation solutions to combat the issues regarding long waits at the latrine, and residents having to walk far in order to access the latrines. It would be better if the latrines were shared by fewer people, two families on average or less. This would make the cleaning easier, and people might get a better sense of ownership, leading to better caretaking of the latrines. Another issue that need to be solved is the high pit filling rates. Going from communal latrines to shared family latrines have proven to reduce open defecation and excreta-related illnesses in Nepal (UNHCR, 2009). If new sanitation solutions are to be implemented in the Kampala slums, there are a few things to consider. They need to be able to withstand flooding, since parts of Kampala are flooded seasonally (The Republic of Uganda, 2010). The high water tables also need to be taken into account, as well as how the latrines should be emptied.

Kayima et al. (2008) suggests the use of EcoSan toilets. EcoSan toilets can be urine-diverting or non-urine-diverting and consist of two chambers built above ground; each chamber is big enough to house the sludge from one to two years of use. Only one chamber is used at a time. When the first chamber is full it is sealed off and the second chamber is used. After one to two years, when the second chamber is full, the first chamber can be emptied. Since the sludge has been decomposing for at least one year, it will be more hygienic and safer to handle than if it were to be emptied directly (UNHCR, 2009). The chambers are emptied by opening hatches on the backside of the chambers and removing the sludge manually. The

sludge can then be used as fertilizer on farmlands. The advantages of these toilets are that they can be used at places with high water tables and they are easier to empty than regular pit latrines since they are above ground. They require no water to be functional and they have proven to be successful in Korba, India and in a refugee camp in Eastern Nepal (WaterAid, 2014; UNHCR, 2009). However, UNHCR (2009) does not recommend the use of EcoSan toilets at places that are seasonally flooded. Other things to consider if EcoSan toilets are to be implemented in Kampala slums are that the toilets must be accepted by the locals, and since the sludge is supposed to be used as manure on farmlands, the farmers need to be willing to use the sludge on their lands. There is also need of monitoring to make sure the users know when to close the first chamber and when to remove the compost (WaterAid, 2014). This is to make sure the system works as intended. Finally, easily accessible stairs should be built to the toilets, to make it easier for everyone to use. If steep ladders are used, it might discourage women and children from using the toilets.

An alternative to EcoSan toilets are raised twin pit latrines. They are like regular raised latrines, except that they have two pits. Only one pit is used at a time, and when the first pit is full it is sealed off and the other pit is used, much like the EcoSan toilets. Nyenje et al. (2013) suggest the use of raised pit latrines in Kampala slums to inhibit pollutant transport to the groundwater. Raised pit latrines are already used in the slums to a certain degree, but some of them are hard to reach for the disabled, women and children (Kwiringira et al., 2014). If more raised pit latrines are built in the area, they should not be built with steep ladders leading up to the latrine. Instead, easily accessible stairs could be built to the latrine. The advantages of these latrines are that they can be used at places that are seasonally flooded and that have high water tables (UNHCR, 2009). Since pit latrines are widely used in the slums the locals should hopefully be used to the technology. One disadvantage of the raised pit latrines is that they are difficult to empty. It has been established beforehand that the emptying of pit latrines in Kampala slums is insufficient (3.1.1). However, since the sludge in the twin pit latrines have decomposed for several months before it is removed, it is safer to handle than from a single pit. This should reduce the health risks associated with pit latrine emptying, and will hopefully encourage more people to participate in the emptying. The sludge can be used as manure on farmlands, if the farmers are willing to use it.

A third option is to install simplified sewerage in the area. Simplified sewerage is a network of pipes that transport household sewage to be treated somewhere else, just like conventional sewerage (Paterson, Mara and Curtis, 2007). Unlike conventional sewerage however, the design in regards to depth, minimum pipe diameter and gradients are not as strict with simplified sewerage. The pipes can be made of vitrified clay or PVC and are connected with simple joints. The construction is flexible, making it ideal in places with unplanned infrastructure (Paterson, Mara and Curtis, 2007). The advantages of this system are that it is cheap and the sewage is transported elsewhere, hence there is no need of emptying. The use of simplified sewerage has been a success at places where the people are poor and where sanitation services commonly have not been found (Paterson, Mara and Curtis, 2007). The construction cost of simplified sewerage is generally higher than the cost of installing a pit latrine (simple or improved), but according to Sinnatamby (1983 cited in Paterson, Mara and Curtis, 2007), the annual cost of simplified sewerage was cheaper than on-site systems at population densities higher than ~160 people/hectare. In Brazil, the use of simplified sewerage is widespread and in some cases it works just as well as conventional sewerage, but costs much less (Watson, 1995). The use of simplified sewerage might not be ideal in Kampala slums however, since Kwiringira et al. (2014) reported that water borne facilities in the slums often were abandoned due to lack of constant water flow and lack of toilet paper

and cleaning supplies. It is not entirely certain that simplified sewerage is optimal at places with high water tables since there is a risk of overflows during floods.

Whatever sanitation system is chosen, the most important factor to consider is that it is accepted by the locals. It must be a system that they are willing to invest in, and it is important that everyone is on board and uses it correctly. It has been shown earlier that the people who clean and use their latrines properly can be discouraged from using their latrines if other residents are littering the area around the latrines with waste (Kwiringira et al., 2014). It is important that the residents are given information on how to properly use and clean their new system, otherwise the systems will be unusable and the residents will have to resort to open defecation again. Most likely there will be need of follow-up during a long period after the instalment of the system. There are also some overall challenges in providing safe sanitation solutions in the slums of Kampala. The work is hampered by the fact that the slums do not have legal statuses, which means that the structures can be demolished at any time. Residents that are renting houses are also unwilling to invest in sanitation solutions since they don't own the houses they live in (Paterson, Mara and Curtis, 2007; Katukiza et al., 2012). In conclusion: sanitation in the slum is a very complex issue, with a lot of factors to consider. There is no obvious solution to this problem, but with proper communication and cooperation with the locals the sanitation situation might be improved. This would lead to improved lives for the locals, and would hopefully reduce the leakage of pharmaceuticals to Nakivubo channel since the practice of open defecation would be diminished.

Only the pharmaceutical load from the slums has been discussed here, but the removal of pharmaceuticals in Bugolobi WWTP should also be studied in future studies. Suggestions could be given on how to treat the wastewater more efficiently in order to reduce the pollution of the pharmaceuticals that weren't effectively removed in the treatment plant. Suggestion should also be given on how the piping systems can be improved so that less untreated wastewater is released into the environment.

5.8 NATURAL REMOVAL OF SELECTED PHARMACEUTICALS IN THE WATER

5.8.1 Results

Four pharmaceuticals, atenolol, sulfamethoxazole, carbamazepine and diclofenac were all detected in the inflow, outflow, channel and wetland. Atenolol was also detected in the Lake Victoria. All pharmaceuticals did not show a clear trend of reduction except for the diclofenac, which decreased between all measurements points (Figure 13).

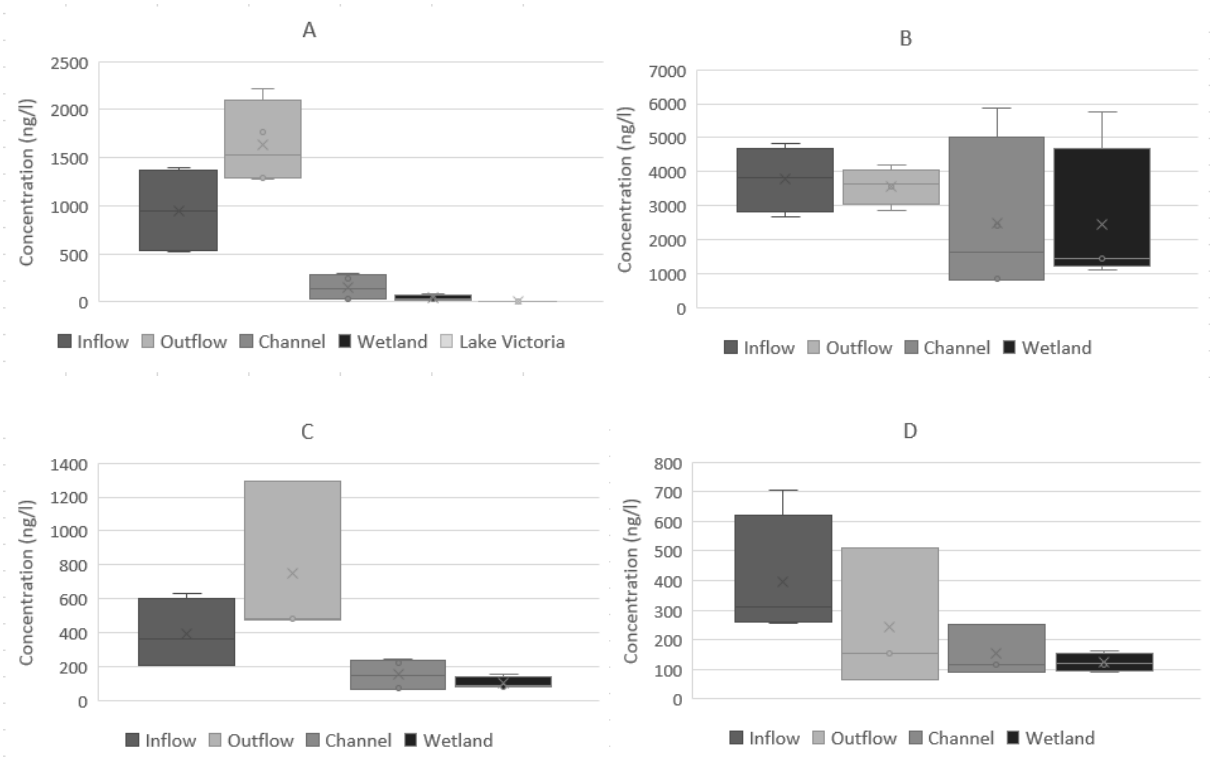

Figure 13. *Concentrations in ng/l for the pharmaceuticals atenolol (A), sulfamethoxazole (B), carbamazepine (C) and diclofenac (D) in the wastewater at the measurement points inflow, outflow, channel, wetland and Lake Victoria for atenolol. The data are from both sample dates, 4/29 and 5/5-15. The minimum and maximum measurement points used are 3 and 4. Average and median values are shown, the cross respective the line in the boxes. The boxes extended between 75 % and 25 % quartile and the standard deviation is presented with a line.*

There was a significant negative correlation between the concentration of carbamazepine in the water samples and total suspended solids (TSS), p-values < 0.05. I.e. concentration of carbamazepine decreased as the TSS concentration increased (Figure 14). All data were normal distributed when using Shapiro-Wilk test. The residuals were randomly spread which indicates that the measurement points were independent of each other. The residuals were also normal distributed when checked with a normal probability plot in Excel (Microsoft, 2013). There existed no significant connection between the concentration of TSS and the other pharmaceuticals most frequently found in the water; sulfamethoxazole, losartan, diclofenac and trimethoprim.

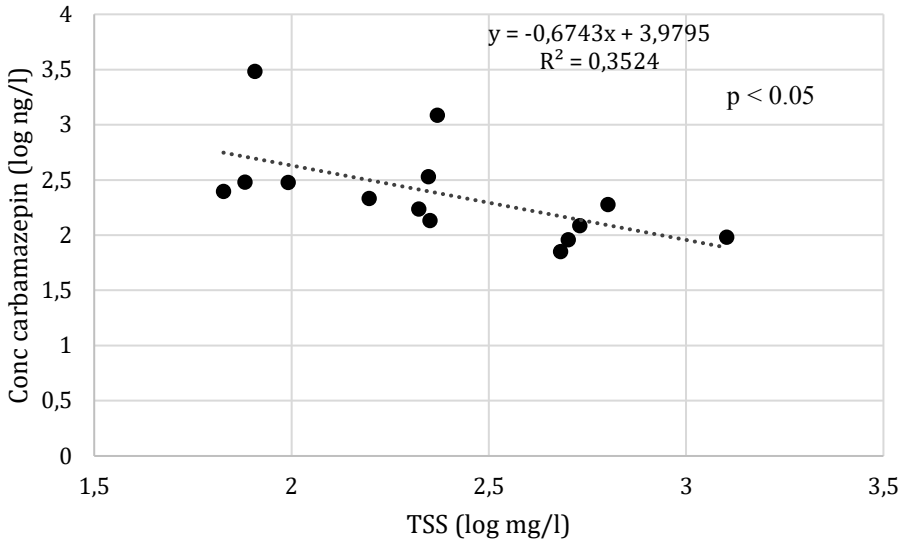

Figure 14. *Linear regression between the logarithm of the concentrations of carbamazepine in the wastewater and the total suspended solids (TSS) was found. The regression line with y = -0.67x + 3.98, R^2 = 0.35 is shown. n = 14.The p-value is below 0.05 which indicate that the correlation is statistically significant.*

5.8.2 Discussion

As expected, the highest concentrations in the wastewater were obtained in the beginning of the pathway of the water, inflow to and outflow from the WWTP, for the pharmaceuticals atenolol, carbamazepine and diclofenac (Figure 13). For all of them, the concentrations were then drastically decreased in the channel and continued to decrease in the wetland and were very low or not-detectable in the Lake Victoria. This result was expected since dilution with rain and channel water and some natural treatment of the wastewater was expected. Unfortunately due to few measurement points, the decline is not statistically significant. For sulfamethoxazole, the decrease is not as clear as for the others. Despite the fluctuations of the concentration in the channel and in the wetland, both the average and the median value have decreased. Some of the concentrations are estimated with linear regression while other are measured directly. It is therefore not optimal to compare the data with each other. Also, it was generally higher concentrations the second day (5 May 2015), which indicate that the concentrations fluctuated between days.

Carbamazepine and diclofenac were expected to adsorb to the sediment of the channel, since both have been detected in river sediments at other places (Yang, Toor and Williams, 2015; Carmona, Andreu and Picó, 2014). Other degradation like photodegradation and microbial degradation is also likely to occur for all pharmaceuticals (Thiele-Bruhn, 2003). E.g. antibiotics (like sulfamethoxazole) has been seen to decompose during transport (Kadlec and Wallace, 2008). This means that factors like how long the water is transported, i.e. the length of the Nakivubo channel and the retention time of the water also have impact on the degradation of the pharmaceuticals as well as the characteristics of the sediment (Kadlec and Wallace, 2008; Kansiime and Maimuna, 1999). It is approximately 3 km between the two measurement points, outflow and channel, and the retention time has roughly been estimated with simulations and it should be about 3.2 days in the central flow path (Kansiime and Maimuna, 1999; Kyambadde et al., 2004). According to Kadlec and Wallace (2008), the longer the retention time, the longer the time for interaction between the pharmaceuticals and the sediment/sun and more degradation will occur.

During the rainy seasons the retention time is expected to decrease due to more runoffs and higher water flow, and hence the degradation would be expected to decrease. With larger water flows it takes less than a day for the water to travel from the city and in dry weather conditions, four days (Kansiime and Maimuna, 1999). At the same time, under high flow conditions the wastewater will be more diluted and hence the concentrations will be lower. During the sunny seasons more photodegradation is expected to occur, which is especially important for diclofenac as it is classified as photodegradable (Matamoros, Sala and Salvadó, 2012). Lower concentrations were seen for diclofenac, sulfamethoxazole and carbamazepine in the channel the second sampling day. This could be a consequence of the rainfall which diluted the wastewater samples. The opposite was seen in the wetland, higher concentrations the second day, which also could be a consequence of the rainfall since the retention time in the wetland is expected to decrease due to higher water flow. But further research is needed to draw any conclusion on correlation between the concentration and the precipitation, more data is needed.

As described in chapter 3 the wetland has in earlier studies been reported to be efficient in treating the wastewater from nutrients and other contaminations before it reaches the Lake Victoria and the drinking water intake. Therefore the wetland was assumed to do some treatment on the wastewater also with regards to pharmaceuticals.

Hydrophilic pharmaceuticals are more treated in constructed wetlands than hydrophobic pharmaceuticals (Lee et al., 2011). As seen in this study the concentration of the hydrophilic pharmaceutical atenolol has decreased (Table 10; Figure 13), which could be because of the wetland. The wetland ought to possess great abilities to treat the wastewater with regards to pharmaceuticals. The high annual temperatures and the almost neutral pH in the area favour microbes and their activity, and provide low seasonality. Therefore, the decomposition rates and oxygen inputs, due to turbulence, are high (Kansiime and Maimuna, 1999). This gives good conditions for biodegradation of the pharmaceuticals. Chen at al. (2013) found that degradation and absorption in the soil were the two major ways for reduction of the pharmaceuticals diclofenac and naproxen.

The soil of the wetland, as described in chapter 3.3, tends to be more anaerobe than aerobic, due to high oxygen consumption. That is supported by the total nitrogen and nitrate measurements done in the diluted wastewater, which showed that the water was anaerobic (5.2.1). It has been seen that anaerobic conditions tend to improve the degradation of some micropollutants like pharmaceuticals (Matamoros, 2008). However, a number of authors have suggested that more developed aerial and underground parts of plants are beneficial for the removing capacity of constructed wetlands. The underground parts enable aerobic conditions, where the pharmaceuticals diclofenac and carbamazepine have been found to be efficiently removed (Verlicchi and Zambello, 2014) (Hijosa-Valsero et al., 2010). This conclusion is also supported by Hijosa-Valsero et al. (2011) who found that diclofenac was more reduced when vegetation was present. The Nakivubo wetland is mainly dominated by papyrus and mischantium grass, which could contribute to the uptake of pharmaceuticals. However the original vegetation has decreased due to increased cultivation of sugar canes and cocoyam (Kyambadde et al., 2004), which could have impact on the pharmaceutical uptake. But to be able to quantify the uptake by the plants, the plants need to be examined in future studies. The soil of the wetland also contains large amounts of organic carbon (Table 8) which mainly adsorb hydrophobic pharmaceuticals, as shown in this thesis by the carbamazepine and pyrimethamine content in the soil samples (Figure 16).

It has been seen by Li et al. (2014), that constructed horizontal subsurface wetlands are very effective in removing some pharmaceuticals such as sulfamethoxazole and trimethoprim. The Nakivubo wetland is classified as a horizontal subsurface wetland and can therefore be assumed to remove these pharmaceuticals from the wastewater. This assumption was largely supported by the results from this thesis, where the concentrations of many of the substances decrease between the channel and the wetland measurement points, but still, the concentration of a few substances stayed essentially the same (Table 10).

It can be seen that the wetland yam soil had accumulated carbamazepine, since measurable concentrations were detected for both the wetland water and the soil (Table 10; Table 13). When the concentrations are compared, the water in ng/ml and the soil in ng/g, it is seen that the soil had higher concentrations and therefore had adsorbed carbamazepine from the water. This also indicated that carbamazepine had accumulated in the soil. There existed a correlation between the total suspended solids (TSS) in the water and the carbamazepine concentrations (Figure 13). When the TSS content increased the carbamazepine content decreased. It was statistically significant, albeit low, however the decrease also depends on a lot of other factors like adsorption to the sediment discussed above. Unfortunately the causality could not be proven scientifically due to poor input data to perform calculation on and due to lack of studies in the same area. The high K_{oc}-value of carbamazepine indicated that carbamazepine easily adsorbs to solids and hence the reason for the correlation with TSS can be hypothesised. The TSS content increases in the channel while the carbamazepine content has decreased probably due to total suspended solid adsorption and a dilution factor (Figure 11; Figure 13). The TSS measurement in the wetland is lower than in the channel but is still expected to be high, since only three measurement points could be used, the wetland is also assumed to contribute to the water treatment. No correlation between TSS and the other pharmaceuticals most frequently detected were seen. It is hard to know why since both sulfamethoxazole and trimethoprim have been found to easy adsorb to solids. The number of measurement points available for the different pharmaceuticals may impact the result. The highest number was seen for atenolol with 18 points. More measurement points makes it more statistically significant to draw any conclusions. The results from the TSS analyse was the must uncertain result and that had impact on the results of the TSS correlation with different pharmaceuticals. If the TSS analysis would had shown more certain results the correlation between TSS and other pharmaceuticals that easy adsorbs to solids, was expected to be more certain and possible to be significant.

The ability of the wetland to reduce the content of heavy metals specifically zinc, copper, cadmium and lead has decreased with approximately 7 percentage points between the years 2006 and 2008 (Mbabazi et al., 2010a). One reason for the decrease is the increased urban wastewater loading to the wetland and reclamation of farming land in the wetland (Mbabazi et al., 2010a). This finding can also be assumed to affect the ability of the wetland to reduce the pharmaceutical content in the wastewater since it is assumed to depend on similar factors, i.e. wastewater loading and adsorption to soil. The wetland receives a lot of wastewater and exchanges water with the Lake Victoria through seiches (Figure 4). The waters behaviour and the hydrology affect the removing capacity of the wetland, for example has it been seen earlier that the retention time affects the removing capacity. The retention time of the wetland is only 0.5-2 days (Emerton, 2005). If the wastewater and the water from Lake Victoria is mixed, the risk for hydrophilic pharmaceuticals to enter the lake will increase, since it has been seen that the wastewater contains a lot of pharmaceuticals. However the pharmaceutical content in Lake Victoria found in this thesis was still low, but that does not ensure that the concentrations will not increase in the future. That also depends on the pharmaceutical

consumption, if it will increase and if the removal capacity of the wetland continues to go down, the concentrations of pharmaceuticals in the lake will increase. According to Emerton (2005), in order to fully utilize the capacity of the wetland to remove contaminants, the retention time needs to increase and the wastewater needs to be equally distributed over a larger area.

To conclude this section, the channel, the wetland and its soil have done some treatment on the wastewater and reduced the pharmaceutical content in the water with regards to several measured pharmaceuticals. One consequence of this is the accumulation in the soil of the pharmaceuticals carbamazepine and pyrimethamine. More research is needed, however to fully understand the treatment capacity of the Nakivubo channel and the wetland.

5.9 PHARMACEUTICALS IN THE SOIL SAMPLES

5.9.1 Results

In total, 11 of 42 pharmaceuticals were detected in the soil samples. The most common of them were pyrimethamine, which was found in all of the soil samples analysed, and carbamazepine. The pharmaceuticals amitriptyline and ketoconazole were found at detectable level in sugar cane soil, and diazepam in the yam soil, only in one of the replicates. The other replicates showed undetectable levels (<LOD) (Figure 15).

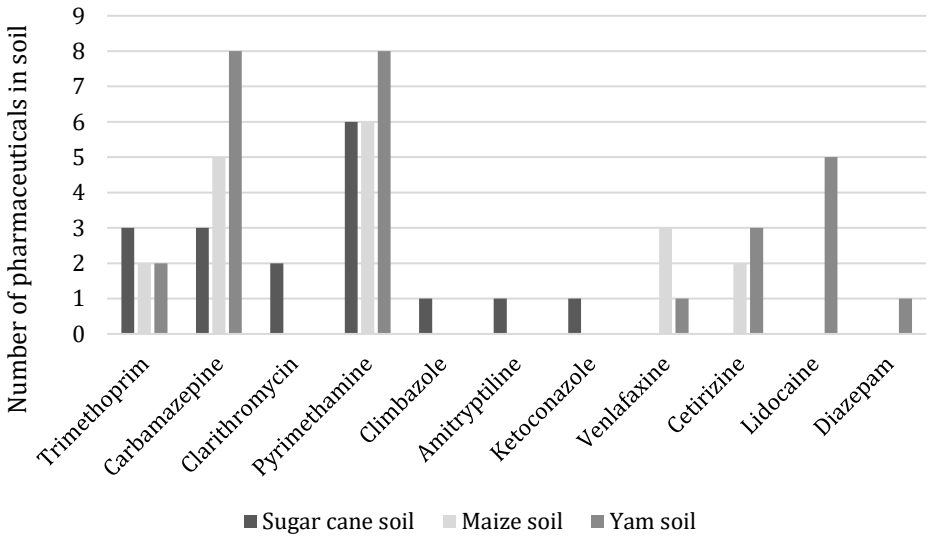

Figure 15. *Number of soil samples with detectable pharmaceutical levels, distributed between the sugar cane soil, the maize soil and the yam soil. Total number of samples for each soil are 6 sugar cane and maize soil samples, and 8 yam soil samples.*

A total of 11 pharmaceutical compounds were identified to occur in the different soil samples (Table 13). Carbamazepine and pyrimethamine were found at detectable levels in all types of soil at ranges of 4.6-9.4 ng/g for carbamazepine and 8.4-10.6 ng/g for pyrimethamine. Cetirizine and clarithromycin were identified to be present in some of samples but could not be quantified due to technical difficulties and thus were labelled as DE (detected).

Table 13. Concentration of pharmaceutical which were identified in yam, sugar cane and maize soils, mean ± standard deviation. The values in italic have been quantified using external calibration curves. The maximum number of samples were 8 in the yam soil and 6 in the sugar cane and the maize soils. n is the number of samples included

Pharmaceutical	Yam soil (ng/g)	Sugar cane soil (ng/g)	Maize soil (ng/g)
Amitriptyline	< LOD (n=8)	1.0 (n=1)	< LOD (n=6)
Carbamazepine	9.4 ± 2.9 (n=8)	4.6 ± 0.7 (n=3)	5.1 ± 1.9 (n=5)
Cetirizine	DE (n=3)	< LOD (n=1)	DE (n=2)
Clarithromycin	< LOD (n=3)	DE (n=2)	< LOD (n=3)
Climbazole	< LOD (n=8)	3.6 (n=1)	< LOD (n=5)
Diazepam	1.5 (n=1)	< LOD (n=6)	< LOD (n=5)
Ketoconazole	< LOD (n=1)	0.06 (n=1)	< LOD (n=2)
Lidocaine	5.8 ± 2.3 (n=4), < LOQ (n=1)	< LOD (n=6)	< LOD (n=6)
Pyrimethamine	*8.4 ± 4.9 (n=8)*	*14.0 ± 10.2 (n=6)*	*10.6 ± 4.4 (n=6)*
Trimethoprim	*< LOQ (n=2)*	*39.6 ± 13.8 (n=3)*	*< LOQ (n=2)*
Venlafaxine	< LOQ (n=1)	< LOD (n=6)	< LOQ (n=3)

Pyrimethamine showed the highest concentration in the soils, particularly in sugar cane soil collected during the second sampling occasion (5 May 2015; Figure 16). The concentrations of carbamazepine and trimethoprim were high, especially in the sugar cane soil 29 April 2015 (Figure 21). Lidocaine was only detected in the yam soil at 5.8 ± 2.3 ng/g. Venlafaxine was detected both in the yam and the maize soil, but the concentration could not be quantified. The results presented are the LOQ value divided by 2, which were quite high (Table 13).

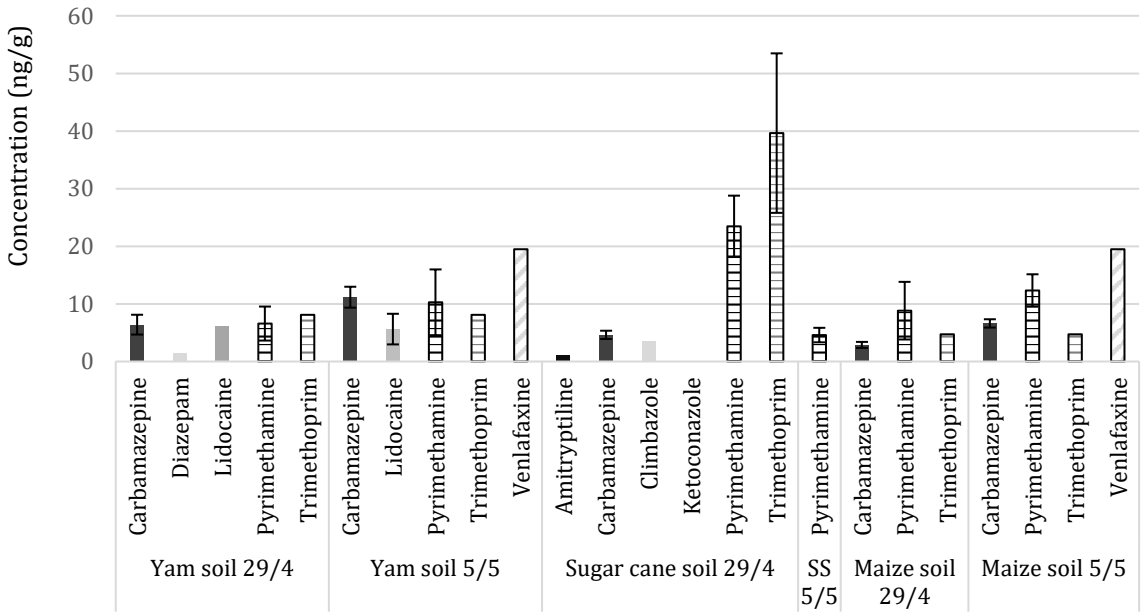

Figure 16. *Average concentrations in ng/g ± standard deviation of the pharmaceuticals identified at detectable levels in the soil samples. For venlafaxine the LOQ value divided by 2 is presented. The concentrations are split between the two sampling dates (4/29-15 and 5/5-15) and between the different soil types. The concentrations calculated by hand are showed with horizontal lines. SS is sugar cane soil.*

LODs and LOQs have been calculated in the samples with detectable pharmaceutical. Values for ketoconazole and carbamazepine in the sugar cane soil were not calculated due to lack of adequate data (Table 14).

Table 14. Limit of detection (LOD) and limit of quantification (LOQ) values in ng/g for different pharmaceuticals in the yam, sugar cane and maize soils. The boxes marked with a line mean no value has been calculated due to lack of data

	LOD			*LOQ*		
	Yam soil (ng/g)	Sugar cane soil (ng/g)	Maize soil (ng/g)	Yam soil (ng/g)	Sugar cane soil (ng/g)	Maize soil (ng/g)
Amitriptyline	2.7	0.3	5.0	9.1	1.0	16.6
Carbamazepine	0.6	0.6	0.4	1.9	1.8	1.3
Cetirizine	53	-	76	176	-	255
Clarithromycin	69	-	69	230	-	230
Climbazole	11.2	3.4	2.6	37.4	11.3	8.6
Diazepam	0.4	0.4	0.2	1.5	1.3	0.8
Ketoconazole	-	-	-	-	-	-
Lidocaine	1.0	1.0	3.8	3.4	3.5	12.5
Pyrimethamine	0.3	0.4	0.2	1.0	1.4	0.6
Trimethoprim	4.9	3.0	2.8	16.2	10.2	9.4
Venlafaxine	12	50	12	39	165	39

In average the recovery percentage was high for the detected pharmaceuticals in the soil, highest percentages are seen for clarithromycin and roxithromycin (Table 15).

Table 15. How much of the pharmaceuticals that have been recovered in percentage in the soil during the extraction. Where the line is seen, no adequate data was available and zero means no pharmaceutical was recovered. A mean value is given between the two recovery samples for each soil

	Yam Soil 2015-04-29 (%)	Maize Soil 2015-04-29 (%)	Sugar cane Soil 2015-04-29 (%)
Acetaminophen	-	-	-
Amitryptiline	-	27.0	9.3
Amlodipine	0	97.9	0
Amoxicillin	0	0	0
Atenolol	131	120	118
Atorvastatin	0	0	0
Bezafibrate	132	131	116
Carbamazepine	142	139	129
Cetirizine	39	440	-
Citalopram	126	113	113
Ciprofloxacin	0	0	0
Clarithromycin	653	730	-
Climbazole	12.8	21.3	18.9
Clotrimazole	0	0	0
Codeine	80.4	98.2	71.6
Diazepam	105	95	101

	Yam Soil 2015-04-29 (%)	Maize Soil 2015-04-29 (%)	Sugar cane Soil 2015-04-29 (%)
Diclofenac	87.9	86.0	91.9
Fluoxetine	69.3	-	-
Furosemide	-	0	0
Gemfibrozil	165	0	179
Hydrochlorothiazide	116	120	107
Irbesartan	121	112	112
Ketoconazole	-	-	0
Lamotrigine	130	105	105
Lidocaine	113	103	102
Losartan	88.4	0	0
Metformin	0	-	0
Metronidazole	84.4	89.2	76.2
Metoprolol	605	384	509
Ofloxacin	0	0	0
Omeprazole	0	0	0
Oxazepam	66.6	74.9	75.3
Pyrimethamine	84	227	167
Ranitidine	0	0	0
Roxithromycin	660	630	-
Salbutamol	134	133	134
Sertraline	0	0	0
Sotalol	241	159	185
Sparfloxacin	0	0	0
Sulfamethoxazole	67.0	72.3	67.9
Trimethoprim	87.7	97.4	82.0
Venlafaxine	109	98	75

There was a significant difference between the carbamazepine concentrations in the yam soil and sugar cane soil, and between the yam soil and the maize soil according to t-test used in the statistical program R, since p-value < 0.05 (Table 16). All data for carbamazepine and pyrimethamine were normal distributed, with p-value > 0.05, using the Shapiro-Wilk test in the statistical program R version 3.1.2. Highest concentration of carbamazepine was in the yam soil while pyrimethamine had highest variations in the sugar cane soil (Figure 17).

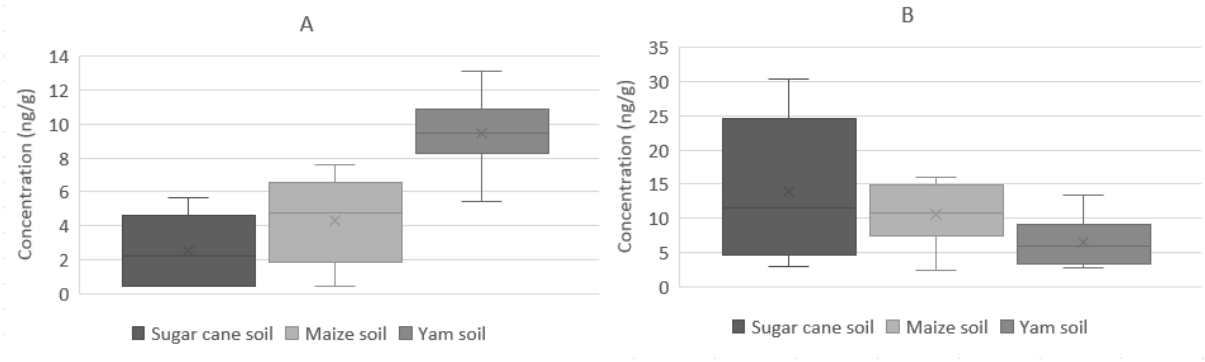

Figure 17. *A: Concentrations of carbamazepine in the sugar cane, maize and yam soil in ng/g. B: Calculated concentrations of pyrimethamine in the sugar cane, maize and yam soil in ng/g. The data are from both sample dates, 29/4 and 5/5-15, and their three replicates. In total 6 measurement points for each soil. Mean and median values are shown, the cross respective the line in the boxes. The boxes extend between 75 % and 25 % quartile and the standard deviation is presented by a line.*

Table 16. Results from the t-test for independent data for the difference in carbamazepine and calculated pyrimethamine concentrations between the yam soil (YS), sugar cane soil (SS) and maize soil (MS). p-value < 0.05 indicates that there is a significant difference in concentration and p-value > 0.05, indicates that there is no concentration difference between the soils

	YS & SS	YS & MS	SS & MS
Carbamazepine	*p < 0.001*	*p < 0.01*	p > 0.05
Pyrimethamine	p > 0.05	p > 0.05	p > 0.05

5.9.2 Discussion

Around 71 % of the analysed pharmaceuticals were detected in the soil recovery samples. The method QuEChERS, used in this thesis, was therefore assumed to be a suitable method for most of the pharmaceuticals that were recovered. The recovery percentage for the pharmaceuticals that were detected in the original samples were overall high (Table 15). Ketoconazole was lowest recovered, only 0.4 % in the sugar cane soil, whereas clarithromycin was recovered most, 650 % and 730 % in the yam respective the maize soil. The method QuEChERS was not optimal for the pharmaceuticals climbazole, amitriptyline and ketoconazole since they were not recovered enough, all below 28 % in all samples (Table 15). The pharmaceuticals that were not detected in any of the recovery samples were amoxicillin, atorvastatin, ciprofloxacin, clotrimazole, ofloxacin, omeprazole, ranitidine, sertraline and sparfloxacin. The pharmaceuticals amlodipine and metformin were only detected in the maize soil, which indicate that this method used for these compounds in the other soils probably was not optimal. Also the pharmaceuticals gemfibrozil, furosemide and losartan were not detected in all the soils. That indicates that this method might not be optimal for these pharmaceuticals as well.

In total 11 of 42 pharmaceuticals were detected in the soil (Figure 15). Considering the wastewater loading of the soils, the result is assumed to be a bit low but at the same time positive for the cultivation as it indicates that the levels probably were not very excessive. Among the most frequently mentioned prescribed pharmaceuticals (Table 9), only cetirizine and ketoconazole, were found at a detectable concentration in the soil (Table 13). Carbamazepine was however the most prescribed pharmaceutical for epilepsy, while pyrimethamine, used as a malaria medicine, found in all soil samples was not mentioned by the pharmacies (Appendix H). One reason for this could be that the parasites causing malaria have developed resistance to pyrimethamine and therefor the use of this medication has gone down in recent years and been replaced by other medicines (Talisuna et al., 2002). The occurrence of carbamazepine despite low consumption, can be explained by the persistence of

the pharmaceutical in the soil and its long half-life (Table 2). It is considered to be the most persistent pharmaceutical in the environment (Kodešová et al., 2016).

In a study made by Fenet et al. (2012), the presence of carbamazepine was determined in irrigated citrus plot soils in Tunisia. They found that the concentration increased when the soil was irrigated, the concentrations found were between 0.28 ng/g and 0.94 ng/g in a 6 months period (Fenet et al., 2012). These concentrations are much lower than the concentrations found in this thesis, which on average were between 9.4 ± 2.9 ng/g and 4.6 ± 0.7 ng/g in the different soil types (Table 13). The organic carbon content in the soil could be a reason for the high concentrations, since carbamazepine adsorbed to the carbon and the highest concentration was found in the yam soil, which also had the highest organic carbon content (Table 8). Anaerobic conditions is also expected to be a major factor since the microbial degradation is less effective in anaerobic conditions. One other explanation could be the time the soil has been irrigated with wastewater. In the study made by Fenet et al. (2012), the soil had only been irrigated for approximately 30 years, while the soil in the Nakivubo wetland has been irrigated with wastewater since year 1969 (Kansiime and Maimuna, 1999). Carbamazepine has been commercially available since year 1965 (Tolou-Ghamari, 2013). Another study made by Durán-Alvarez et al. (2009) looked at two different kind of soils (Phaeozom and Leptosol) that had been irrigated with wastewater for 90 years (Durán-Alvarez et al., 2009). They found concentrations of carbamazepine in the soils similar to the concentrations found in this thesis, 6.48 ng/g (in Phaeozem) and 5.14 ng/g (in Leptosol). They made the conclusion that carbamazepine is more persistent in soil than acidic pharmaceuticals like gemfibrozil and diclofenac, which were found in the soil in concentrations less than 1 ng/g. In this thesis, these two pharmaceuticals were not found in the soil at a detectable level. Carbamazepine has also been found in higher concentrations, up to 163 ± 56.4 ng/g but in biosolids in U.S (McClellan and Halden, 2010).

A notable difference between the sampling days was seen in the sugar cane soil. The samples taken 5 May 2015 only contain the pharmaceutical pyrimethamine, while the samples taken 29 April 2015 contains pyrimethamine, carbamazepine, amitriptyline, climbazole, ketoconazole and trimethoprim (Figure 16). It is hard to explain the difference, something could have gone wrong in the extraction, but this risk is assumed to be low since the samples were prepared at the same time and in the same way. It is unlikely that only these three samples were handled improperly, and not the other samples. As soil is heterogenic, an important source of variation is that the samples were not taken at the exact same spot on the two sample days. Furthermore, on the second occasion it had been raining, which could have had impact on the result. One other reason could be a combination of the rain and the soils organic content, 7.0 % of TS (Table 8). It could be that both the pharmaceuticals that attach to the organic matter and the ones that are more hydrophilic have been in the water phase. On the other hand the opposite can almost be seen in the yam and maize soil, were there was higher concentrations in samples from 5 May 2015 (Figure 16). These differences make the results more uncertain. More measurement points would be needed to know whether it could be a trend for rainy days or just variations between sample days.

Pyrimethamine was detected in the samples at higher concentrations than carbamazepine (Figure 16). These concentrations have been estimated using linear regression (4.5.6) ($R^2 = 1$, Table 6), which make the results more uncertain and are assumed to be an underestimate of the real concentrations. There is a lack of studies made on pyrimethamine in soil, therefor the concentrations found in this thesis is hard to compare with others. Pyrimethamine was detected in all samples and the highest concentration was detected in the sugar cane soil 29

April 2015 at 23.5 ng/g, however the lowest concentration was also detected in the sugar cane soil 5 May 2015 at 4.6 ng/g. This makes the result a bit uncertain with a high standard deviation. Pyrimethamine is a neutral compound and has a relatively high K_{oc}-value (1 569 l/kg) (Table 2). That indicates that the compound would attach strongly to the soil and be less mobile. The compound is also more hydrophobic and has low solubility in water. All of that indicates that pyrimethamine is more likely to exist in the solid phase, e.g. in the soil. Venlafaxine was detected in the two soils, maize and yam, but not in a quantifiable level (Table 13). The concentrations are somewhere below the limit of quantification.

Lidocaine was only detected in the yam soil. The compound is similar to pyrimethamine, in that it attaches to organic material and is slightly hydrophobic. However, compared to pyrimethamine, lidocaine is slightly more soluble in water and does not attach to the organic carbon content as hard as pyrimethamine (Table 2). The yam soil had a much higher organic carbon content than the other two soils, i.e. 35.7 % of TS compared with 4.9 % of TS (maize) and 7.0 % of TS (sugar cane). One explanation could then be that lidocaine was flushed out with the water and not attached in the soil. Another explanation could be that the yam soil is more exposed to the wastewater, due to the soil is soaked in diluted wastewater, than the other two soils and hence is expected to contain more contaminants. Trimethoprim was only found at a quantifiable level in the sugar cane soil samples 29 April 2015 but with a high variation in concentration (standard deviation is 13.8 ng/g) (Figure 16). The results have been calculated with help from linear regression and that will make them more uncertain. The compound tends to be hydrophilic and therefor dissolves in water and if protonated adsorbed to suspended solids in the water.

5.9.3 Differences in the Soil

The carbamazepine concentration, in the yam soil, was the only pharmaceutical content that was significant different from the content in the other soils (Table 16). This difference was expected to be due to the unequal contact between the water and the soil, sample variations, the different soil types, TOC and tot-C content and characteristics of the soil. According to Kodešová et al. (2016), the occurrence and behaviour of pharmaceuticals in soil are largely controlled by the distribution coefficient $K_{d.}$ They even propose that the soil type is the factor that affects pharmaceuticals persistence in soils the most. Unfortunately, in this thesis the soil type was not determine further than TOC and Tot-C, and the K_d coefficient for the studied pharmaceuticals in the different soils were not calculated, due very uncertain results for the total carbon content in the soils, since K_d depend on the results (Equation 2). The Tot-C was almost the same as TOC, most probably due to losses of inorganic carbon in the drying and freezing process and emissions as CO_2. This is recommended as to investigate the soil type for further studies to analyse, since it is important to understand the occurrence of pharmaceuticals in soil.

5.10 DISTRIBUTION OF PHARMACEUTICALS BETWEEN WATER AND SOIL

Selected pharmaceuticals with different physicochemical characteristics that were detected in the wetland wastewater but not in the wetland soil will be discussed further. The two blood pressure pharmaceuticals losartan and irbesartan have similar physicochemical characteristics. Both have high K_{oc}-values, 910 000 l/kg and 8.15x107 l/kg respectively (Table 2). This indicates that they should adsorb strongly to organic particles or to suspended solids in the water, and their mobility in soil is expected to be almost non-existent. Both of them are weak acids according to their pKa-values and will attach to organic material (Table 2). Losartan was only detected in the yam recovery soil samples while irbesartan was recovered in all the recovery soil samples. This indicates that the method used in this thesis may not be optimal

for extraction of losartan and that could have an impact on the result. One other reason for not finding losartan in the soil is that it could have adsorbed really strong to the soil layers first in contact with the wastewater. This is most likely for the maize and sugar cane soils. Irbesartan was found in lower concentrations than losartan in the wastewater and is therefore assumed to be adsorbing to the suspended particles in the wetland water. Irbesartan was not on the list for most prescribed pharmaceuticals which together with the results found in the wastewater and the soil, could indicate that the pharmaceutical recently has started to be used and therefore only recently begun to accumulate in the soil. According to Musinguzi and Nuwaha (2013) and Kayima et al. (2015) hypertension (high blood pressure) is common for adults in Uganda but the awareness of it is low. Kayima reported that only 13.7 % of the 553 hypertensive participants were aware of their condition. The awareness needs to increase and when it does, the medication use is expected to increase as well, provided that the medicines can be afforded.

The anti-inflammatory pharmaceutical diclofenac was detected in the wetland water with the average concentration 123 ± 31 ng/l (Table 10). Despite the relatively large concentration, diclofenac was not detected in any of the soil samples. It was only recovered in one of the two replicate recovery samples for each soil. Diclofenac has been seen in a previous study not to accumulate in soils even if they have been irrigated with wastewater for 90 years (Dalkmann et al., 2012).

Diclofenac has pKa-value 4.2, which indicates that the compound is present in its negative form in the soil. The compound is therefore expected to have low adsorption to soils with pH ≥ 7 (Graouer-Bacart, Sayen and Guillon, 2016). The examined soils in this thesis had all pH near 7 (Table 8), and diclofenac was therefore expected to adsorb poorly to the soils. Various results on the adsorption ability of diclofenac to soils have been reported. The variance is believed to depend on the soils used (e.g. soil pH, organic matter and clay content) and the experimental conditions the studies had. K_d and K_{oc} are much dependent on the soil types and will vary a lot. Since the K_{oc}-value varies a lot in different studies, it is not the best measure of diclofenac's adsorption ability to soils. Graouer-Bacart, Sayen and Guillon (2016) concluded that diclofenac generally does not adsorb to soils and instead moves downwards. The mobility increases significantly with absence of organic content in the soils and can possess increased risk for groundwater contamination (Graouer-Bacart, Sayen and Guillon, 2016). The groundwater and wetland water exchange in the Nakivubo wetland was assumed to be almost none existent, but it was not completely determined in the agricultural parts of the wetland (3). Risks of leaching to groundwater were therefore neglected. Diclofenac had also been proved to be really degradable in aerobic agricultural soils. Variances occurred with soil temperature, type of soil and anaerobic or aerobic conditions, however the half-life did not increase remarkably at lower temperatures and saturated soils (Al-Rajab et al., 2010). Al-Rajab, et al. (2010) estimated half-life of diclofenac in soil to be around 5 days. The average annual air temperature is high, 23 °C, (Figure 3) and the temperature of the water had been found to vary between 21.1 and 27.1 °C in Nakivubo (Kyambadde et al., 2004). That diclofenac was not found in the soils could hence depend on the compounds low adsorption ability and on its short half-life and fast biodegradation in soils.

Of all pharmaceuticals detected in the wetland water, the antibiotic sulfamethoxazole was found at second highest concentration, 2 460 ± 2 200 ng/l (n=3) (Table 10). In spite of this the compound was not detected in the soil. Sulfamethoxazole has been found in soil, irrigated with wastewater at various concentrations depending on the time the soil had been irrigated. The highest accumulated concentration was seen after approximately 23 years and was around

6 ng/g. Dalkmann et al. (2012) concluded that around this time the soil had reached a steady-state condition and the concentration would not increase. On the other hand Liu et al. (2010) found that sulfamethoxazole has a short half-life of two or seven days, in aerobic or anoxic conditions respectively. They concluded that biodegradation plays a major role in the degradation of sulfamethoxazole in the soil (Liu et al., 2010). It has been seen that microbes can adapt to sulfamethoxazole and then more effectively degrade the compound (Martínez-Hernández, Meffe, Herrera López and de Bustamante, 2016). According to the physiochemical properties of the compound, the compound is hydrophilic and dissolves in water. Its hydrophilic nature and fast biodegradation in the soil could be an explanation to why the compound was not detected in the soil in this thesis despite high concentrations in the wastewater.

The β-blocker atenolol was another pharmaceutical detected in the wetland water but not in the wetland soil. Its physicochemical properties indicate that atenolol is hydrophilic and soluble in water, and the K_{oc}-value is low, indicates that atenolol does not adsorb to solid material and hence has high mobility (Table 2). According to its pKa-value, 9.60, atenolol mostly exists in a cationic form. Atenolol has been shown to be the least persistent pharmaceutical in soil of carbamazepine, trimethoprim and sulfamethoxazole among others. The chemical half-life was reported to experimentally be 2.8-30 days and the half-live was decreased with increased sorption, whereas the opposite was shown for sulfamethoxazole (Kodešová et al., 2016). Considering the persistence of atenolol in the soil, it is not surprising that it was not detected.

5.11 PHARMACEUTICALS IN THE CROP SAMPLES

5.11.1 Results

Most of the pharmaceuticals analysed, 39 of 42, were not found in any of the crop samples. Only three compounds were found at detectable concentrations in the yam and those were lidocaine, pyrimethamine and trimethoprim (Table 17). These compounds were neither found in detectable concentrations in the maize nor in the sugar cane.

Table 17. Concentrations (means ± standard deviations) of pharmaceuticals detected in the yam, maize and sugar cane. The n value shows the number of replicates in which the compound was found identified, six being the highest. Values marked in italics have been calculated

Pharmaceutical	Yam (ng/g)	Maize (ng/g)	Sugar cane (ng/g)
Lidocaine	1.2 (n = 1), < LOD (n = 5)	< LOD (n = 6)	< LOD (n = 6)
Pyrimethamine	*1.8 ± 0.8* (n = 4), < LOD (n = 2)	< LOD (n = 6)	< LOD (n = 6)
Trimethoprim	*2.2 ± 1.2* (n = 4), < LOQ (n = 2)	< LOD (n = 6)	< LOD (n = 6)

Trimethoprim had the highest mean concentration and lidocaine had the lowest. The standard deviations for pyrimethamine and trimethoprim were fairly high. LODs and LOQs for the compounds found in the yam were calculated. Pyrimethamine had the lowest LOD while trimethoprim had the highest (Table 18).

Table 18. The limits of detection (LODs) and limits of quantification (LOQs) of the compounds found in the yam samples

Pharmaceutical	LOD (ng/g)	LOQ (ng/g)
Lidocaine	0.3	1.1
Pyrimethamine	0.1	0.2
Trimethoprim	0.4	1.5

The recovery for the crop samples ranged between 0.5 % (amitriptyline) and 469 % (metroprolol) (Table 19). Amoxicillin, ciprofloxacin, clotrimazole, furosemide, losartan, metformin, ofloxacin, omeprazole, sertraline and sparfloxacin had 0 % recovery in all of the crop samples. The recovery was fairly high, 21 of the pharmaceuticals had a recovery above 90 % in at least one of the crops.

Table 19. The average recovery in % for the compounds in the crop samples based on values from crops collected the 29 April 2015. The values were calculated using equation (5). The recovery could not be calculated for some of the compounds since the data was inadequate; these values have therefore been marked with a stroke

Pharmaceutical	Recovery in yam samples (%)	Recovery in maize samples (%)	Recovery in sugar cane samples (%)
Acetaminophen	-	-	32
Amitriptyline	13	0.5	34
Amlodipine	0	0	83
Amoxicillin	0	0	0
Atenolol	121	114	109
Atorvastatin	113	0	0
Bezafibrate	136	131	106
Carbamazepine	152	139	122
Cetirizine	-	-	433
Citalopram	124	144	97
Ciprofloxacin	0	0	0
Clarithromycin	160	174	207
Climbazole	9.7	1.5	18
Clotrimazole	0	0	0
Codeine	50	96	79
Diazepam	103	109	105
Diclofenac	99	51	0
Fluoxetine	-	0	104
Furosemide	-	-	0
Gemfibrozil	53	0	0
Hydrochlorothiazide	110	105	101
Irbesartan	128	734	117
Ketoconazole	-	-	0.9
Lamotrigine	116	126	108
Lidocaine	123	114	115
Losartan	-	-	0
Metformin	-	0	-
Metronidazole	51	85	76
Metroprolol	469	233	330
Ofloxacin	0	0	0
Omeprazole	0	0	0
Oxazepam	66	70	30
Pyrimethamine	-	0	0

Pharmaceutical	Recovery in yam samples (%)	Recovery in maize samples (%)	Recovery in sugar cane samples (%)
Ranitidine	103	89	79
Roxithromycin	136	64	156
Salbutamol	143	118	107
Sertraline	0	0	0
Sotalol	178	139	153
Sparfloxacin	0	0	0
Sulfamethoxazole	71	77	61
Trimethoprim	-	0	0
Venlafaxine	112	115	93

5.11.2 Discussion

No compounds were found at detectable concentrations in the edible parts of maize or sugar cane (Table 17). This can likely be explained, at least partly, by the fact that the edible parts of these crops are above ground, unlike the yam which grows in the soil. The pharmaceuticals would have to be translocated a lot further to reach the edible parts of the sugar cane and maize crops. This conclusion is supported by Herklotz et al. (2010) who found a higher concentration of pharmaceuticals in the crop parts that were closer to the ground. Another contributing factor to the non-detected levels of pharmaceuticals in the sugar cane and maize would be that they were not irrigated with the channel water as often as the yam. The yam field is always soaked with channel water, but the maize's and sugar cane's main water source is rainwater[7]. Excess water from the wetland is led away through channels to the maize and sugar cane fields where the crops are grown on windrows, which means that they are not in as direct contact with the channel water in the same way the yam is[8].

Trimethoprim, pyrimethamine and lidocaine were found in the yam soil and were also taken up by the yam. The concentrations in the crops were lower than those found in the soil however, showing that the pharmaceuticals had not bio-accumulated (Table 13; Table 17). The compounds that were absorbed all had log K_{ow} values within the range of 0.5-3 (Table 2) where they can be taken up by crops according to Duarte-Davidson and Jones (1996), so this was not surprising. Cetirizine and venlafaxine were also found in the yam soil, but they had either too low or too high log K_{ow} values to be taken up by the crops (Table 2). Diazepam on the other hand, had a log K_{ow} value that ought to enable uptake by plants, but still it was not found in detectable concentrations in the yam. This is likely due to the fact that diazepam was found in a lower concentration and to a lesser extent than the other pharmaceuticals. As reported before, Dolliver, Kumar and Gupta (2007) found a lower uptake of pharmaceuticals in the plant when the concentration in the manure was lower. Surprisingly enough, carbamazepine was not found in detectable concentrations in any of the yam replicates.

Carbamazepine had the highest mean concentration in the yam soil and was found in all yam soil samples (Table 13). It has a log K_{ow} that ought to enable uptake by crops and is also unionized which should result in a large uptake. A possible explanation could be that the yam crop has a very low lipid content, only about 0.31 % of the dry weight (Agbor Egbe and

[7] Sahar Dalahmeh, Department of Energy and Technology, Swedish University of Agricultural Sciences. 24-05-2016

[8] Allan John Komakech, Department of Agriculutural & Bio systems Engineering, Makerere University, Kampala. Email 17-05-2016

Treche, 1984). The lipid content affects the uptake of hydrophobic compounds, i.e. compounds with higher log K_{ow} values. Carter et al. (2014) studied the uptake of pharmaceuticals in radish and ryegrass and found a lower uptake of carbamazepine in the radish, which also has a very low lipid content. This can not entirely explain the lack of carbamazepine in the yam however, since both lidocaine and pyrimethamine which have a similar log K_{ow} value to carbamazepine, was taken up by the yam. A possible explanation for the lack of carbamazepine could instead be that the compound was transported to the leaves of the yam. Shenker et al. (2011) studied the uptake of carbamazepine in cucumber plants. They found the largest concentration of the pharmaceutical in the leaves of the plant. Only the edible part of the yam was studied in this thesis, so therefore the carbamazepine would not be detected in the samples, if it was mainly in the leaves.

Some of the concentration values for the pyrimethamine and trimethoprim were calculated manually and were therefore more uncertain than the concentrations that were given directly. In general, the calculated values seemed to underestimate the concentrations, meaning that the real concentrations in the yam might be higher. This is based on comparisons with the real concentrations found in the crops. The concentrations are still lower than those in the soil, which means that they did not bio-accumulate.

Seven of the compounds studied were not recovered in any of the crop samples (Table 19). These were amoxicillin, ciprofloxacin, clotrimazole, ofloxacin, omeprazole, sertraline and sparfloxacin. Climbazole and ketoconazole hade low recoveries (< 30 %) in all three crop samples. These results suggest that another method would have been more suitable to extract these compounds. The recovery for the other compounds were generally very high, a lot of the compounds had a recovery above 100 %. For example, twelve of the compounds had a recovery above 100 % in all of the crops. These compounds were atenolol, bezafibrate, carbamazepine, clarithromycin, diazepam, hydrochlorothiazide, irbesartan, lamotrigine, lidocaine, metroprolol, salbutamol and sotalol. These positive results indicate that the method was good at extracting most of the compounds.

5.12 UNCERTAINTIES IN LAB RESULTS

The largest uncertainty factor is the preparation of the samples, i.e. extraction and lab work performed. According to Azzouz and Ballesteros (2012) that step is the most critical during the whole procedure. The analysis and the machine used also gave some error, but much lesser than the sample preparation. Another very large uncertainty, especially for the soil and the water samples, is the sampling process which was performed before the start of this thesis. The soil is heterogenic and usually 5-15 samples are taken to try to even out the heterogeneity. In this thesis only three soil samples were mixed together. The same problem applies for the water since the water samples are dissimilar due to heterogeneous flow.

The results which have been calculated manually with help of calibration curves and linear regressions are even more uncertain than the ones which have been calculated directly by the machine. The manually calculated results seemed to generally give underestimations of the real concentrations, the exception was trimethoprim in the wastewater samples. This was seen when the equations used were tested on real data. Trimethoprim at the inflow and outflow seem to be overestimated instead, since the height in the chromatogram was a bad approximation of the response for those measurements. Even if some pharmaceuticals were not detected, this does not mean that they were not there. It is possible that the pharmaceuticals were present, but their concentrations were too low to be detected. All these factors make it hard to say how certain the results are.

During the wastewater extraction, no pH-adjustment was made which could have some impact on which pharmaceuticals that were extracted. The Oasis HLB cartridges used, can effectively extract neutral, acidic and basic compounds at all pH levels, 0-14 (WATERS, 2016). Hao et al. (2005), extracted pharmaceuticals at three different pH levels using Oasis HLB. They found that carbamazepine, trimethoprim and gemfibrozil were better recovered at a higher pH, around 7 or 9 than at pH 2.5. Bezafibrate was best recovered at a pH around 2 (Hao et al., 2005). The pH of the water in this thesis was 8 for all samples (5.1.6). In this thesis, bezafibrate had good recovery in all of the different types of samples, in spite of the high pH values in the water samples. This indicates that the majority of all targeted pharmaceuticals could be extracted without the pH being adjusted.

The LODs and LOQs couldn't be determined directly, but were instead calculated from the signal to noise ratio. The signal to noise ratios given were overrated which makes the results uncertain. The LODs and LOQs should not be seen as absolutely correct values, but can instead be compared with each other in order to give an indication of which pharmaceuticals had the most interferences during the analysis.

The lab results should be interpreted with caution and no big conclusions should be drawn from the results since only measurements from two days and a few samples were analysed. Longer measurement periods at more measurement points would have provided a better understanding of the pharmaceutical conditions in the water, soil and crops. Still these measurements greatly increase the knowledge base of pharmaceuticals in wastewater, soil and crops in the Nakivubo wetland in Uganda, as the previous knowledge on this is very small.

5.13 RISK ASSESSMENT

5.13.1 Results

The risk assessment only included atenolol, furosemide, lidocaine, pyrimethamine and trimethoprim. These were found in the yam and/or in Lake Victoria. Lidocaine had the highest ADI while pyrimethamine had the lowest (Table 20).

Table 20. The lowest therapeutic dose, safety factor and the acceptable daily intake (ADI) of the pharmaceutical compounds found in the crops and Lake Victoria. The ADI was calculated using equation (7)

Pharmaceutical	Use	Source	Lowest therapeutic dose (mg/day)	ADI (μg/kg/day)
Atenolol	β-blocker	Water from Lake Victoria	25[a]	0.42
Furosemide	Diuretics	Water from Lake Victoria	20[b]	0.33
Lidocaine	Local anaesthetic	Yam	420[c]	7.00
Pyrimethamine	Antimalarial	Yam	3.6[d]	0.06
Trimethoprim	Antibiotic	Water from Lake Victoria, Yam	200[e]	3.33

[a](Drugs.com, 2016a), [b](Drugs.com, 2016b), [c]Maximum recommended dose (Drugs.com, 2016c), [d](Drugs.com, 2016d), [e](Drugs.com, 2016e)

The EDI for all the compounds were quite low, and all the hazard quotient were < 0.1 (Table 21).

Table 21. The estimated daily intake (EDI) and hazard quotient for the compounds found in the yam and in Lake Victoria. The EDI was calculated using equation (8). Pyrimethamine and trimethoprim in the yam were found in several different replicates and the EDIs and hazard quotients were calculated for each individual value. Their results are presented as mean values with standard deviations

Pharmaceutical	Source	EDI (μg/kg/day)	Hazard quotient (EDI/ADI)
Atenolol	Lake Victoria	1.6×10^{-4}	3.8×10^{-4}
Furosemide	Lake Victoria	5.6×10^{-3}	0.02
Lidocaine	Yam	6.3×10^{-4}	9.0×10^{-5}
Pyrimethamine	Yam	$9.7 \times 10^{-4} \pm 4.2 \times 10^{-4}$	$0.02 \pm 6.90 \times 10^{-3}$
Trimethoprim	Lake Victoria	1.2×10^{-4}	3.6×10^{-5}
Trimethoprim	Yam	$1.0 \times 10^{-3} \pm 5.4 \times 10^{-4}$	$3.1 \times 10^{-4} \pm 1.6 \times 10^{-4}$

Trimethoprim in Lake Victoria had the lowest hazard quotient with 3.6×10^{-5}. Atenolol's and trimethoprim's EDIs in Lake Victoria were calculated by substituting the concentration with the LOQ. Given the concentrations found in the yam and water in this thesis, the people of Kampala can eat a lot of yam and drink a lot of water before they should suffer any consequences of the pharmaceuticals in yam and water (Table 22). The exception is the yam containing pyrimethamine, if people eat more than 0.5 kg daily they are at risk.

Table 22. The amount of yam and water that can be consumed daily without exceeding a hazard quotient of 0.1. The numbers are approximations based on a small number of measurements and should not be seen as guidelines

Pharmaceutical	Source	Amount that can be consumed daily
Atenolol	Lake Victoria	119 litre
Furosemide	Lake Victoria	5 litre
Lidocaine	Yam	119 kg
Pyrimethamine	Yam	0.5 kg
Trimethoprim	Lake Victoria	1 257 litre
Trimethoprim	Yam	17 kg

5.13.2 Discussion

Eating yam grown in Nakivubo wetland and drinking water from Lake Victoria does not seem to pose any significant risk to the people of Kampala, according to the risk assessment made (Table 21). The risk assessment concerning drinking water was made on raw water from Lake Victoria, but since people drink the water after it has been treated in Gaba treatment plant, the risk of drinking the water should be lower than what was shown in the risk assessment. The water is treated in different treatment steps at the Gaba water treatment plant. First, there is a coagulation and flocculation step, followed by lamella plate clarifiers. The water is then transported through rapid sand filters before it is chlorinated and sent to the residents of Kampala (Water technology, 2016). According to WHO (n.d.a), the concentration of pharmaceuticals in treated drinking water is generally much lower than those found in raw water and since the drinking water is undergoing extensive treatment in Gaba the pharmaceutical concentrations should decrease in the treatment plant. With that in mind, it is the pyrimethamine in the yam that poses the greatest risk for the people in Kampala that eat yam from the wetland. It had a hazard quotient of 0.016 ± 0.007 and if the emissions of pyrimethamine are larger in the future, the hazard quotient might exceed the limit of 0.1. This is not deemed very likely however, since the parasites that cause malaria has become resistant against pyrimethamine (Talisuna et al., 2002). Furthermore, the yam is peeled and cooked before it is eaten, which may reduce the concentration of pharmaceuticals in it (H A L Talwana, 2009). The hazard quotients were very small, and even if the locals increase their intake of yam and drinking water, the pharmaceuticals shouldn't impact them negatively. As long as the locals don't eat more than 0.5 kg yam and drink more than 5 litres of water a day, the risk of negative effects of the pharmaceuticals should be acceptable (Table 22). However, the leaves of the yam are eaten as vegetables, and if they contain carbamazepine (as discussed in 5.11.2) they will be an additional source of pharmaceuticals in the locals' diet. Since carbamazepine had the highest concentration in the yam soil, there is a risk that there is a significant uptake of carbamazepine in the yam leaves and thus it might be harmful to eat them. This ought to be analysed in future studies.

The individual compounds do not pose a risk at their current concentrations, but the effect of the mixture of the pharmaceuticals may be harmful. A mixture of pharmaceuticals may have a negative impact on humans, even at low concentrations. The joint toxicity of the mixture can be higher than the toxicity of the individual pharmaceuticals, even if the pharmaceuticals have such low concentrations that they wouldn't be toxic individually (Backhaus, 2014). The effects of mixtures were not studied in this thesis, but it is important to keep this in mind when discussing the toxicity of pharmaceuticals.

It is also worth noting that trimethoprim is an antibiotic. Since it is persistent enough to be found in all measurement points (excluding the maize and sugar cane) there is a risk that it

can cause antibiotic resistance. Even if the concentrations in the yam and Lake Victoria are not harmful to humans, measures should still be taken to reduce the concentration of trimethoprim and the other pharmaceuticals in the water and subsequently in the soil and crops. The concentration of the two antibiotics trimethoprim and sulfamethoxazole were the two pharmaceuticals that had the highest concentrations in the water which was very alarming. Lowering the release of pharmaceuticals into the environment would decrease the risk of antibiotic resistance, and would also be beneficial for other reasons. For example, Boxall et al. (2006) found in their study that veterinary pharmaceuticals could reduce plant growth. Herklotz et al. (2010) also found that the disinfectant triclosan could hinder plant growth and survival. High soil concentrations (> 4 mg/kg) of carbamazepine lead to burnt edges on zucchini leaves, and a decrease in biomass both above and below ground. The concentrations of pharmaceuticals found in the soil were not at all that high, but it is still good to keep in mind that the food production can be affected by the pharmaceuticals. The people that live near the wetland also eat a lot of fish from it (Kansiime and Maimuna, 1999). Since the wetland had detectable concentrations of a lot of different pharmaceuticals, there is a risk that there are some pharmaceuticals in the fishes. This thesis has not studied the uptake from fishes, so it is hard to say if this poses a risk for the people of Kampala or not. It is however clear that the reduction of pharmaceuticals in the water would be beneficial for many different reasons, even if the pharmaceuticals found in the crops and drinking water do not pose a risk for the people of Kampala at their current concentrations.

5.14 REDUCING PHARMACEUTICALS IN CROPS

As seen in the risk assessment, eating the crops does not pose any health risk with regards to their pharmaceutical content today. The cocoyam was the crop that had largest uptake of pharmaceuticals and hence was the one that possess the highest risk of being harmful to the people in the area. It has been concluded that tuber vegetables, such as carrots and radish, possess a higher risk of pharmaceutical uptake in edible parts than other types of crops (Wu et al., 2015). On the other hand, according to Kansiime and Maimuna (1999) cocoyam is ideal for farming in the Nakivubo wetland since the yams have high water tolerance, eliminate weed by shading the ground and have high resistance against pests and diseases (Kansiime and Maimuna, 1999). However plants with high translocation potential may also have high concentrations of pharmaceuticals in the fruits and leaves (Wu et al., 2015). The crops studied in this thesis have different growing periods, where sugar cane is the one with the longest growth period, 15-24 months (3.6). No studies have been made on the duration of growth for different crops and linked it to uptake of pharmaceuticals. Physicochemical properties of the pharmaceuticals, soil characteristics and characteristics of the plants e.g. transpiration rates and lipid content are of greater importance (Wu et al., 2015; Sundstøl Eriksen et al., 2009). It has however been seen that the pharmaceutical ibuprofen has been taken up by celery stalks within 24 hours. Within an hour, ibuprofen had even reached the leaves (Schroeder et al., 2015). This implies that the duration of growth is not the most important factor, although more studies are needed on the plants allocation and metabolism.

A suitable crop for cultivation is hard to suggest since it depends on many factors e.g. cultivation methods, soil properties, environmental conditions, vermins, nutrition content, getting plant material and so on (3.6). As seen in this study, sugar cane and maize did not have any detectable uptake in the edible parts (Table 17) and hence are more suitable for crop cultivation in the wetland with regards to pharmaceutical uptake. Also the weather conditions in Uganda are ideal for cultivate sugar cane, maize and yam (Figure 3; 3.6). But a major difference in the cultivation is the wastewater logging, since the cocoyam field is annually soaked with wastewater and the other fields are not. One suggestion on a crop that could be

suitable to grow is rice. The Ugandan rice production has increased rapidly in recent years, between year 2004 and 2009, the production has increased 2.5 times (Mohapatra, 2009). Rice is also seen as a high value crop in Uganda and is favourable to consume (CGIAR, 2013). Wetlands as well as irrigated ecology are suitable for rice production in Africa (Oteng and Sant' Anna, 1997). This means that the Nakivubo wetland could be suitable for rice production. Pharmaceutical uptake in rice has not been studied much but since the grains are above ground, the concentration of pharmaceuticals are expected to be lower than the concentration in tuber crops, like cocoyam. It has been seen by Goh and Lee (1999) that aluminium inhibits root growth of rice in acidic soils. The agricultural soils in the Nakivubo wetland contained high amounts of aluminium (33 700-59 300 mg/kg TS) but had neutral pH around 7 (Table 8). This means that aluminium uptake from the soils is limited as long as the pH of the soils are neutral or basic.

To conclude, a crop with high steam and low translocation that can grow in saturated soils and at the same time can be satisfying to eat for the people, that crop would be ideal to grow in the wetland. Such a crop might be rice and one recommendation could be to try growing rice, to see if it grows well, and to verify that no pharmaceuticals will be taken up by the crop.

6 CONCLUSIONS

The concentrations of the water quality parameters were above the Uganda's maximum permissible limits in almost all of the measurement points in the outflow of Bugolobi WWTP and Nakivubo channel. It is clear that the Nakivubo channel is polluted, and that more treatment of the water is needed.

In the thesis, 42 compounds and 24 % of the most prescribed and sold pharmaceuticals in Kampala were analysed. Further studies need to examine the presence and quantity of the pharmaceuticals not studied, since it is probable that they also are in the environment.

The highest concentrations of pharmaceuticals were found at the inflow and outflow of Bugolobi WWTP. The most commonly found pharmaceuticals were: atenolol, carbamazepine, cetirizine, sulfamethoxazole and trimethoprim. Those pharmaceuticals were among the most persistent and the most sold in the area. The measured concentrations of atenolol, carbamazepine, codeine, hydrochlorothiazide, irbesartan, lidocaine, losartan, pyrimethamine, salbutamol and trimethoprim pharmaceuticals were higher at the outflow of the WWTP than at the inflow, showing that it has problems removing those. It is true however, that the WWTP was not working properly on one of the sampling days and the result might have been different if it was functional. Only sulfamethoxazole and diclofenac had a lower concentration at the outflow than at the inflow, showing removal rates of 5 % and 39 % respectively. The concentrations of the compounds were generally lower in the channel, indicating that there are no point sources of pharmaceuticals there. The dilution made it difficult to detect pharmaceuticals in Lake Victoria.

Five pharmaceuticals were detected in the maize soil and seven in the cocoyam and sugar cane soils. In total, eleven different pharmaceuticals were detected in the soil, despite that 29 pharmaceuticals were detected in the wastewater. The most commonly detected pharmaceuticals were the ones that were more prone to adsorb to the soil and that were hard to degrade. Carbamazepine and pyrimethamine were identified in highest concentrations. The difference between the soils is assumed to depend on the pharmaceuticals physicochemical properties and the soils different composition, especially its TOC, which largely determines the pharmaceuticals ability to adsorb to the soil. The different wastewater loadings to the fields are also crucial for the pharmaceutical content in the soils.

The uptake of pharmaceuticals in the crops was low, and no bioaccumulation of pharmaceuticals in the crops was found. The yam was the only crop for which an uptake of pharmaceuticals was detected (lidocaine, pyrimethamine and trimethoprim, at 1.2-2.2 ng/g). No pharmaceuticals could be detected in the edible parts of maize and sugar cane, presumably because the edible parts of those crops are above the ground, far from the roots, unlike the cocoyam. The uptake of pharmaceuticals in yam is probably affected by the concentration of the pharmaceutical in the soil, as well as its log K_{ow} value.

The channel, the wetland and its soil have contributed to the treatment of the wastewater and reduced the content of selected pharmaceuticals in the water. One consequence of this is accumulation in the soil of carbamazepine and pyrimethamine. The retention time, in both the channel and the wetland, the length of the channel, the content of suspended solids in the water, the vegetation, the soils TOC content and the weather conditions have all contributed to the treatment of the wastewater. The treatment was also affected by the soil characteristics

and different physicochemical properties of the pharmaceutical. A statistically significant correlation was found between the concentration of carbamazepine and total suspended solids in the water. When the content of total suspended solids increased the concentration of carbamazepine decreased. More research is needed, however to fully understand the treatment capacity of the Nakivubo channel and wetland.

More toilets ought to be installed in the slums in order to diminish the practice of open defecation. This might help reduce the pollution of pharmaceuticals and nutrients in Nakivubo channel since the faeces will be contained instead of washed down into the channel when it rains. In future studies, the removal of pharmaceuticals in Bugolobi WWTP ought to be studied, and suggestions given on how to improve the treatment of the water. Suggestions could also be given for improvement of the piping system, in order to reduce the amount of untreated wastewater that is released into the channel and the environment.

According to the risk assessment, eating yam or drinking water from Lake Victoria does not give any significant risk with the pharmaceutical concentrations found in this thesis. The risk is also reduced when the yam crop is cooked before eating. In future studies, the uptake of carbamazepine in the yam leaves ought to be studied in order to determine if they pose a health risk or not. The uptake of pharmaceuticals in fish living in the wetland can also be studied to make sure that eating the fish is not hazardous for the people living near the Nakivubo wetland.

A suitable crop to grow, with no detectable uptake of pharmaceuticals, suitable for the current surrounding environmental conditions and at the same time attractive for the people as food is hard to find. A crop with eatable parts above ground that does not translocate pharmaceuticals, like the maize, would be a good choice. Trials with growing rice would be interesting. This is recommended for further studies to look into more.

REFERENCES

Aber, J.S., Pavri, F. and Aber, S., 2012. *Wetland Environments: A Global Perspective*. John Wiley & Sons.

African Development Bank, 2016. *Kampala sanitation program*. [online] Available at: <http://www.afdb.org/en/projects-and-operations/project-portfolio/project/p-ug-e00-008/> [Accessed 9 Feb. 2016].

Agbor Egbe, T. and Treche, S., 1984. Varilability in the chemical composition of yams grown in Cameroo. In: *Tropical Root Crops - production and uses in Africa*. [online] Ottawa, Canada: International Development Research Centre. Available at: <http://horizon.documentation.ird.fr/exl-doc/pleins_textes/pleins_textes_5/b_fdi_12-13/14555.pdf>.

Alcorn, K., 2011. *HIV & AIDS Information :: UNAIDS: Treat 15 million by 2015*. [online] Available at: <http://www.aidsmap.com/UNAIDS-Treat-15-million-by-2015/page/1825513/> [Accessed 3 Jun. 2016].

Al-Rajab, A.J., Sabourin, L., Lapen, D.R. and Topp, E., 2010. The non-steroidal anti-inflammatory drug diclofenac is readily biodegradable in agricultural soils. *Science of The Total Environment*, 409(1), pp.78–82.

Anastassiades, M., Maštovská, K. and Lehotay, S.J., 2003. Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. *Journal of Chromatography A*, 1015(1-2), pp.163–184.

Apoteket, 2005. *Läkemedel och miljö: en skrift med fakta och tankar om hur läkemedel och läkemedelsrester kan påverka vår miljö och därmed vår hälsa*. Stockholm: Apoteket.

Ashley, R.O., 2016. *Corn Maturity and Ensiling Corn*. [online] Available at: <https://www.ag.ndsu.edu/archive/dickinso/agronomy/cornmaturity.htm> [Accessed 18 May 2016].

Atkins, P. and Jones, L., 2007. *Chemical principles: The quest for insight*. 5th ed. United states of America: Macmillan.

Aus der Beek, T., Weber, F.-A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A. and Küster, A., 2016. Pharmaceuticals in the environment—Global occurrences and perspectives. *Environmental Toxicology and Chemistry*, 35(4), pp.823–835.

AVERT, 2015. *HIV and AIDS in Uganda | AVERT*. [online] Available at: <http://www.avert.org/professionals/hiv-around-world/sub-saharan-africa/uganda> [Accessed 29 May 2016].

Aznar, R., Sánchez-Brunete, C., Albero, B., Rodríguez, J. and Tadeo, J., 2014. Occurrence and analysis of selected pharmaceutical compounds in soil from Spanish agricultural fields. *Environmental Science and Pollution Research*, 21(6), pp.4772 – 4782.

Azzouz, A. and Ballesteros, E., 2012. Combined microwave-assisted extraction and continuous solid-phase extraction prior to gas chromatography–mass spectrometry determination of pharmaceuticals, personal care products and hormones in soils, sediments and sludge. *Science of The Total Environment*, 419, pp.208–215.

Backhaus, T., 2014. Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. *Philosophical transactions of the Royal Society B-Biological sciences*, [online] 369(1656). Available at: <http://apps.webofknowledge.com.ezproxy.its.uu.se/full_record.do?product=WOS&search_mode=GeneralSearch&qid=3&SID=Z1tDGyZtL1nVccg2rs8&page=1&doc=1>.

Balirwa, E.K., 1992. *Maize research and production in Uganda*. Available at: <http://pdf.usaid.gov/pdf_docs/Pnabt623.pdf> [Accessed 18 May 2016].

Bodelier, P.L.E. and Dedysh, S.N., 2013. Microbiology of wetlands. *Frontiers in Microbiology*, [online] 4. Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3617397/> [Accessed 22 Feb. 2016].

Bonnet, U., Bingmann, D., Wiltfang, J., Scherbaum, N. and Wiemann, M., 2010. Modulatory effects of neuropsychopharmaca on intracellular pH of hippocampal neurones in vitro. *British Journal of Pharmacology*, 159(2), pp.474–483.

Borton, C. and Tidy, C., 2014. *Lipid-regulating Drugs (including Statins)*. [online] Available at: <http://patient.info/doctor/lipid-regulating-drugs-including-statins> [Accessed 18 May 2016].

Boxall, A.B.A., Johnson, P., Smith, E.J., Sinclair, C.J., Stutt, E. and Levy, L.S., 2006. Uptake of Veterinary Medicines from Soils into Plants. *Journal of Agricultural and Food Chemistry*, 2006(54), pp.2288–2297.

Brodin, T., Fick, J., Jonsson, M. and Klaminder, J., 2013. Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations. *Science*, 339(6121), pp.814–815.

Brooks, B.W. and Huggett, D.B. eds., 2012. *Human pharmaceuticals in the environment: current and future perspectives*. Emerging topics in ecotoxicology. [online] New York, NY: Springer. Available at: <ub.uu.se>.

Carmona, E., Andreu, V. and Picó, Y., 2014. Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water. *Science of The Total Environment*, 484, pp.53–63.

Carter, L.J., Harris, E., Williams, M., Ryan, J.J., Kookana, R.S. and Boxall, A.B.A., 2014. Fate and Uptake of Pharmaceuticals in Soil-Plant Systems. *Journal of Agricultural and Food Chemistry*, 62(4), pp.816–825.

CGIAR, 2013. *Benefits and burdens of new rice varieties in Uganda.* Available at: <http://www.cgiar.org/consortium-news/benefits-and-burdens-of-new-rice-varieties-in-uganda/> [Accessed 20 Jun. 2016].

ChemIDplus Advanced, 2016. *ChemIDplus Advanced - Chemical information with searchable synonyms, structures, and formulas*. [online] Available at: <http://chem.sis.nlm.nih.gov/chemidplus/> [Accessed 22 May 2016].

Chen, W., Xu, J., Lu, S., Jiao, W., Wu, L. and Chang, A.C., 2013. Fates and transport of PPCPs in soil receiving reclaimed water irrigation. *Chemosphere*, 93(10), pp.2621–2630.

CHROMacademy, n.d. *Sample Preparation - Liquid/Liquid Extraction Tecniques*. Available at: <http://www.chromacademy.com/lms/sco59/Sample_Preparation_Liquid-Liquid_Extraction_Techniques.pdf>.

CIPLA Quality Chemicals Industries Limited, 2014. *Products*. [online] CIPLA Quality Chemicals Industries Limited. Available at: <http://www.ciplaqcil.co.ug/products/> [Accessed 16 May 2016].

Colaneri, K., 2014. *What is the long-term health impact of pharmaceuticals in our water?* [online] Available at: <https://stateimpact.npr.org/pennsylvania/2014/05/15/what-is-the-long-term-health-impact-of-pharmaceuticals-in-our-water/> [Accessed 1 Jun. 2016].

Cowardin, L.M., Carter, V., Golet, F.C. and LaRoe, E.T., 1979. *Classification of Wetlands and Deepwater Habitats of the United States - Classification-of-Wetlands-and-Deepwater-Habitats-of-the-United-States.pdf*. [online] U.S Department of the Interior, U.S. Fish & Wildlife Service, Publication. Available at: <http://www.fws.gov/wetlands/Documents/Classification-of-Wetlands-and-Deepwater-Habitats-of-the-United-States.pdf> [Accessed 3 Jun. 2016].

Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E. and Siemens, J., 2012. Accumulation of Pharmaceuticals, Enterococcus, and Resistance Genes in Soils Irrigated with Wastewater for Zero to 100 Years in Central Mexico. *PLoS ONE*, [online] 7(9). Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3458031/> [Accessed 26 May 2016].

Daughton, C.G. and Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental health perspectives*, 107(Suppl 6), p.907.

Dolliver, H., Kumar, K. and Gupta, S., 2007. Sulfamethazine Uptake by Plants from Manure-Amended Soil. *Journal of Environmental Quality*, 36(4), pp.1224–1230.

Drechsel, P., Scott, C.A., Raschid-Sally, L., Redwood, M. and Bahri, A., 2010. *WASTEWATER IRRIGATION AND HEALTH Assessing and Mitigating Risk in Low-income Countries*. [online] Earthscan, IWMI, IDRC. Available at: <https://www.idrc.ca/en/book/wastewater-irrigation-and-health-assessing-and-mitigating-risk-low-income-countries> [Accessed 30 May 2016].

Drugs.com, 2016a. *Atenolol Dosage*. [online] Available at: <http://www.drugs.com/dosage/atenolol.html> [Accessed 24 May 2016].

Drugs.com, 2016b. *Furosemide Dosage*. [online] Available at: <http://www.drugs.com/dosage/furosemide.html> [Accessed 24 May 2016].

Drugs.com, 2016c. *Lidocaine*. [online] Available at: <http://www.drugs.com/pro/lidocaine.html> [Accessed 24 May 2016].

Drugs.com, 2016d. *Pyrimethamine Dosage*. [online] Available at: <http://www.drugs.com/dosage/pyrimethamine.html> [Accessed 24 May 2016].

Drugs.com, 2016e. *Trimethoprim Dosage*. [online] Available at: <http://www.drugs.com/dosage/trimethoprim.html> [Accessed 24 May 2016].

Duarte-Davidson, R. and Jones, K.C., 1996. Screening the environmental fate of organic contaminants in sewage sludge applied to agricultural soils: II. The potential for transfers to plants and grazing animals. *Science of the Total Environment*, 185(1-3), pp.59–70.

Durán-Alvarez, J.C., Becerril-Bravo, E., Castro, V.S., Jiménez, B. and Gibson, R., 2009. The analysis of a group of acidic pharmaceuticals, carbamazepine, and potential endocrine disrupting compounds in wastewater irrigated soils by gas chromatography–mass spectrometry. *Talanta*, 78(3), pp.1159–1166.

Emerton, L., 2005. *Values and rewards: counting and capturing ecosystem water services for sustainable development*. [online] IUCN. Available at: <https://www.google.com/books?hl=sv&lr=&id=Z-DfqYh2w3MC&oi=fnd&pg=PA1&dq=Values+and+Rewards:+Counting+and+Capturing+Ecosystem+Water+Services+for+Sustainable+Development+&ots=8QFUZUIQdt&sig=qoJM0YIDTOIqFkLgDnqg9FICbZY> [Accessed 25 Jan. 2016].

Encyclopædia Britannica, 2016a. *Analgesic*. [online] Available at: <http://academic.eb.com/EBchecked/topic/22403/analgesic> [Accessed 18 Jan. 2016].

Encyclopædia Britannica, 2016b. *Anesthetic*. [online] Available at: <http://academic.eb.com.ezproxy.its.uu.se/EBchecked/topic/24338/anesthetic?anchor=ref65007> [Accessed 18 May 2016].

Encyclopædia Britannica, 2016c. *Antibiotic (chemical compound)*. [online] Available at: <http://academic.eb.com.ezproxy.its.uu.se/EBchecked/topic/27751/antibiotic> [Accessed 18 May 2016].

Encyclopædia Britannica, 2016d. *Antidiabetic drug*. [online] Available at: <http://academic.eb.com.ezproxy.its.uu.se/EBchecked/topic/1912820/antidiabetic-drug> [Accessed 18 May 2016].

Encyclopædia Britannica, 2016e. *Antifungal drug*. [online] Available at: <http://academic.eb.com.ezproxy.its.uu.se/EBchecked/topic/27986/antifungal-drug> [Accessed 18 May 2016].

Eriksson, J., Nilsson, I., Simonsson, M. and Wiklander, L., 2005. *Marklära*. Lund: Studentlitteratur.

Fenet, H., Mathieu, O., Mahjoub, O., Li, Z., Hillaire-Buys, D., Casellas, C. and Gomez, E., 2012. Carbamazepine, carbamazepine epoxide and dihydroxycarbamazepine sorption to soil and occurrence in a wastewater reuse site in Tunisia. *Chemosphere*, 88(1), pp.49–54.

Fent, K., Weston, A. and Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology*, 76(2), pp.122–159.

Fortune of Africa Uganda, 2016. *Sugar Cane*. [online] Fortune of Africa Uganda. Available at: <http://fortuneofafrica.com/ug/sugar-cane-in-uganda/> [Accessed 18 May 2016].

Franco, A., Fu, W. and Trapp, S., 2009. Influence of soil pH on the sorption of ionizable chemicals: Modeling advances. *Environmental Toxicology and Chemistry*, 28(3), pp.458–464.

Frishman, W.H., Cheng-Lai, A. and Chen, J., 2000. *Current cardiovascular drugs*. [online] Philadelphia, Pa.: Current Medicine. Available at: <https://books.google.se/books?id=y3R1Vd3NHqcC&pg=PA152&dq=mode+of+action+of+beta+blockers&hl=sv#v=onepage&q&f=false> [Accessed 20 Jan. 2016].

Fuhrimann, S., Winkler, M.S., Schneeberger, P.H., Niwagaba, C.B., Buwule, J., Babu, M., Medlicott, K., Utzinger, J. and Cissé, G., 2014. Health risk assessment along the wastewater and faecal sludge management and reuse chain of Kampala, Uganda: a visualization. *Geospatial health*, 9(1), pp.241–245.

Gao, H., Bai, J., Wang, Q., Huang, L. and Xiao, R., 2010. Profile distribution of soil nutrients in unrestored and restored wetlands of the Yellow River Delta, China. *Procedia Environmental Sciences*, 2, pp.1652–1661.

Goh, C.-H. and Lee, Y., 1999. Aluminum uptake and aluminum-induced rapid root growth inhibition of rice seedlings. *Journal of Plant Biology*, 42(2), pp.151–158.

Göbel, A., McArdell, C.S., Joss, A., Siegrist, H. and Giger, W., 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of The Total Environment*, 372(2-3), pp.361–371.

Graouer-Bacart, M., Sayen, S. and Guillon, E., 2016. Adsorption and co-adsorption of diclofenac and Cu(II) on calcareous soils. *Ecotoxicology and Environmental Safety*, 124, pp.386–392.

Gros, M., Petrović, M. and Barceló, D., 2006. Multi-residue analytical methods using LC-tandem MS for the determination of pharmaceuticals in environmental and wastewater samples: a review. *Analytical and Bioanalytical Chemistry*, 386(4), pp.941–952.

Gros, M., Petrović, M., Ginebreda, A. and Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. *Environment International*, 36(2010), pp.15–26.

Gunnarsson, D.R., 2002. *Korrelationsanalys och regressionsanalys*. [online] Available at: <http://infovoice.se/fou/bok/statmet/10000053.shtml> [Accessed 2 Jun. 2016].

Günther, I., Horst, A., Lüthi, C., Mosler, H.-J., Niwagaba, C. and Tumwebaze, I., 2011. *Where do Kampala's Poor 'go'? Urban sanitation conditions in Kampala's low–income areas*. [Research for policy] Zürich, Switzerland. Available at: <https://www.ethz.ch/content/dam/ethz/special-interest/gess/department-dam/D-GESS%20DAM%20General/Dokumente/Sonstige%20Artikel/policy_brief_kampala_sanitation_1_2011.pdf>.

H A L Talwana, A.K.S., 2009. Production Status and Prospects of Cocoyam (Colocasia esculenta (L.) Schott.) in East Africa. *Journal of Root Crops*, 35, pp.98–107.

Hao, C., Lissemore, L., Nguyen, B., Kleywegt, S., Yang, P. and Solomon, K., 2005. Determination of pharmaceuticals in environmental waters by liquid chromatography/electrospray ionization/tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 384(2), pp.505–513.

Harvey, P., Rambeloson, Z. and Dary, O., 2008. *Determining the Dietary Patterns of Ugandan Women and Children*. Available at: <https://www.spring-nutrition.org/sites/default/files/a2z_materials/508-uganda_food_consumption_survey_final_08152011.pdf>.

Hasunira, R., 2008. *Access To And Use Of Medicines By Households In Uganda*. Available at: <http://apps.who.int/medicinedocs/documents/s16374e/s16374e.pdf>.

Healthcare Waste Management Technical Working Group, 2009. *National Health Care Waste Management Plan, 2009/10- 2011/12*. Available at: <http://www-

wds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2010/02/23/000333038_20100223014556/Rendered/INDEX/E23680v70P11151C10AFR1HCWMP1P111556.txt> [Accessed 30 May 2016].

Herklotz, P.A., Gurung, P., Vanden Heuvel, B. and Kinney, C.A., 2010. Uptake of human pharmaceuticals by plants grown under hydroponic conditions. *Chemosphere*, 78(11), pp.1416–1421.

Hijosa-Valsero, M., Matamoros, V., Pedescoll, A., Martin-Villacorta, J., Becares, E. and Garcia, J., 2011. Evaluation of primary treatment and loading regimes in the removal of pharmaceuticals and personal care products from urban wastewaters by subsurface-flow constructed wetlands. *Intern. J. Environ. Anal. Chem.*, 91(7), pp.632–653.

Hijosa-Valsero, M., Matamoros, V., Sidrach-Cardona, R., Martín-Villacorta, J., Bécares, E. and Bayona, J.M., 2010. Comprehensive assessment of the design configuration of constructed wetlands for the removal of pharmaceuticals and personal care products from urban wastewaters. *Water Research*, 44(12), pp.3669–3678.

Isabirye, M., Raju, D.V.., Kitutu, M., Yemeline, V., Deckers, J., Poese, J., 2013. Sugarcane Biomass Production and Renewable Energy, in: Matovic, M.D. (Ed.), Biomass Now - Cultivation and Utilization. InTech.

IUPAC, (1997). Compendium of Chemical Terminology, 2nd ed. (the ”Gold Book”). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M. Nic, J. Jirat, B. Kosata: updates compiled by A. Jenkins. ISBN 0-9678550-9-8. doi: 10.1351/goldbook.

IVL - Swedish Environmental Research Institute, 2015. *Pharmaceutical residues and other emerging substances in the effluent of sewage treatment plants - Review on concentrations, quantification, behaviour, and removal options*. [online] Stockholm. Available at: <http://sjostad.ivl.se/download/18.15a855cb14c38029e641382/1430315031950/B2226.pdf>.

Jenkins, M.W., Cumming, O. and Cairncross, S., 2015. Pit Latrine Emptying Behavior and Demand for Sanitation Services in Dar Es Salaam, Tanzania. *International Journal of Environmental Research and Public Health*, 12(3), pp.2588–2611.

Kadlec, R.H. and Wallace, S., 2008. *Treatment Wetlands, Second Edition*. [online] CRC Press. Available at: <https://books.google.se/books?hl=sv&lr=&id=hPDqfNRMH6wC&oi=fnd&pg=PP1&dq=Treatment+wetlands.+2nd+ed.+New+York&ots=k6O-bXbWaN&sig=YfPWnwIpPaF-lY3fPFRPD7t8MYQ&redir_esc=y#v=onepage&q&f=false>.

Kansiime, F. and Maimuna, N., 1999. *Wastewater treatment by a natural wetland: the Nakivubo swamp, Uganda*. [online] CRC Press. Available at: <https://www.google.com/books?hl=sv&lr=&id=i5IVXoP-geYC&oi=fnd&pg=PP11&dq=Wastewater+Treatment+by+a+Natural+Wetland:+the+Nakivubo+Swamp,+Uganda&ots=jrRqVLT2KR&sig=VW0gZ9oCTM3fTlyr89fPTCI5mEQ> [Accessed 20 Jan. 2016].

Kasanvu slum's appalling hygiene. 2011. [video online] Directed by Urban TV Uganda. Available at: <https://www.youtube.com/watch?v=LE19wY4xy_8> [Accessed 3 Jun. 2016].

Katukiza, A.Y., Ronteltap, M., Niwagaba, C.B., Foppen, J.W.A., Kansiime, F. and Lens, P.N.L., 2012. Sustainable sanitation technology options for urban slums. *Biotechnology Advances*, 30(2012), pp.964–978.

Kayima, J., Kyakula, M., Komakech, W. and Echimu, S.P., 2008. A study of the degree of Pollution in Nakivubo Channel, Kampala, Uganda. *Journal of Applied Sciences and Environmental Management*, 12(2), pp.93–98.

Kayima, J., Nankabirwa, J., Sinabulya, I., Nakibuuka, J., Zhu, X., Rahman, M., Longenecker, C.T., Katamba, A., Mayanja-Kizza, H. and Kamya, M.R., 2015. Determinants of hypertension in a young adult Ugandan population in epidemiological transition—the MEPI-CVD survey. *BMC Public Health*, 15, p.830.

Kibbey, T.C.G., Paruchuri, R., Sabatini, D.A. and Chen, L., 2007. Adsorption of Beta Blockers to Environmental Surfaces. *Environmental Science & Technology*, 41(15), pp.5349–5356.

Kinney, C.A., Furlong, E.T., Werner, S.L. and Cahill, J.D., 2006. Presence and distribution of wastewater-

derived pharmaceuticals in soil irrigated with reclaimed water. *Environmental Toxicology and Chemistry*, 25(2), pp.317–326.

Koch, A., 2015. *Fate of pharmaceuticals and perfluoroalkyl substances during source separated wastewater treatment*. [Avancerad nivå, A2E] Available at: <http://stud.epsilon.slu.se/8671/> [Accessed 30 May 2016].

Kodešová, R., Kočárek, M., Klement, A., Golovko, O., Koba, O., Fér, M., Nikodem, A., Vondráčková, L., Jakšík, O. and Grabic, R., 2016. An analysis of the dissipation of pharmaceuticals under thirteen different soil conditions. *Science of The Total Environment*, 544, pp.369–381.

K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H. and Demeestere, K., 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere*, 149(2016), pp.238–244.

Kulabako, N.., Nalubega, M. and Thunvik, R., 2007. Study of the impact of land use and hydrogeological settings on the shallow groundwater quality in a peri-urban area of Kampala, Uganda. *Science of The Total Environment*, 381(1-3), pp.180–199.

Kümmerer, K. ed., 2008. *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks*. 3., rev. and enl. ed ed. [online] Berlin: Springer. Available at: <ub.uu.se>.

Küster, A. and Adler, N., 2014. Pharmaceuticals in the environment: scientific evidence of risks and its regulation. *Philosophical transactions of the Royal Society of London*, [online] 369(1656). Available at: <http://apps.webofknowledge.com.ezproxy.its.uu.se/full_record.do?product=UA&search_mode=GeneralSearch&qid=5&SID=W2fFmXFTpvWm15uHjR5&page=1&doc=9>.

Küster, A., Alder, A.C., Escher, B.I., Duis, K., Fenner, K., Garric, J., Hutchinson, T.H., Lapen, D.R., Péry, A., Römbke, J., Snape, J., Ternes, T., Topp, E., Wehrhan, A. and Knacker, T., 2010. Environmental risk assessment of human pharmaceuticals in the European Union: A case study with the β-blocker atenolol. *Integrated Environmental Assessment and Management*, 6(S1), pp.514–523.

Kwiringira, J., Atekyereza, P., Niwagaba, C. and Günther, I., 2014. Descending the sanitation ladder in urban Uganda: evidence from Kampala Slums. *BMC Public Health*, [online] 14(634). Available at: <http://apps.webofknowledge.com.ezproxy.its.uu.se/full_record.do?product=WOS&search_mode=GeneralSearch&qid=1&SID=R1OVatrvsOgP4gd9vPY&page=1&doc=1>.

Kyambadde, J., Kansiime, F., Gumaelius, L. and Dalhammar, G., 2004. Hydraulic loading, stability and water quality of Nakivubo wetland, Uganda. *African Journal of Aquatic Science*, 29(2), pp.213–220.

Lee, S., Kang, S.-I., Lim, J.-L., Huh, Y.J., Kim, K.-S. and Cho, J., 2011. Evaluating controllability of pharmaceuticals and metabolites in biologically engineered processes, using corresponding octanol–water distribution coefficient. *Ecological Engineering*, 37(10), pp.1595–1600.

Levén, L., Calvo, M.G., Dalahmeh, S., Ljung, E., Lundin, G., Ahrens, L., Wiberg, K., Jönsson, H. and Eveborn, D., (in press). *Läkemedel i källsorterat klosettvatten och latrin – behandling och risker*. Uppsala: JTI – Institutet för jordbruks- och miljö teknik.

Lin, K. and Gan, J., 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. *Chemosphere*, 83(3), pp.240–246.

Liu, F., Ying, G.-G., Yang, J.-F., Zhou, L.-J., Tao, R., Wang, L., Zhang, L.-J. and Peng, P.-A., 2010. Dissipation of sulfamethoxazole, trimethoprim and tylosin in a soil under aerobic and anoxic conditions. *Environmental Chemistry*, 7(4), pp.370–376.

Livsmedelsverket, 2016. *Dricksvatten*. [online] Available at: <http://www.livsmedelsverket.se/matvanor-halsa--miljo/miljo/miljosmarta-matval2/dricksvatten-miljo/> [Accessed 17 May 2016].

Li, Y., Zhu, G., Ng, W.J. and Tan, S.K., 2014. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: design, performance and mechanism. *Science of the Total Environment*, 468, pp.908–932.

Luque de Castro, M.D. and Priego-Capote, F., 2010. Soxhlet extraction: Past and present panacea. *Journal of Chromatography A*, 1217(16), pp.2383–2389.

Manyele, S.V. and Anicetus, H., 2006. Management of medical waste in Tanzanian hospitals. *Tanzania Health Research Bulletin*, 8(3), pp.177–182.

Martínez-Hernández, V., Meffe, R., Herrera López, S. and de Bustamante, I., 2016. The role of sorption and biodegradation in the removal of acetaminophen, carbamazepine, caffeine, naproxen and sulfamethoxazole during soil contact: A kinetics study. *Science of The Total Environment*, 559, pp.232–241.

Martin, G. and Wane, W., 2013. *Education and health services in Uganda : data for results and accountability*. [online] The World Bank, pp.1–16. Available at: <http://documents.worldbank.org/curated/en/2013/11/18523074/education-health-services-uganda-data-results-accountability> [Accessed 2 Feb. 2016].

Matamoros, V., García, J. and Bayona, J.M., 2008. Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. *Water Research*, 42(3), pp.653–660.

Matamoros, V., Sala, L. and Salvadó, V., 2012. Evaluation of a biologically-based filtration water reclamation plant for removing emerging contaminants: A pilot plant study. *Bioresource Technology*, 104, pp.243–249.

Mbabazi, J., Kwetegyeka, J., Ntale, M. and Wasswa, J., 2010a. Ineffectiveness of Nakivubo wetland in filtering out heavy metals from untreated Kampala urban effluent prior to discharge into Lake Victoria, Uganda. *African Journal of Agricultural Research*, 5(24), pp.3431–3439.

Mbabazi, J., Wasswa, J., Kwetegyeka, J. and Bakyaita, G.K., 2010b. Heavy metal contamination in vegetables cultivated on a major urban wetland inlet drainage system of Lake Victoria, Uganda. *International Journal of Environmental Studies*, 67(3), pp.333–348.

McClellan, K. and Halden, R.U., 2010. Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. *Water Research*, 44(2), pp.658–668.

Medical Dictionary, 2008. *Antiulcer Drugs*. [online] Available at: <http://medical-dictionary.thefreedictionary.com/Antiulcer+Drugs> [Accessed 18 May 2016].

Merriam-Webster, 2016. *Lipid*. [online] Available at: <http://www.merriam-webster.com/dictionary/lipid> [Accessed 1 Jun. 2016].

Middleditch, B.S., 1989. *Analytical artifacts: GC, MS, HPLC, TLC, and PC*. Journal of chromatography library. Amsterdam ; New York: Elsevier.

Ministry of Health, Uganda, 2015. *National medicines policy 2015*. Available at: <http://www.health.go.ug/sites/default/files/National%20Medicines%20Policy%202015%20%286%29.pdf> [Accessed 16 May 2016].

Mohapatra, S., 2009. Uganda's rice revolution. *Scribd, Rice today*, pp.22–23.

Mompelat, S., Le Bot, B. and Thomas, O., 2009. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. *Environment International*, 35(2009), pp.803–814.

Mugambe, R.K., Ssempebwa, J.C., Tumwesigye, N.M., van Vliet, B. and Adedimeji, A., 2012. Healthcare waste management in Uganda: management and generation rates in public and private hospitals in Kampala. *Journal of Public Health*, 20(3), pp.245–251.

Mukiibi, J.K., 2001. *Agriculture in Uganda, Volume II Crops*. Kampala: Fountain Publishers Ltd.

Musinguzi, G. and Nuwaha, F., 2013. Prevalence, Awareness and Control of Hypertension in Uganda. *PLoS ONE*, [online] 8(4). Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3629133/> [Accessed 27 May 2016].

Mwakikagile, G., 2009. *Uganda: the land and its people*. [online] Intercontinental Books. Available at: <https://www.google.com/books?hl=sv&lr=&id=T9k0IkER6KYC&oi=fnd&pg=PA5&dq=Mwakikagile,+2009+uganda+the+land+and+its+people&ots=0Tb4eNW9Ex&sig=mizQPBrZwOaETQx1NwBt4q9347g> [Accessed 29 Jan. 2016].

Nakagiri, A., Kulabako, R.N., Nyenje, P.M., Tumuhairwe, J.B., Niwagaba, C.B. and Kansiime, F., 2015. Performance of pit latrines in urban poor areas: A case of Kampala, Uganda. *Habitat International*, 49(2015), pp.529–537.

Nationalencyklopedin, 2016a. *Alluvium*. [online] Available at: <http://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/alluvium> [Accessed 2 Jun. 2016].

Nationalencyklopedin, 2016b. *Anoxi*. [online] Available at: <http://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/anoxi-(2)> [Accessed 2 Jun. 2016].

Nationalencyklopedin, 2016c. *Antidepressiva läkemedel*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/antidepressiva-l%C3%A4kemedel> [Accessed 18 May 2016].

Nationalencyklopedin, 2016d. *Antiepileptikum*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/antiepileptikum> [Accessed 18 May 2016].

Nationalencyklopedin, 2016e. *Antihistaminer*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/antihistaminer> [Accessed 18 May 2016].

Nationalencyklopedin, 2016f. *Antihypertensiva medel*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/antihypertensiva-medel> [Accessed 17 May 2016].

Nationalencyklopedin, 2016g. *Diuretika*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/diuretika> [Accessed 17 May 2016].

Nationalencyklopedin, 2016h. *Lakustrin*. [online] Available at: <http://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/lakustrin> [Accessed 2 Jun. 2016].

Nationalencyklopedin, 2016i. *Majs*. [online] Available at: <http://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/majs> [Accessed 18 May 2016].

Nationalencyklopedin, 2016j. *Malaria*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/malaria> [Accessed 18 May 2016].

Nationalencyklopedin, 2016k. *Matris*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/matris-(kemi)> [Accessed 19 Apr. 2016].

Nationalencyklopedin, 2016l. *Metabolit*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/metabolit> [Accessed 1 Jun. 2016].

Nationalencyklopedin, 2016m. *Protolys*. [online] Available at: <http://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/protolys> [Accessed 30 May 2016].

Nationalencyklopedin, 2016n. *Regressionsanalys*. [online] Available at: <http://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/regressionsanalys> [Accessed 2 Jun. 2016].

Nationalencyklopedin, 2016o. *Vätskeextraktion*. [online] Available at: <http://www.ne.se.ezproxy.its.uu.se/uppslagsverk/encyklopedi/l%C3%A5ng/v%C3%A4tskeextraktion>

[Accessed 20 Apr. 2016].

National Water and Sewerage Corporation, 2016. *Sewer Services*. [online] Available at: <https://www.nwsc.co.ug/index.php/home-mobile/itemlist/category/26-sewer> [Accessed 9 Feb. 2016].

NEMA, 1999. *The National Environment (Standards For Discharge of Effluent into Water or on Land) Regulations, 1999*. Available at: <http://faolex.fao.org/docs/pdf/uga40868.pdf>.

Nollet, L.M.L. and Rathore, H.S., 2015. *Biopesticides Handbook*. CRC Press.

Nyenje, P.M., Foppen, J.W., Kulabako, R., Muwanga, A. and Uhlenbrook, S., 2013. Nutrient pollution in shallow aquifers underlying pit latrines and domestic solid waste dumps in urban slums. *Journal of Environmental Management*, 122(2013), pp.15–24.

Nyenje, P.M., Havik, J.C.N., Foppen, J.W., Muwanga, A. and Kulabako, R., 2014. Understanding the fate of sanitation-related nutrients in a shallow sandy aquifer below an urban slum area. *Journal of Contaminant Hydrology*, 164(2014), pp.259–274.

Oteng, J.. and Sant' Anna, R., 1997. *Rice production in Africa: current situation and issues*. Available at: <http://www.fao.org/docrep/003/x2243t/x2243t05.htm> [Accessed 20 Jun. 2016].

Park, N., Vanderford, B.J., Snyder, S.A., Sarp, S., Kim, S.D. and Cho, J., 2009. Effective controls of micropollutants included in wastewater effluent using constructed wetlands under anoxic condition. *Ecological Engineering*, 35(3), pp.418–423.

Paterson, C., Mara, D. and Curtis, T., 2007. Pro-poor sanitation technologies. *Geoforum*, 38(5), pp.901–907.

Pavlović, D.M., Babić, S., Horvat, A.J.M. and Kaštelan-Macan, M., 2007. Sample preparation in analysis of pharmaceuticals. *TrAC Trends in Analytical Chemistry*, 26(11), pp.1062–1075.

Peng, X., Wang, Z., Yang, C., Chen, F. and Mai, B., 2006. Simultaneous determination of endocrine-disrupting phenols and steroid estrogens in sediment by gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1116(1–2), pp.51–56.

Pérez-Carrera, E., Hansen, M., León, V., Björklund, E., Krogh, K., Halling-Sørensen, B. and González-Mazo, E., 2010. Multiresidue method for the determination of 32 human and veterinary pharmaceuticals in soil and sediment by pressurized-liquid extraction and LC-MS/MS. *Analytical and Bioanalytical Chemistry*, 398(3), pp.1173 – 1184.

Peysson, W. and Vulliet, E., 2013. Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry. *Journal of Chromatography. A*, 1290, pp.46–61.

Pickford, J. and Shaw, R., 2005. Technical Brief No. 54: Emptying pit latrines. *Waterlines*, 23(3), pp.15–18.

Pontolillo, J. and Eganhouse, R.P., 2001. *The search for reliable aqueous solubility (Sw) and octanol-water partition coefficient (Kow) data for hydrophobic organic compounds: DDT and DDE as a case study*. [online] US Department of the Interior, US Geological Survey Reston, Virginia. Available at: <http://pubs.usgs.gov/wri/wri014201/> [Accessed 11 Apr. 2016].

Prosser, R.S. and Sibley, P.K., 2015. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. *Environment International*, 75(2015), pp.223–233.

PubChem, 2016. *The PubChem Project*. [online] Available at: <https://pubchem.ncbi.nlm.nih.gov/> [Accessed 22 May 2016].

Rakkiyappan, P., Thangavelu, S., Bhagyalakshmi, K.V. and Radhamani, R., 2007. Uptake of nitrogen, phosphorus and potassium by some promising mid late maturing sugarcane clones. *Sugar Tech*, 9(1), pp.23–27.

Runnqvist, H., Bak, S.A., Hansen, M., Styrishave, B., Halling-Sørensen, B. and Björklund, E., 2010. Determination of pharmaceuticals in environmental and biological matrices using pressurised liquid extraction—Are we developing sound extraction methods? *Journal of Chromatography a*, 1217(16), pp.2447–2470.

Ryan, J.A., Bell, R.M., Davidson, J.M. and O'Connor, G.A., 1988. Plant uptake of non-ionic organic chemicals from soils. *Chemosphere*, 17(12), pp.2299–2323.

Salvia, M.-V., Vulliet, E., Wiest, L., Baudot, R. and Cren-Olivé, C., 2012. Development of a multi-residue method using acetonitrile-based extraction followed by liquid chromatography–tandem mass spectrometry for the analysis of steroids and veterinary and human drugs at trace levels in soil. *Journal of Chromatography A*, 1245, pp.122–133.

Sanchez-Prado, L., Garcia-Jares, C. and Llompart, M., 2010. Microwave-assisted extraction: Application to the determination of emerging pollutants in solid samples. *Journal of Chromatography A*, 1217(16), pp.2390–2414.

Sangster, J., 1997. *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*. [online] Montreal, Canada: John Wiley and Sons Ltd. Available at: <http://eu.wiley.com/WileyCDA/WileyTitle/productCd-0471973971.html> [Accessed 5 Apr. 2016].

Santos, H.M. and Capelo, J.L., 2007. Trends in ultrasonic-based equipment for analytical sample treatment. *Talanta*, 73(5), pp.795–802.

Schroeder, L.M., Blackwell, B., Klein, D. and Morse, A.N., 2015. Rate Uptake of Three Common Pharmaceuticals in Celery, Apium Graveolens. *Water, Air, & Soil Pollution*, 226(4), pp.1–20.

Shenker, M., Harush, D., Ben-Ari, J. and Chefetz, B., 2011. Uptake of carbamazepine by cucumber plants – A case study related to irrigation with reclaimed wastewater. *Chemosphere*, 82(6), pp.905–910.

da Silva, B.F., Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M. and Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. *Chemosphere*, 85(8), pp.1331–1339.

Simonsen, F. and Lindegren, R., 2005. *Analysteknik: instrument och metoder*. Lund: Studentlitteratur.

Smith, R.M., 2003. Before the injection—modern methods of sample preparation for separation techniques. *Journal of Chromatography A*, 1000(1-2), pp.3–27.

Statista 2016, 2016. *Pharmaceutical market worldwide revenue 2001-2014 | Statistic*. [online] Statista. Available at: <http://www.statista.com/statistics/263102/pharmaceutical-market-worldwide-revenue-since-2001/> [Accessed 30 May 2016].

Sundstøl Eriksen, G., Amundsen, C.E., Bernhoft, A., Eggen, T., Grave, K., Halling-Sørensen, B., Källqvist, T., Sogn, T. and Sverdrup, L., 2009. *Risk assessment of contaminants in sewage sludge applied on Norwegian soils - Opinion from the Panel on Contaminants in the Norwegian Scientific Committee for Food Safety*.

Svenska FN-förbundet, 2016. *Befolkningsantal, Total befolkning i tusental.* Available at: <http://www.globalis.se/Statistik/Befolkningsantal> [Accessed 29 Jan. 2016].

Sylvia, D.M., 2005. *Principles and Applications of Soil Microbiology*. Pearson Prentice Hall.

Tadeo, J.L., Sánchez-Brunete, C., Albero, B. and García-Valcárcel, A.I., 2010. Application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples. *Journal of Chromatography A*, 1217(16), pp.2415 – 2440.

Talisuna, A.O., Langi, P., Bakyaita, N., Egwang, T., Mutabingwa, T.K., Watkins, W., Marck, E.V. and D'Alessandro, U., 2002. Intensity of malaria transmission, antimalarial-drug use and resistance in Uganda: what is the relationship between these three factors? *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 96(3), pp.310–317.

The Republic of Uganda, 2010. *The national policy for disaster preparedness and management*. Available at:

<http://www.ifrc.org/docs/IDRL/Disaster%20Policy%20for%20Uganda.pdf>.

The World Bank, 2014a. *GDP per capita (current US$)*. [online] Available at: <http://data.worldbank.org/indicator/NY.GDP.PCAP.CD> [Accessed 1 Feb. 2016].

The World Bank, 2014b. *Uganda*. [online] Available at: <http://data.worldbank.org/country/uganda> [Accessed 1 Feb. 2016].

Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils – a review. *Journal of Plant Nutrition and Soil Science*, 166(2), pp.145–167.

Tolls, J., 2001. Sorption of veterinary pharmaceuticals in soils: a review. *Environmental science & technology*, 35(17), pp.3397–3406.

Tolou-Ghamari, Z., Zare, M., Habibabadi, J.M. and Najafi, M.R., 2013. A quick review of carbamazepine pharmacokinetics in epilepsy from 1953 to 2012. *Journal of research in medical sciences*, pp.81-85.

Trapp, S., 2000. Modelling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. *Pest Management Science*, 56(9), pp.767–778.

Trautmann, N.M. and National Science Teachers Association eds., 2001. *Assessing toxic risk*. Student ed ed. Cornell scientific inquiry series. [online] Arlington, Va: NSTA Press. Available at: <http://static.nsta.org/files/PB162X1Sweb.pdf>.

Uganda National Meteorological Authority, 2016. *Home | Uganda National Meteorological Authority (UNMA) - Weather and Climate for All - UNMA - Uganda National Meteorological Authority*. [online] Available at: <https://www.unma.go.ug/> [Accessed 1 Jun. 2016].

UNHCR, 2009. Chapter 6: Strategies for difficult situations. In: *Excreta Disposal in Emergencies: A Field Manual*. [online] UNHCR, pp.93–116. Available at: <http://www.unhcr.org/4a3391c46.html>.

U.S. Environmental Protection Agency, 1996. *Soil screening guidance technical background document*. [online] Washington: Office of Emergency and Remedial Response. Available at: <https://scholar.google.se/scholar?hl=sv&q=Soil+Screening+Guidance%3A+Technical+Background+Document&btnG=> [Accessed 5 Apr. 2016].

US EPA, O., 2015. *Constructed Wetlands*. [Overviews and Factsheets] Available at: <https://www.epa.gov/wetlands/constructed-wetlands> [Accessed 30 May 2016].

Verlicchi, P., Galletti, A., Petrovic, M. and Barceló, D., 2010. Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options. *Journal of Hydrology*, 389(3), pp.416–428.

Verlicchi, P. and Zambello, E., 2014. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. *Science of The Total Environment*, 470–471, pp.1281–1306.

Walpole, S.C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G. and Roberts, I., 2012. The weight of nations: an estimation of adult human biomass. *BMC Public Health*, [online] 12(439). Available at: <http://www.biomedcentral.com/content/pdf/1471-2458-12-439.pd>.

Walters, E., McClellan, K. and Halden, R.U., 2010. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. *Water Research*, 44(20), pp.6011–6020.

Water technology, 2016. *Kampala Water Supply and Sanitation Expansion Programme, Uganda.* [online] Available at: <http://www.water-technology.net/projects/kampala/> [Accessed 31 July 2016].

WaterAid, 2014. *EcoSan toilets, an alternative to conventional sanitation in vulnerable locations*. [online] Available at: <http://www.wateraid.org/news/news/ecosan-toilets> [Accessed 15 May 2016].

WATERS, 2016. *Oasis Sample Extraction Products : Waters*. [online] Available at: <http://www.waters.com/waters/en_SE/Oasis-Sample-Extraction-Products/nav.htm?locale=en_SE&cid=513209> [Accessed 29 May 2016].

Watson, G., 1995. *Good sewers cheap? Agency–customer interactions in low-cost urban sanitation in Brazil*. Available at: <http://waterfund.go.ke/safisan/Downloads/Global%20Good%20Sewers%20Cheap.pdf>.

Watts, C., Maycock, D., Crane, M. and Fawell, J., 2007. *Desk based review of current knowledge of pharmaceuticals in drinking water and estimation of potential levels*. [online] Department of Environment, Food and Rural Afairs- Drinking Water Inspectorate. Available at: <http://dwi.defra.gov.uk/research/completed-research/reports/dwi70-2-213.pdf>.

Weatherbase, 2016. *Kampala, Uganda Travel Weather Averages (Weatherbase)*. [online] Weatherbase. Available at: <http://www.weatherbase.com/weather/weather.php3?s=636800&cityname=Kampala-Central-Region-Uganda,> [Accessed 1 Jun. 2016].

Welsch, D.J., Smart, D.L., Boyer, J., Minkin, P., Smith, H.C. and McCandless, T.L., 1995. *Forested wetlands*. [online] U.S. Department of Agriculture, Forest Service, Northeastern Area. Available at: <http://www.na.fs.fed.us/Spfo/Pubs/n_resource/wetlands/index.htm> [Accessed 17 May 2016].

WHO, n.d.a *Information sheet: Pharmaceuticals in drinking-water*. [online] Available at: <http://www.who.int/water_sanitation_health/emerging/info_sheet_pharmaceuticals/en/> [Accessed 30 May 2016a].

WHO, n.d.b *Fact sheet on environmental sanitation 3.4 - Simple pit latrines*. Available at: <http://www.who.int/water_sanitation_health/hygiene/emergencies/fs3_4.pdf>.

WHO, 2006. *Medecine Prices in Uganda*.

WHO/UNICEF Joint Water Supply and Sanitation Monitoring Programme, World Health Organization and UNICEF, 2015. *Progress on sanitation and drinking water - 2015 update and MDG assessment.*

Wood, A., Dixon, A. and McCartney, M., 2013. *Wetland management and sustainable livelihoods in Africa*. [online] Routledge. Available at: <https://www.google.com/books?hl=sv&lr=&id=VTbNpVioirEC&oi=fnd&pg=PP2&dq=wetland+management+and+sustainable+livelihoods+in+africa&ots=oJJ3N6cByj&sig=mT649v2tU8GrheF1mjAxbCsX5Ro> [Accessed 20 Jan. 2016].

Wu, X., Dodgen, L.K., Conkle, J.L. and Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. *Science of The Total Environment*, 536, pp.655–666.

Xu, J., Wu, L. and Chang, A.C., 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. *Chemosphere*, 77(10), pp.1299–1305.

Yang, Y.-Y., Toor, G.S. and Williams, C.F., 2015. Pharmaceuticals and organochlorine pesticides in sediments of an urban river in Florida, USA. *Journal of Soils and Sediments*, 15(4), pp.993–1004.

Zuccato, E., Calamari, D., Natangelo, M. and Fanelli, R., 2000. Presence of therapeutic drugs in the environment. *The Lancet*, 355(9217), pp.1789–1790.

APPENDIX A – DETAILED DESCRIPTION OF WATER QUALITY PARAMETERS ANALYSIS

PHOSPHATE

The phosphorus as phosphate concentration was determined using the Spectroquant® Phosphate Test by Merck, Germany (Appendix D). 5.0 ml of each water sample was pipetted with an automatic pipette into test tubes and 5.0 ml Milli-Q water was added to one test tube, this would serve as the blank. Five drops of reagent 1 was added to each test tube, and then the samples were mixed. One level microspoon of reagent 2 was added to the test tubes, and afterwards they were mixed until the reagent had dissolved completely. The microspoon was a small spoon that came with the measuring kit. The samples were left to stand for five minutes, after which they were added to 1 ml cuvettes. A Spectroquant® NOVA 60 photometer by Merck, Germany (Appendix D) then measured the PO_4-P concentration in mg/l.

TOTAL PHOSPHORUS

The total phosphorus concentration was determined using the Spectroquant® Crack Set 10 by Merck, Germany (Appendix D). 10.0 ml of each of the diluted (DF: 2) water samples were pipetted with an automatic pipette into test tubes and 10.0 ml Milli-Q water added to an additional test tube, serving as a blank. One drop of reagent 1 was added to each test tube and then the samples were mixed. One dose of reagent 2 was then added to each test tube with the help of a special dose-metering cap that came with the measuring kit. The test tubes were sealed with caps and then mixed thoroughly until the reagent had dissolved completely, after which they were put in an incubator and heated at 120 °C for 60 minutes. The incubator was a Spectroquant® TR 420 by Merck, Germany (Appendix D). After the hour had passed, the test tubes were taken out of the incubator and left in a test tube rack to cool for about 30 minutes. When the test tubes had reached room temperature, the samples underwent the same procedure as in section PHOSPHATE.

NITRATE

The nitrite concentration was determined with the help of the Spectroquant® nitrate test by Merck, Germany (Appendix D). The analysis was performed in a fume hood. First 4.0 ml of reagent 1 was pipetted into a dry glass test tube according to the instructions provide. Thereafter 0.5 ml of each water sample was pipetted into the test tubes. Also one blank was created with 0.50 ml Milli-Q water instead of wastewater. 0.5 ml of reagent 2 was then added carefully and the test tubes were mixed. The solutions were then placed to stand for at least 10 minutes before they were measured in the spectrophotometer Spectroquant® NOVA 60 by Merck, Germany in the unit mg/l.

TOTAL NITROGEN

The total nitrogen concentration was determined with the help of the Spectroquant® Crack Set 20 by Merck, Germany (Appendix D). All water samples were diluted with 50 % Milli-Q water, due to expected high values. The analysis was performed in a fume hood. According to the crack set 20's instructions, 10 ml of the pre-treated water samples were pipetted into test tubes made of glass. One blank sample was created with 10 ml Milli-Q water pipetted instead of sample water. Thereafter were one level of blue microspoon from reagent 1 added to all test tubes. The tubes were then mixed until the reagent had been totally dissolved. 6 drops of reagent 2 were added to the tubes, which also were sealed tightly before mixing. Later, all

tubes were put in the incubator, Spectroquant® TR 420 by Merck, Germany, in 120 °C for 60 minutes. When the time had passed all tubes were put to cool down to room temperature in a test-tube rack for approximately 30 minutes. The samples thereafter went through the same procedure described in the NITRATE chapter.

CHEMICAL OXYGEN DEMAND

The chemical oxygen demand (COD) concentration was determined with the help of the Spectroquant® COD test by Merck, Germany (Appendix D). Two different ranges were used, one lower with the range 10-150 mg/l and a higher one with the range 100-1500 mg/l. Following the instructions, 2.0 ml of water were carefully pipetted into the ready reaction cell's reagent. The reaction cell was supplied with the analysis test. Three blanks were created using only Milli-Q water. The screw caps were then tightly attached to the cells and the samples were vigorously mixed. Thereafter all cells were put in the incubator, Spectroquant® TR 420 by Merck, Germany, at 148 °C for 120 minutes. The cells were then put into a test-tube rack for cooling down in 10 minutes before they were shaken. They were left standing in the test-tube racks to cool to room temperature for about 30 minutes before they were finally measured in the spectrophotometer Spectroquant® NOVA 60 by Merck, Germany, in the unit mg/l.

TOTAL ORGANIC CARBON

The concentration of TOC was determined using two Spectroquant® TOC cell tests (Merck, Germany) with both a high and a low detection range (Appendix D). 1.0 ml of each of the water samples were added to test tubes and then diluted with 9.0 ml of Milli-Q water and mixed. The blank comprised of 10.0 ml of Milli-Q water. Two drops of reagent 1 was added to each test tube and then the test tubes were mixed. The test tubes were then left in a platform shaker, Rotamax 120 by Heidolph, Germany (Appendix D), to be stirred for 10 minutes at medium speed. 3.0 ml of each of the stirred samples were then pipetted into reaction cells that came with the measuring kit. One microspoon of reagent 2 was added to each of the reaction cells. The microspoon was a small spoon that came with the measuring kit. According to the instructions, the cells were then supposed to be sealed with aluminium caps and left to stand upside down in the incubator for 120 minutes at 120 °C. Aluminium caps were unavailable during this test however, so the cells were instead sealed with plastic caps and they were left to stand bottom down in the incubator. The incubator was a Spectroquant® TR 420 by Merck, Germany (Appendix D). After the two hours had passed, the cells were taken out of the incubator and were left to stand bottom down in a test tube rack for 60 minutes until they had reached room temperature. After the cells had cooled down, the concentration of TOC had to be measured within 10 minutes. The TOC concentration in mg/l was measured with a Spectroquant® NOVA 60 photometer by Merck, Germany (Appendix D).

TOTAL SOLIDS AND TOTAL SUSPENDED SOLIDS

In the total solid analysis approximately 30 ml water from each sample were placed into a weighed crucible and put in the oven at 105 °C for approximately 360 minutes. When the samples had been totally dried, the crucibles were taken out from the oven and weighed again. The difference in weight is the weight of the total solids in the water. The unit was transformed from mg to mg/l with help from the added volume.

For the total suspended solids the water were filtered through 0.7 µm glass microfiber filters GF/F with a diameter of 47 mm with help from a small vacuum pump (Appendix D). The volume of water added to the filtration depended on the content of solids in the water samples. The volume varied between 50 and 100 ml (Figure 18).

Figure 18. *Filtration of a water sample. The sample can be seen in the plastic bottle to the right. The blue boxes in the middle of the picture are the vacuum pumps and the filtration unit is to the left. The filtered sample was collected in the big glass jar and was later transferred to plastic bottles.*

The filters were weighed prior to the filtration and thereafter put into crucibles and put in the oven at 105 °C for 60 minutes to dry. The filters were thereafter put in a desiccator to cool down. The dry and cooled filters were then weighed again and the difference in weight represents the total suspended solids in the water in mg. The unit was transformed from mg to mg/l with help from the added volume.

APPENDIX B – PHARMACEUTICALS STUDIED

Table 23. The pharmaceuticals that were studied in this thesis with their corresponding internal standard

Pharmaceutical	Use	Internal standard
Acetaminophen	Analgesic	Acetaminophen
Codeine	Analgesic	Codeine
Amoxicillin	Antibiotic	Amoxicillin
Ciprofloxacin	Antibiotic	Ciprofloxacin
Clarithromycin	Antibiotic	Azithromycin
Metronidazole	Antibiotic	Metronidazole
Ofloxacin	Antibiotic	Ofloxacin
Roxithromycin	Antibiotic	Azithromycin
Sparfloxacin	Antibiotic	Ofloxacin
Sulfamethoxazole	Antibiotic	Sulfamethoxazole
Trimethoprim	Antibiotic	Trimethoprim
Amitriptyline	Antidepressant	Carbamazepine
Citalopram	Antidepressant	Citalopram
Diazepam	Antidepressant	Diazepam
Fluoxetine	Antidepressant	Fluoxetine
Oxazepam	Antidepressant	Oxazepam
Sertraline	Antidepressant	Sertraline
Venlafaxine	Antidepressant	Venlafaxine
Metformin	Anti-diabetic	Metformin
Carbamazepine	Antiepileptic	Carbamazepine
Lamotrigine	Antiepileptic	Lamotrigine
Climbazole	Antifungal agent	Metronidazole
Clotrimazole	Antifungal agent	Ketoconazole
Ketoconazole	Antifungal agent	Ketoconazole
Cetirizine	Antihistamine	Amlodipine
Amlodipine	Antihypertensive	Amlodipine
Irbesartan	Antihypertensive	Irbesartan
Losartan	Antihypertensive	Losartan
Diclofenac	Anti-inflammatory	Diclofenac
Ibuprofen[a]	Anti-inflammatory	-
Naproxen[a]	Anti-inflammatory	-
Salbutamol	Anti-inflammatory	Salbutamol
Lumefantrine[a]	Antimalarial	-
Pyrimethamine	Antimalarial	Trimethoprim
Omeprazole	Antiulcer agent	Omeprazole
Ranitidine	Antiulcer agent	Ranitidine
Atenolol	β-blocker	Atenolol
Metroprolol	β-blocker	Atenolol
Sotalol	β-blocker	Atenolol

Pharmaceutical	Use	Internal standard
Furosemide	Diuretics	Furosemide
Hydrochlorothiazide	Diuretics	Hydrochlorothiazide
Atorvastatin	Lipid regulator	Atorvastatin
Bezafibrate	Lipid regulator	Bezafibrate
Gemfibrozil	Lipid regulator	Diclofenac
Lidocaine	Local anaesthetic	Lidocaine

[a] Were not included in the instrumental analysis with the mass spectrometer

APPENDIX C – DETAILED DESCRIPTION OF THE CLEANING OF THE LAB WARE

All plastic equipment were first rinsed with 96 % pure ethanol (Appendix D) and then washed in the dishwasher Miele Professional G7804 with 30 % potassium hydroxide neodisher® LaboClean FLA as the detergent, for 33 minutes. Thereafter they were rinsed with 99.9 % pure methanol (Appendix D) or 96 % ethanol three times and stored, if necessary, in a clean area before use. For example were 20 plastic cans that holds 1 litre, washed and rinsed and thereafter sealed with an inner and an outer lid. They were used for storing the wastewater samples after they had been filtered. Reservoirs used in the wastewater extraction section 4.5.4, were dish washed in the machine G7804 and then rinsed with methanol three times before every new use.

All glass and ceramic materials like test tubes, beakers, pistons, crucible and so on were also washed in the dishwasher G7804 and then placed in the oven over the night (240 minutes) in 400 ºC, to make sure that all contaminations were gone. All the openings of the material were thereafter covered in aluminium foil until use. Amber glass vials and glass Pasture pipettes were only used once and then disposed. They were not washed, only burned in 400 ºC in the oven over night before use. Glass microfiber filters GF/F (Appendix D) for raw water filtration were also burned at 400 ºC over night and then stored in aluminium foil packages, to minimize the risk of contaminations, before use.

Materials such as plastic caps for test tubes, metal and plastic needles for N_2 evaporation, Solid Phase Extraction (SPE)-equipment like valves and adapters were cleaned by using the B5510 Ultrasonic Cleaner Sonicator from Branson. They were first put in small beakers with methanol or ethanol and then placed in the sonicator bath for 15 minutes. The procedure was performed twice for the SPE-equipment with new methanol/ethanol added to each new sonication. Reduction-explosion cycles used in the sonicator release adsorbed substances from the surface of the material and in that way clean the equipment (Santos and Capelo, 2007).

Before each new laboratory analysis started, all test-tubes were carefully marked to minimize the risk of mixing the samples with each other.

APPENDIX D – COMPLETE LIST OF THE CHEMICAL SUBSTANCES, EQUIPMENT AND DEVICES USED IN THESIS

CHEMICAL SUBSTANCES

Table 24. Complete list of the chemical compounds used in this Master's thesis

Name	Solvent/powder	Specification	Manufacturer
Acetic acid	Solvent	ACS reagent, ≥99.7 %	SIGMA-ALDRICH®
Acetonitrile	Solvent	LiChrosolv®, 1.00029, hypergrade for LC-MS. Purity (GC): ≥99.9 %	Merck
EDTA-Na_2	Solvent	0.1 mol/l	SIGMA-ALDRICH®
Ethanol	Solvent	96 % vol	VWR International
Methanol	Solvent	LiChrosolv®, 1.06035, hypergrade for LC-MS	Merck
Methanol	Solvent	LiChrosolv®, 1.06007, gradient grade for liquid chromatography. Purity (GC): ≥99.9 %	Merck
QuEChERS extract pouch	Powder	AOAC method	Agilent Technologies

DEVICES

Table 25. Complete list of the devices used in this Master's thesis

Name	Type of device	Manufacturer
Analog Vortex Mixer	Shaker	VWR International
B5510 Ultrasonic Cleaner	Sonicator	Branson
Centrifuge 5810	Centrifuge	Eppendorf
N-EVAP™112	Nitrogen evaporator	Organomation Associates, Inc.
NewClassic ML	Analytical balance	METTLER TOLEDO
Pioneer™	Analytical balance	OHAUS
Rotamax 120	Platform shaker	Heidolph
Spectroquant® NOVA 60	Photometer	Merck
Spectroquant® TR 420	Incubator	Merck

EQUIPMENT

Table 26. Complete list of the equipment used in this Master's thesis

Name	Type of equipment	Specification	Manufacturer
5 ml NORM-JECT® syringe	Syringe	HPLC-certified	Henke-Sass, Wolf GmbH
25 mm syringe filter	Syringe filter	With 0.45 µm Polypropylene Membrane, European Article No. 514-0065	VWR International
Glass microfiber filters GF/F	Glass microfiber filters 0.7 µm	cat No. 1825-047, Ø47	GE Healthcare, Life Sciences, Whatman™
Neolus® hypodermic needle	Hypodermic needle	0.7 x 50 mm	Terumo
Oasis® HLB	SPE cartridge	-	Waters
Spectroquant® COD Cell Test	Standard kit	1.09772.0001, detection range 10-150 mg/l	Merck
Spectroquant® COD Cell Test	Standard kit	1.09773.0001, detection range 100-1500 mg/l	Merck
Spectroquant® Crack Set 10	Standard kit	1.14687.0001, detection range 0.05-5.0 mg/l	Merck
Spectroquant® Crack Set 20	Standard kit	1.14963.0001, detection range 1.0-25 mg/l	Merck
Spectroquant® Nitrate Test	Standard kit	1.09713.0002, detection range 0.1-25.0 mg/l	Merck
Spectroquant® Phosphate Test	Standard kit	1.14848.0001, detection range 0.01-5.0 mg/l	Merck
Spectroquant® TOC Cell Test	Standard kit	1.14878.0001, detection range 5.0-80.0	Merck
Spectroquant® TOC Cell Test	Standard kit	1.14879.0001, detection range 50-800	Merck

APPENDIX E – DETAILED DESCRIPTION OF THE PREPARATION AND EXTRACTION OF PHARMACEUTICALS FROM THE LIQUID SAMPLES

PREPARATION

The water samples were taken from the freezer and placed in 1 or 2 litres burned beakers for thawing. The caps were opened a little bit and the beakers act like holders for minimizing leakage. They were placed in a refrigerated room at 8 degrees over the weekend. When all liquid had melt, the filtration started according to (Appendix A) but without weighting of the filters. 500 ml of each sample were filtered and additionally 100 ml for two recovery test. Two blanks were also prepared, one with 500 ml Milli-Q water without EDTA and one for recovery with 100 ml Milli-Q water with EDTA.

EXTRACTION

All the 20 liquid samples (Table 3) were analysed individually, along with one blank made of Milli-Q water and three recovery samples. 50 µl of each of the pharmaceutical internal standards with concentration 1 ng/µl were added to the filtered water samples. The recovery samples were injected with 200 µl internal standard with concentration 1 ng/µl. 15 ml of 0.1 M EDTA-Na_2 were added to the samples. Before the start of the extraction, all of the bottles containing the water samples were weighed.

The SPE manifold was put in a fume hood and connected to a vacuum pump. The SPE cartridges used were Waters hydrophilic-lipophilic balanced (HLB) from Oasis (Appendix D). They were first labelled and then connected to the manifold via valves. The cartridges were pre-treated with 6 ml methanol, and after it had gone through the cartridge, 6 ml of Milli-Q water was added to the cartridge. This step was called conditioning of the cartridges and no vacuum was used. The adapters and reservoirs were connected to the cartridges and small amounts of the water samples were added to the reservoirs (Figure 19). Multiple cartridges were used for each water sample since the samples kept clogging up the filters in the cartridges. Vacuum was used to make the water go through the cartridges faster, but the flow was not higher than one drop per second, and the under pressure was no higher than 5 inHg, or 16 932 Pa.

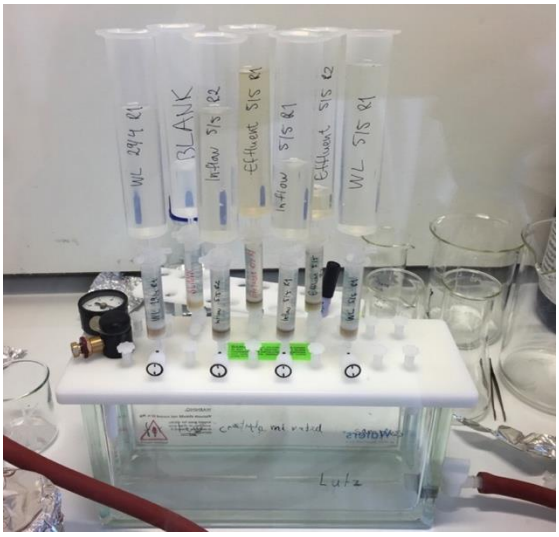

Figure 19. *Setup of the SPE equipment. The manifold is at the bottom of the picture, and the valves are the units with the black circles. The cartridges are connected to the valves, and the reservoirs are connected to the cartridges via adapters. The water was added to the reservoirs and then flowed through the cartridges into the manifold.*

When the total volume of 500 ml had flown through the cartridges, or when the filters were so clogged that the cartridges needed to be replaced, the reservoirs and adapters were disconnected from the cartridges and 6 ml of Milli-Q water was added directly to the cartridge. After the Milli-Q water had gone through the cartridge, the valve was closed and the cartridge was stored in a fridge at 2° C until centrifugation. When the extraction was completed the cartridges were taken out from the fridge and put into centrifuge vials and then centrifuged at 2000 rpm for 2 minutes to remove excess water. The empty bottles that had contained the wastewater samples were weighed again in order to determine exactly how much wastewater had flown through the cartridges.

When the cartridges had been centrifuged, they were eluted. The manifold and the valves were cleaned again, as described in 4.5.2. The SPE cartridges were connected to the manifold via valves again, but this time clean glass tubes were put underneath each cartridge (Figure 20).

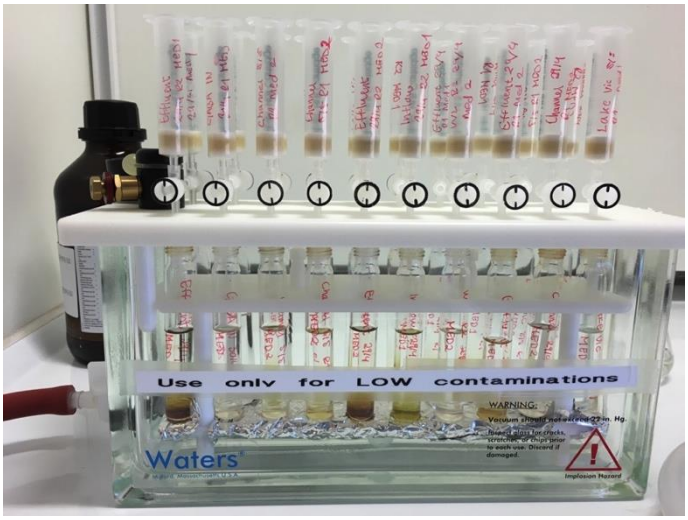

Figure 20. *The setup for elution of the SPE cartridges. The glass tubes inside the manifold collected the methanol that ran through the cartridges.*

4 ml methanol was added to the cartridge that was collected by the glass tube underneath. The methanol dripped by gravity, but occasionally vacuum was used to start the flow. After the

methanol had gone through the cartridge, the vacuum was turned on for one minute and after that an additional 4 ml of methanol was added to the cartridge. The vacuum was once more turned on for one minute after the last of the methanol had gone through the cartridge. At this point, about 8 ml of methanol had been added to each glass tube. The glass tubes were put in an N-EVAP™112 nitrogen evaporator (Appendix D) and the extracts were evaporated using N_2. When there was about 0.5 ml extract left in the tubes, the extracts that originated from the same water sample were transferred to one glass tube using glass Pasteur pipettes. To make sure that no sample was stuck on the walls of the empty glass tubes, they were cleaned with about 200 µl methanol three times and then the methanol was transferred to the new glass tube (Figure 21). A different Pasteur pipette was used for each different sample to prevent cross contamination.

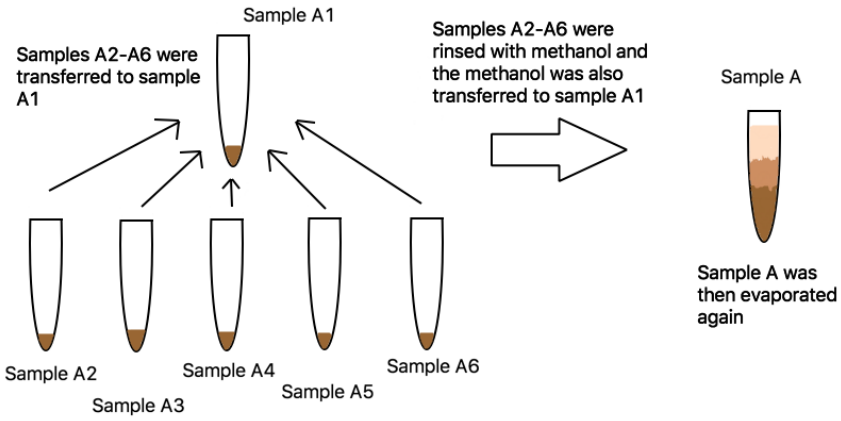

Figure 21. *A simple sketch explaining how extracts originating from the same water sample were transferred to one test tube.*

When all of the extracts had been transferred and there was only one glass tube left for each sample the extracts were evaporated again until there was about 200 µl left. The remaining content in the glass tubes were filtered using 5 ml NORM-JECT® syringes, 25 mm syringe filters and Neolus® hypodermic needles (Appendix D). Afterwards they were transferred to amber glass HPLC vials. The glass tubes were cleaned three times with approximately 200 µl methanol each time, and then the methanol was also filtered into the amber glass HPLC vial. Glass Pasteur pipettes were used to transfer the samples from the glass tubes to the syringes. A different Pasteur pipette was used for each different sample to prevent cross contamination. The content in the HPLC vials were then evaporated to dryness in the nitrogen evaporator. When all the vials were empty, 50 µl methanol and 450 µl Milli-Q water was added to the glass vials, and then they were shaken for 30 seconds in an Analog Vortex Mixer (Appendix D). The samples were stored in a freezer at -20° C until HPLC analysis.

APPENDIX F – DETAILED DESCRIPTION OF THE PREPARATION AND EXTRACTION OF PHARMACEUTICALS FROM THE SOLID SAMPLES

PREPARATION

Only the dry substance was examined for the solid samples. The preparations started with cleaning of 60 new 50 ml and 120 new 15 ml PP tubes. They were all rinsed with methanol three times, the bigger tubes with 1 ml and the smaller tubes with 0.5 ml each time. Thereafter they were placed to dry over the night in a plastic box. The box was earlier washed in the dishwasher Miele Professional G7804 and rinsed with ethanol. 0.900 g $MgSO_4$ and 0.150 g PSA were weighed up on two different calibrated scales with three and six decimals accuracy and added to the sixty 15 ml PP tubes.

The preparation of the crop samples were performed in a fume hood and started with that approximately 5 grams from the original crop samples were weighed on the scale NewClassic ML. They were then crushed with a mortar to powder to homogenise the sample (Figure 22).

Figure 22. *The picture to the left shows yam before and after the crush, the one in the middle shows sugar cane before and after the crushing. To the right is maize sample uncrushed and crushed.*

The samples from the same sampling date were mixed three and three, in total all fields ended up with 8 mixed crop samples and 8 mixed soil samples for each field (including recoveries). 1 gram from the mixed samples were then added to the 50 ml PP tubes. The remaining powder was put into small plastic bags and then back into the freezer.

During the work the samples were placed in ice boxes to remain cool and the leftover were quickly put back in the freezer. All surfaces and equipment were washed once or twice with methanol or ethanol between the different samples to avoid the samples being mixed with each other. The same procedure was performed for the soil samples.

EXTRACTION

After 1 g of each soil and crop sample had been transferred to the 50 ml PP tubes, 50 µl of each internal standard was added to all the samples, except the recovery samples. The recovery samples were injected with 200 µl internal standard instead. The samples were then shaken in an Analog Vortex Mixer (Appendix D) at high speed in order to homogenize them. After the samples had been mixed, 7.5 ml of EDTA-Na_2 0.1 M (Appendix D) solution was added to the samples after which they were shaken for 30 seconds. Another 7.5 ml of acetonitrile with acetic acid 1 % solution was added to the samples and they were shaken for another 30 seconds. 1.5 g NaOC and 6 g $MgSO_4$ were added to the samples from ready pouches, QuEChERS extract pouches (Appendix D). The samples were then shaken manually for 30 seconds, shaken in the shaker for 60 seconds at high speed and then centrifuged at 3500 rpm or 2465 g-forces for 15 minutes in a Centrifuge 5810 (Appendix D). The samples were taken out of the centrifuge and about 6 ml of the acetonitrile layers were taken up with an

automatic pipette and transferred to the 15 ml PP tubes with salts in, described in the section above. The small PP tubes were then shaken for 30 seconds at low speed and 60 seconds at high speed in the shaker. After the solids had settled the acetonitrile layer was measured and then the PP tubes were centrifuged at 3500 rpm for 15 minutes in the Centrifuge 5810. The acetonitrile layer was measured once again after the centrifugation, and then the layer was taken up by an automatic pipette and transferred into glass tubes with caps.

The glass tubes were put in an N-EVAP™112 nitrogen evaporator (Appendix D). The caps were removed from the tubes and the contents were evaporated until approximately 200 µl was left. The remaining extracts were transferred to amber glass vials via glass Pasteur pipettes. The glass tubes were cleaned three times with about 200 µl acetonitrile. The acetonitrile was then transferred to the glass vials. This was to ensure that there were no extracts left in the old PP tubes. More acetonitrile was added to the new glass vials until it was approximately 1 ml. A new Pasteur pipette was used for each new sample to avoid cross contamination. Afterwards, the extracts were frozen for at least one hour at -20° C. After the extracts had been taken out of the freezer, they were centrifuged at 3500 rpm for five minutes in a Centrifuge 5810 (Appendix D). The liquids in each of the vials were then transferred to new amber glass vials, but a small layer of the liquid was left at the bottom of the old vials to make sure that none of the salts used in the extraction would be transferred to the new vials. The new vials were then evaporated to dryness in the N-EVAP™112 nitrogen evaporator (Appendix D). When all the vials were dry, 150 µl methanol and 350 µl Milli-Q water were added to them. During this step, a lot of the samples became turbid. These samples were filtered using 5 ml NORM-JECT® syringes, 25 mm syringe filters and Neolus® hypodermic needles (Appendix D) into new vials. Methanol was used to rinse the old vials, and the methanol was then filtered into the new vial as well. Some of the samples had to be filtered two or three times in order for them to become clear again. The vials were then put in the nitrogen evaporator and evaporated to dryness. After the vials were dry, 150 µl methanol and 350 µl Milli-Q water was added again which resulted in some of the samples becoming murky once more. These samples were filtered once again, but they were not evaporated and refilled with the methanol/Milli-Q solvent.

APPENDIX G – DETAILED DESCRIPTION OF THE INSTRUMENTAL ANALYSIS

Pharmaceuticals were analysed using an Acquity Ultra-Performance Liquid Chromatography (UPLC) system (Waters Corporation, USA) coupled to a quadrupole-time-of-flight (QTOF) mass spectrometer (QTOF Xevo G2S, Waters Corporation, Manchester, UK). Extracts were injected twice, since some compounds were analysed under positive electrospray ionization (PI) and the others under negative electrospray ionization (NI). Chromatographic separation took place using an Acquity HSS T3 column (100 mm x 2.1 mm i.d., 1.8 µm particle size) for the compounds analysed by PI and an Acquity BEH C18 column (50 mm × 2.1 mm i.d., 1.7 m particle size) for the substances determined under NI. Both columns were purchased from Waters Corporation. The mobile phases used in PI mode were A) 5 mM ammonium formate buffer with 0.01 % formic acid and B) acetonitrile with 0.01 % formic acid and in NI mode they were A) 5 mM ammonium acetate buffer with 0.01 % ammonia and B) acetonitrile with 0.01 % ammonia. The chromatographic flow rate used was 0.5 ml/min, the total run time was 21 min in both positive and negative electrospray ionization and the injection volume was 5 µl. The column temperature was set at 40 °C and the sample manager temperature at 15 °C. The resolution of the TOF mass spectrometer was around 30 000 at full width and half maximum (FWHM) at m/z 556. MS data were acquired over a m/z range of 100–1200 in a scan time of 0.25 s. Capillary voltages of 0.35 and 0.4 kV were used in positive and negative ionization modes, respectively. A cone voltage of 30 V was applied, the desolvation gas flow rate was set at 700 l/h and the cone gas flow was set to 25 l/h. The desolvation temperature was set to 450 °C and the source temperature to 120 °C. Samples were acquired with MSE experiments in the resolution mode. In this type of experiments, two acquisition functions with different collision energies were created: the low energy (LE) function with a collision energy of 4 eV, and the high energy (HE) function with a collision energy ramp ranging from 10 to 45 eV. Calibration of the mass-axis from m/z 100 to 1200 was conducted daily with a 0.5 mM sodium formate solution prepared in 90:10 (v/v) 2-propranolol/water. For automated accurate mass measurement, the lock-spray probe was employed, using a lock mass leucine encephalin solution (2 mg mL-1) in ACN/water (50/50) with 0.1 % formic acid. The solution was pumped at 10 µl/min through the lock-spray needle.

APPENDIX H – MOST COMMONLY SOLD PHARMACEUTICALS FOR DIFFERENT TYPES OF DISEASES IN KAMPALA

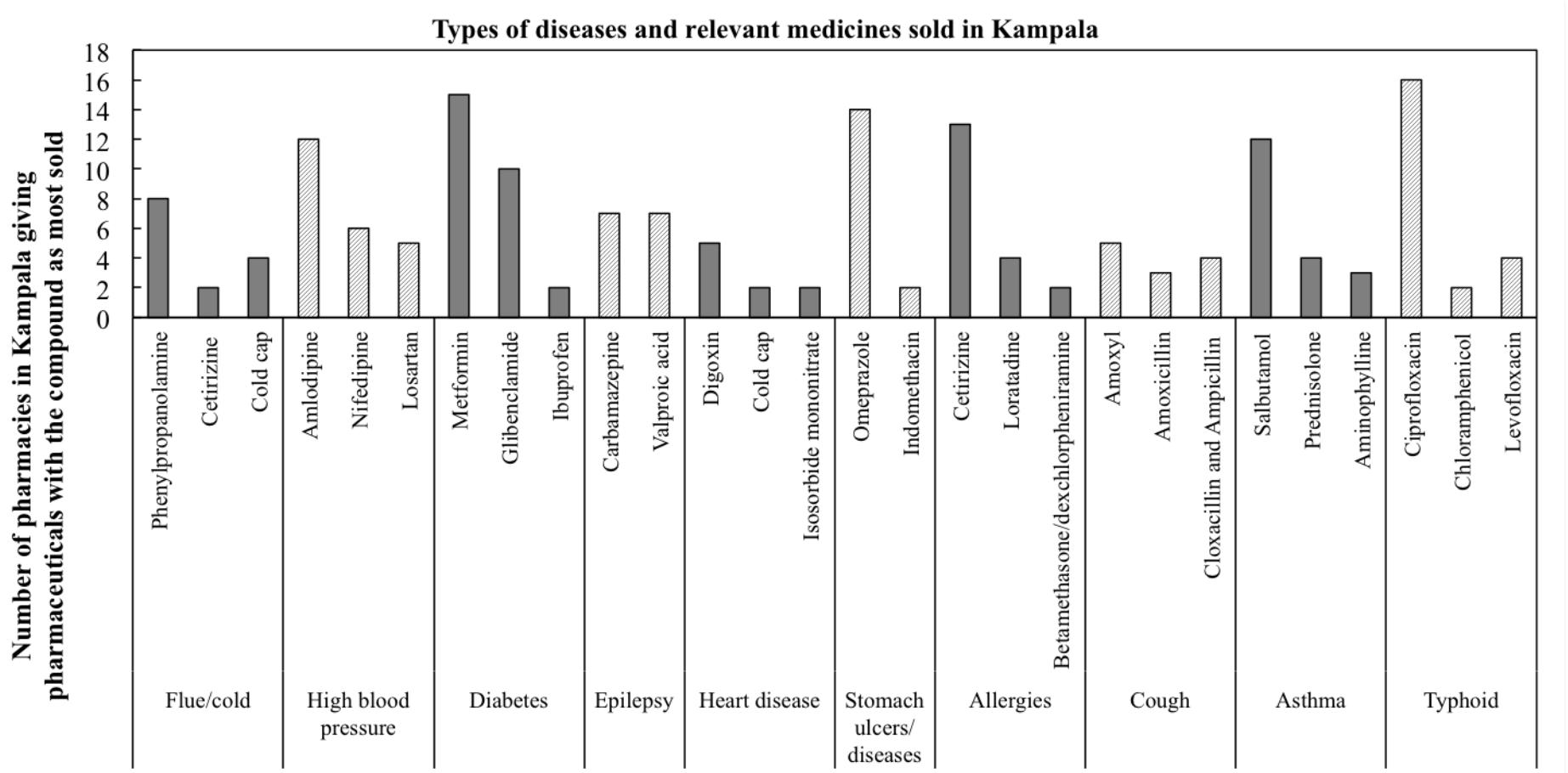

Figure 23. *Number of pharmacies in Kampala giving pharmaceuticals with the compound as most sold.*

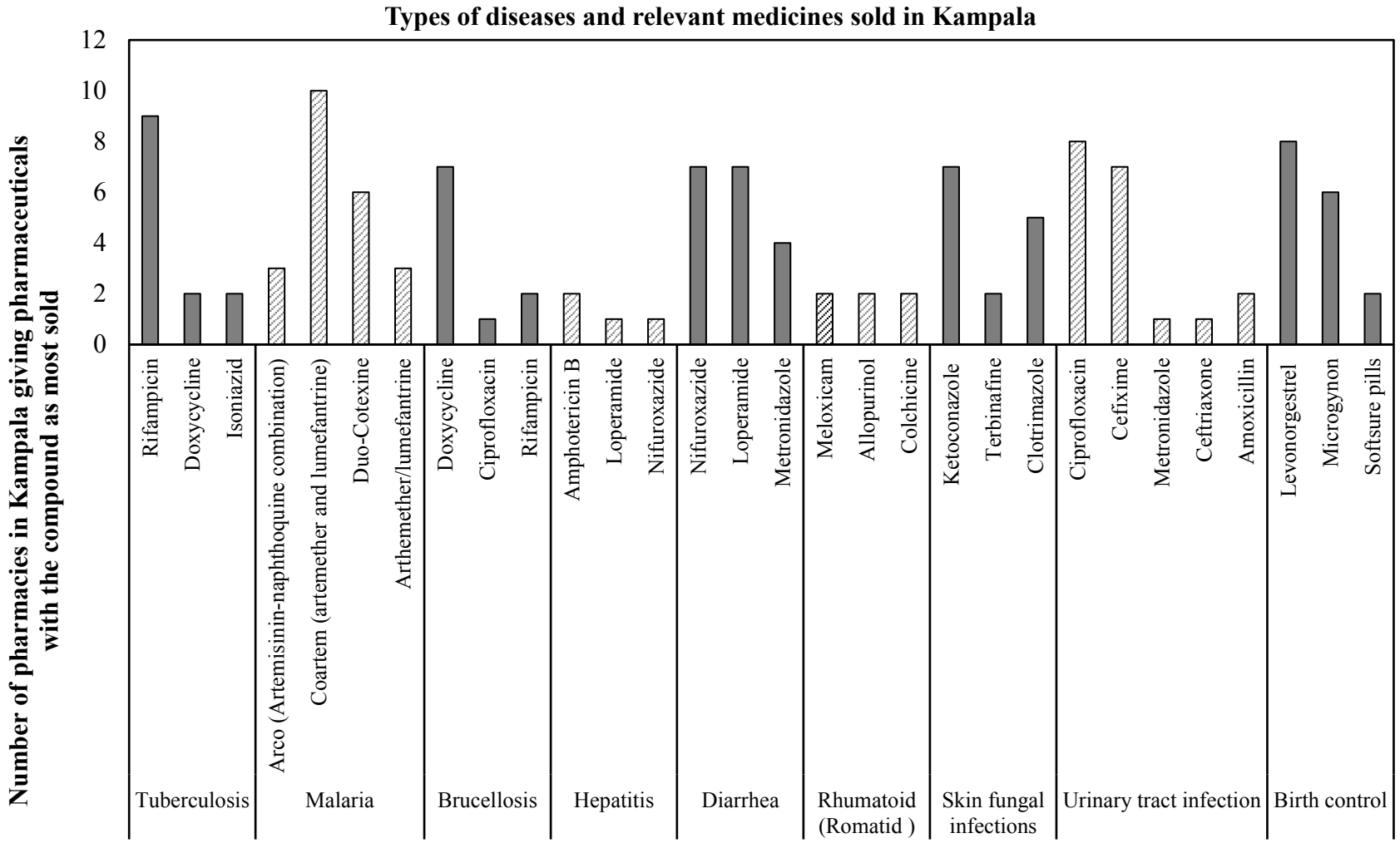

Figure 24. *Number of pharmacies in Kampala giving pharmaceuticals with the compound as most sold.*