**Master’s Thesis**

# Occurrence of selected antibiotics and antiretroviral pharmaceuticals in Lusaka, Zambia

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This research focuses on the occurrence of selected antibiotics and antiretrovirals in the Lusaka region and the general sanitation situation and attitude towards the use of urine as fertilizer in rural areas of Zambia. Samples were taken from groundwater, wastewater and surface water, and urine collected from dry toilets in the region. The sampling area was the Madimba area on the outskirts of Lusaka, the Machiachi and Chunga wastewater treatment plants, and the Chunga River near the Chunga wastewater treatment plant. The sanitation situation and attitude towards the use of urine as fertilizer in rural areas of Zambia were analyzed based on a survey conducted by the Finnish Global Dry Toilet Association. The sampling study was conducted by collecting six urine samples from dry toilets, two groundwater samples from the Madimba region, three wastewater samples and one groundwater sample from the Manchinchi wastewater treatment plant, two surface water samples and three wastewater samples from the Chunga wastewater treatment plant. The samples were analyzed for the presence of selected antibiotics and antiretroviral drugs. The samples were prepared for transport to Finland in the University of Zambia laboratory. Final analyses were performed in the University of Jyväskylä laboratory using a liquid chromatography tandem mass spectrometer LC-MS/MS. The highest detected pharmaceutical concentration in groundwater samples was nevirapine (151 ng/L), the highest detected pharmaceutical concentration in wastewater samples was lamivudine (3170 ng/L), the highest detected pharmaceutical concentration in surface water samples was erythromycin (1021 ng/L) and the highest detected pharmaceutical concentration in urine samples was lamivudine (48 211.5 ng/L). Based on the results of the analysis, information was obtained on the presence of drugs in the Lusaka area and on the possibility and attitude to using urine separated from dry toilets as fertilizer for non-edible crops. The survey analysis provided a picture of the sanitation situation across Zambia and information on attitudes towards the use of urine as fertilizer. Of the respondents, 69% were interested in using urine as fertilizer.

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Hakusanat: antibiootit, antiretroviraalit, lannoite, LC-MS/MS, sanitaatio

Tämä tutkimus keskittyy valittujen antibiottien ja antiretroviraalien esiintymiseen Lusakan alueella ja yleiseen sanitaatiotilanteeseen sekä asenteeseen virtsan käytöstä lannoitteena Sambian maaseutualueilla. Näytteitä otettiin alueen pohjavedestä, jäte- ja pintavedestä sekä kuivakäymälästä erotellussa virtsasta. Näytteenoton tutkimusalueena oli Lusakan laitamilla sijaitseva Madimban alue, Machiachin ja Chungan jätevedenpuhdistamot sekä Chungan jätevedenpuhdistamon läheisyydessä sijaitseva Chunga joki. Sanitaatio tilannetta sekä asennetta virtsan käyttöön lannoitteena Sambian maaseutualueilla analysointiin Suomen Global Dry Toilet Associationin tekemän kyselytutkimuksen perusteella. Näytteenotto tutkimus suoritettiin keräämällä kuusi virtsanäytettä kuivakäymälästä erotetusta virtsasta, kaksi pohjavesinäytettä Madimban alueelta, kolme jätevesinäytettä ja yksi pohjavesinäyte Manchinchin jätevedenpuhdistamolta, kaksi pintavesinäytettä ja kolme jätevesinäytettä Chungan jätevedenpuhdistamon alueelta. Näytteistä analysoitiin valittujen antibioottien ja antiretroviraalisten lääkkeiden esiintyminen. Näytteet valmisteltiin Suomeen kuljetettaviksi University of Zambian laboratoriossa. Lopulliset analyysit tehtiin Jyväskylän yliopiston laboratoriossa nestekromatografisella tandemmassaspektrometrillä LC-MS/MS. Korkein havaittu lääkeainepitoisuus pohjavesinäytteissä oli nevirapiinia (151 ng/L), korkein havaittu lääkeainepitoisuus jätevesinäytteissä oli sulfametoksatsolia (3170 ng/L), korkein havaittu lääkeainepitoisuus pintavesinäytteissä oli lamivudiinia (1021 ng/L) ja korkein havaittu lääkeainepitoisuus virtsanäytteissä oli tetrasykliiniä (48 221.5 ng/L). Analyysin tulosten perusteella saatiin tietoa lääkeaineiden esiintymisestä Lusakan alueella ja mahdollisuudesta sekä asenteesta käyttää kuivakäymälästä erotettua virtsaa syötäväksi kelpaamattomien viljelykasvien lannoitteena. Kyselyanalyysin perusteella saatiin kuvaa sanitaatio tilanteesta eri puolilla Sambiaa sekä tietoa asenteesta virtsan käyttöön lannoitteena. Kyselyyn vastanneista 69 % oli kiinnostunut virtsan käyttämisestä lannoitteena.

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# ABBREVIATIONS

| | |
|---|---|
| **3TC** | Lamivudine |
| **ABC** | Abacavir |
| **ACL** | Acyclovir |
| **AIDS** | Acquired immune deficiency syndrome |
| **ARB** | Antibiotic resistance bacteria |
| **ART** | Antiretroviral therapy |
| **ARVD** | Antiretroviral drug |
| **AMX** | Amoxicillin |
| **CIP** | Ciprofloxacin |
| **GDTF** | Global Dry Toilet Association of Finland |
| **EFV** | Efavirenz |
| **ETC** | Erythromycin |
| **HLB** | Hydrophilic lipophilic balance |
| **HIV** | Human immunodeficiency virus |
| **Kd** | Sorption coefficient |
| **Kow** | Octanol-water partition coefficient |
| **LC-MS/MS** | Liquid-chromatography-tandem-mass-spectrometry |
| **LOD** | Limit of detection |
| **LWSC** | Lusaka Water and Sewerage Company |
| **NECOS** | Network of Environmental Concerns and Solutions |
| **NGO** | Non-governmental Organization |
| **NVP** | Nevirapine |
| **OFL** | Ofloxacin |
| **OTL** | Oxytetracycline |
| **PPCPs** | Pharmaceuticals and Personal Care Products |
| **SDZ** | Sulfadiazine |
| **SDX** | Sulfadoxine |
| **SMP** | Sulfamethoxypyrazine |
| **SMX** | Sulfamethoxazole |
| **SMZ** | Sulfamethazine |
| **SPE** | Solid-phase extraction |
| **RAL** | Raltegravir |
| **RFP** | Rifampicin |
| **TAF** | Tenofovir alafenamide |
| **TDF** | Tenofovir Disoproxil umarate |
| **TET** | Tetracycline |
| **TMP** | Trimethoprim |
| **VAL** | Valacyclovir |
| **WWTP** | Wastewater treatment plant |
| **ZDV** | Zidovudine |
| **XTC** | Lamivudine or Emtricitabine |

# 1 INTRODUCTION

Most of the used pharmaceuticals end up in the environment with urine (Malmqvist et al. 2023). In this master's thesis, the presence of selected antibiotics and antiretrovirals in the Lusaka area was particularly investigated. Active pharmaceutical components, especially antibiotics, are micropollutants that can promote antibiotic resistance in the environment and cause toxicity in sensitive species (Tuvo et al. 2023). The consumption of antibiotics has increased significantly around the world (Klein et al., 2018). Reasons for increased consumption include increased availability, especially over-the-counter prescriptions, and increased resistance of pathogenic bacteria to available antimicrobial agents and commonly used antibiotics are no longer as effective (Gelband et al. 2015; Klein et al., 2018; Van Boeckel et al. 2014). Antibiotic use is high in Zambia due to its easy availability and the prevalence of HIV (Zambia Demographic and Health Survey 2018).

The definition of the term "pharmaceutical" varies in the official laws and regulations of different countries. In this text, the term "pharmaceutical" is defined according to the WHO definition:

> Pharmaceutical products - also known as medicines or drugs - are special preparations used in modern and traditional medicine. They are essential for the prevention and treatment of diseases, and protection of public health (WHO 2024).

Worldwide, we are constantly more aware of the effects and harms of pharmaceuticals on the environment (Sammut Bartolo et al. 2020). In order to understand the real effects of pharmaceuticals on the environment, we must understand and take into account the entire life cycle of the pharmaceutical, which starts with its manufacture and ends with its disposal (Figure 1) (Riikonen et al. 2024). Emissions into the environment are already generated during the manufacture of the pharmaceuticals (Riikonen et al. 2024). During manufacturing, pharmaceuticals can get into wastewater systems (Peake 2015). Finished pharmaceuticals are transported for use in e.g. to hospitals and health centres (Peake 2015). In addition, it is possible to buy pharmaceuticals over-the-counter from pharmacies, supermarkets, gas stations and grocery stores (Peake 2015). The pharmaceuticals used can be taken either as a tablet or as a suspension (Peake 2015). Taken pharmaceuticals are excreted from the body with urine and faeces either in their unchanged (parent) form or in their metabolic form (Garza et al. 2023). Finally, the pharmaceuticals into the wastewater system (Massima Mouele et al. 2021).

Most of the time, not all purchased pharmaceuticals are used. Expired and unused pharmaceuticals that are stored indefinitely are usually disposed of by pouring them down the toilet or sink (Kümmerer 2009). Pharmaceuticals disposed of in this way enter the wastewater in their original form (Peake 2015).

Another way to dispose of unwanted pharmaceuticals is to collect them in a waste container if they are transported to be buried in a landfill or burned at a waste incineration plant (Peake 2015). Pharmaceuticals buried in a landfill can seep into the soil and eventually reach groundwater (Kümmerer 2009). The leachate in the groundwater can potentially enter the drinking water reservoirs and contaminate the drinking water reserves (Peake 2015). Pharmaceuticals burned in a properly equipped incineration plant are oxidized into completely harmless compounds, such as carbon dioxide and water, which are released (Peake 2015).

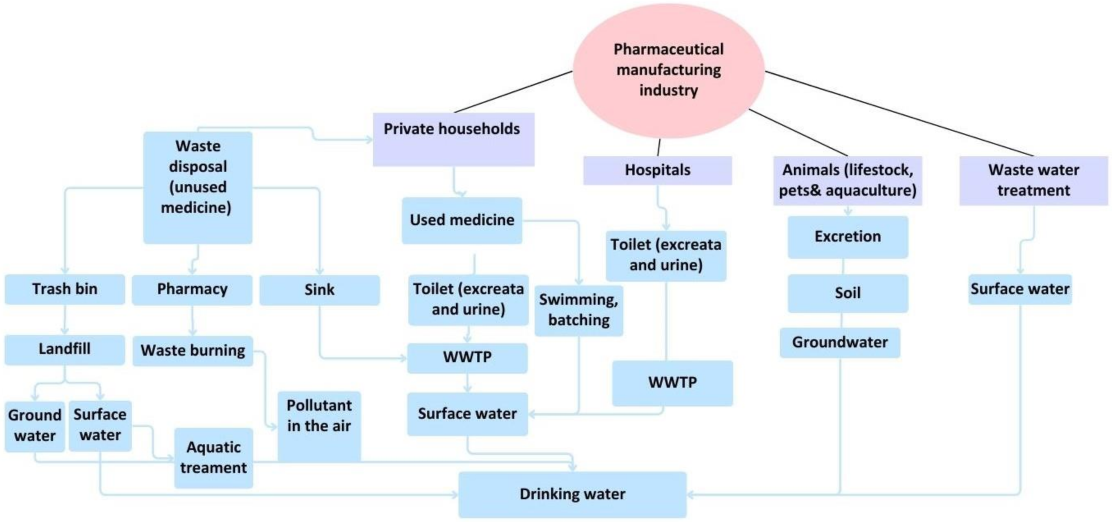

Figure 1. The figure shows the movement of pharmaceutical substances from the factory to drinking water. The image was made with the Canva.com using the sources presented in the paragraph.

## 1.1 Presence of antibiotics and antiretrovirals in surface water and wastewater in Africa

As background information for the study, this chapter presents other studies on the presence of antibiotics and antiretrovirals in surface water and wastewater. Pharmaceuticals in surface water studies have done in Lake Victoria Uganda, Pretoria South Africa, Juja Kenya, Mitheu River Kenya, Nairobi Kenya and around South Africa. Pharmaceuticals in wastewater carried out in Nairobi Kenya, Yaoundé Cameroon, and Pretoria South Africa (Table 1).

Lake Victoria samples were collected in Murchison Bay, Waiya Bay, Napoleon Gulf and Thurston Bay (Nantaba et. all. 2020). Samples were collected in the months July to September 2018 (Nantaba et. all. 2020). Pretoria, South Africa sampling was conducted on different days from December 2016 to March 2018 at the Daspoort Wastewater Treatment Works (Mhuka et al. 2020). Juja town

Kenya is a peri-urban area, about 30 km North of Nairobi, Kenya (Muriuki et. all, 2020). Sampling was done during the dry month of August 2019 (Muriuki et. all, 2020). Mitheu River, Kenya sampling was done in January 2019, which is usually a dry month preceding short rains (Kairigo et al. 2020). Nairobi, Kenya samples were collected in three rivers in the Nairobi River Basin, along the Athi River, and from the WWTP effluents (Ngumba, et al. 2016). Samples were collected in October 2014 during the dry season (Ngumba et al. 2016). South Africa samples have been collected from different parts of South Africa (Wood et al. 2015). The samples were collected in the winter of 2013 and in the summers of 2011 and 2014 (Wood et al. 2015). Cameroon sample was collected on the 11th of May 2015 in waste waters of the University Hospital Center of Yaoundé (UHCY) (Tembe-Fokunang 2018).

TABLE 1. The occurrence of antibiotics and antiretrovirals in surface water and wastewater in relevant studies in different parts of Africa (ng/L)

n.a = not applicable

| Location | Sample type | 3TC | TMP | ZDV | OFL | CIP | SMX | NVP | Year | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Lake Viktoria, Uganda | Surface water | n.a | 89 | n.a | n.a | 41 | 5600 | n.a | 2018 | (Nantaba et. all. 2020) |
| Pretoria, South Africa | Surface water | 9 | 115 | n.a | 5 | nd | 7 | 237 | 2016–2018 | (Mhuka, et al. 2020) |
| Juja, Kenya | Surface water | 70300 | 7200 | 8900 | n.a | 75200 | nd | nd | 2018 | (Muriuki et. all, 2020) |
| Mitheu River, Kenya | Surface water | n.a | 200 | n.a | n.a | 1300 | 497 | nr | 2019 | (Kairigo, et al. 2020) |
| South-Africa | Surface water | 200 | n.a | 1000 | n.a | n.a | n.a | 1500 | 2011–2014 | (Wood, et al. 2015) |
| Nairobi, Kenya | Surface water | 5428 | 2650 | 7684 | n.a | 509 | 13765 | 4859 | 2014 | (Ngumba, et al. 2016) |
| Naironi, Kenya | WWTP, influent | 3985 | 66 | 513 | n.a | 67 | 3336 | 1357 | 2014 | (Ngumba, et al. 2016) |
| Yaoundé, Cameroon | Waste water | n.a | 265 | n.a | n.a | 24000 | 162 | n.a | 2015 | (Tembe-Fokunang, 2018) |
| Pretoria, South Africa | WWTP, influent | 1001 | 578 | n.a | 68 | 77 | 2405 | 26 | 2016–2018 | (Mhuka, et al. 2020) |
| Pretoria, South Africa | WWTP, effluent | 323 | 137 | n.a | 87 | 6 | 81 | 81 | 2016–2018 | (Mhuka, et al. 2020) |

nd= not detected; n.a = not applicable

The highest concentrations of pharmaceuticals in surface water samples were found compared to the results in wastewater samples. The highest concentration of pharmaceuticals found in surface water samples was lamivudine in Juja, Kenya (70,300 ng/L). The highest concentration found in wastewater samples was ciprofloxacin in Yaoundé, Cameroon (24,000 ng/L).

## 1.2 Presence of antibiotics and antiretrovirals in surface water and wastewater in other parts of word

This chapter presents the results of other similar studies on the presence of antibiotics and antiretrovirals in other parts of the world (Table 2). Lake Honghu samples were collected connecting river in November 2015 and May 2016 (Wang et al. 2017). Nansi Lake is located in southwestern Shandong Province and is divided into upper and lower lakes (Zhang et al. 2020). Nansi Lake connecting rivers were sampled in May 2017 (Zhang et al. 2020). Laizhou Bay included samples from the Yellow River, Guangli River, Zimai River, Xiaoqing River, Bailang River, Dihe River, Yuhe River, Weihe River, Jiaolai River and the Dajiawa drainage system (Zhang et al. 2012). Rivers were sampled in September 2009 (Zhang et al. 2012). The River Arno was sampled at Rignano sull'Arno (Zuccato et al. 2010). River was sampled in various periods in 2007 and 2008 (Zuccato et al. 2010). Lugano Switzerland and Milan, Italy wastewater treatment plants were sampled in various periods in 2007 and 2008 (Zuccato et al. 2010). The Seine was sampled at Paris conurbation and in Paris downtown (Nantaba et al. 2020). The river was sampled in December 2009 (Nantaba et. al. 2020). Charmoise river was sampled in 2009 (Dinh et al. 2011). Chao Phraya wastewater treatment plant of Bangkok was sampled in various periods in June and September 2011 and January 2012 (Tewari et al. 2013).

TABLE 2. Comparison of the results with other similar studies in Europe and Asia (ng/L).

| Location | Sample type | TMP | ZDV | OFL | CIP | SMX | NVP | Year | References |
|---|---|---|---|---|---|---|---|---|---|
| Lake Honghu, China | Lake | n.a | n.a | 69 | 33 | 169 | n.a | 2014 | (Wang , et al. 2017) |
| Laizhou Bay, China | River | 13,600 | 513 | 45 | 346 | 527 | n.a | 2009 | (Zhang et al. 2012) |
| Nansi Lake, China | River | 0.5 | n.a | 11 | 0.5 | 62 | n.a | 2017 | (Zhang et al., 2020) |
| River Arno, Italy | River | n.a | n.a | 5 | 19 | 5 | n.a | 2007-2008 | (Zuccato et al. (2010). |
| Seine, France | River | BDL | n.a | 18 | 17 | 18 | n.a | 2009 | (Nantaba et. all. 2020) |
| Charmoise, France | River | BDL | n.a | 4 | 4 | 6 | n.a | 2009 | (Dinh et al., 2011) |
| Thailand | Influent | 221 | n.a | n.a | 382 | 31 | n.a | 2011-2012 | Tewari et al. 2013) |
| Thailand | Effluent | 25 | n.a | n.a | 231 | 89 | n.a | 2011-2012 | Tewari et al. (2013) |
| Lugano, Switzerland | Influent | n.a | n.a | 0.3 | 5 | 33 | n.a | 2007-2008 | (Zuccato et al. (2010). |
| Lugano, Switzerland | Effluent | n.a | n.a | BDL | BDL | 15 | n.a | 2007-2008 | (Zuccato et al. (2010). |
| Milan, Italy | Influent | n.a | n.a | 8 | 32 | 40 | n.a | 2007-2008 | (Zuccato et al. (2010). |
| Milan, Italy | Effluent | n.a | n.a | 5 | 24 | 16 | n.a | 2007-2008 | (Zuccato et al. (2010). |

n.a = not applicable
BDL = below detection limit

The concentrations in the study results are lower compared to studies conducted in Africa. The concentrations of pharmaceuticals in surface water samples were the highest compared to the results in wastewater samples. The highest concentration of pharmaceuticals found in surface water samples was trimethoprim in Laizhou Bay, China (13,600 ng/L). The highest concentration found in wastewater samples was ciprofloxacin in Thailand (382 ng/L).

## 1.3 Commercialization of urine as fertilizer

Farmers in Sub-Saharan Africa struggle with the availability and high cost of fertilizers (Amankwah et al. 2024). However, the use of human urine as fertilizer is not a common practice in Africa (Simpson-Hebert 2004). Although the use of human urine as fertilizer reduces the need for commercial fertilizers, which in turn reduces environmental pollution (Christofaro 2022). Commercial fertilizers pollute groundwater and significantly contribute to climate change (Christofaro 2022). In addition, for environmental and health reasons, the world is increasingly interested in organic farming methods (Karak and Bhattacharyya 2011). It is also known that traditional intensive farming practices can introduce pollutants into the food chain, which have adverse effects on the environment (Karak and Bhattacharyya 2011). Organic farming offers a more environmentally friendly and sustainable alternative to food production (Karak and Bhattacharyya, 2011).

However, the biggest problem in the profitability of organic farming is the reduction of organic raw materials in composite production (Karak and Bhattacharyya 2011). To this end, researchers are looking at various organic sources that are abundant in nature and available at low or free prices (Karak and Bhattacharyya 2011). One possible alternative to this is human urine, which has gained popularity in organic farming (Karak and Bhattacharyya 2011). The use of urine in agriculture has many possibilities. It can be used as a fertilizer without fear of the spread of antibiotic resistance (Davis 2020). Urine is rich in nutrients, nitrogen and phosphorus, which are essential for plants (Patel et al. 2020). In addition, separating the source of urine can reduce the environmental burden of pharmaceuticals while enhancing nutrient recovery (Davis 2020).

People have known for a long time that their urine is an excellent source of nitrogen, phosphorous and potassium for crops (Yirka 2022). On average, an adult person produces 1.6–1.7 g of phosphorus daily, and about 60% of this phosphorus is excreted in urine (Pathy et al. 2021). Based on world population (7.5 billion) 25 tons of nitrogen per year, 1.8 tons of phosphorus per year with urine every year (Pathy et al. 2021). The recovery of these nutrients could have a market value of 20 billion USD/year (Pathy et al. 2021).

As a definition, human urine is a liquid produced by the kidneys in the metabolic process (Patel et al. 2020). This is known as urinalysis (Ajiboye et al. 2022). Many things affect urine composition, including diet, body size, water intake, environmental conditions, and general health (Mir et al. 2020). On average, the human body excretes approximately 1.3 l (1.0–1.5 l) of urine daily (Larsen et al. 2013). Human urine is composed of a variety of compounds, including potassium, phosphorus, nitrogen, and sodium, as well as creatine, which are many of the same ingredients found in commercial fertilizers (Patel et al. 2020). In addition, urine contains other ions necessary for plant growth, such as Na+, Cu2+, Mg2+ and Cl− (Viskari et al. 2018). Also, human urine contains heavy metals (Viskari et al. 2018). However, the number of heavy metals is lower than in industrial fertilizers and animal urine (Ajiboye et al. 2022). Table 3 shows the average composition of human urine.

TABLE 3. Composition of human urine (Sarigul et al. 2019).

| Composition of human urine | | | |
|---|---|---|---|
| Property and composition | Molar Mass (g/mol) | Normal range in humans | Molarity (mmol/1.5L) |
| Volume | | 0.8-2 L | |
| pH | | 4.5-8.0 | |
| Specific gravity (SG) | | 1.002-1.030 g/ml | |
| Osmolality | | 150-1150 mOsm/kg (>1) | |
| Urea | 60.06 | 10-35 g/d | 249.750 |
| Uric Acids | 168.11 | <750 mg/d (>16) | 1.487 |
| Creatinine | 113.12 | Males: 955-2936 mg/d<br>Females 601-1689 mg/d | 7.791 |
| Citrate | 192.12 | 221-1191 mg/d | 2.450 |
| Sodium | 22.99 | 41-227 mmol/d | 92.625 |
| Potassium | 39.10 | 17-77 mmol/d | 31.333 |
| Ammonium | 18.05 | 15-56 mmol/d | 23.667 |
| Calcium | 40.08 | Males <259 mg/d<br>Females <200 mg/d | 1.663 |
| Magnesium | 24.31 | 51-269 mg/d | 4.389 |
| Chloride | 35.45 | 40-224 mmol/d | 88.000 |
| Oxalate | 88.02 | 0.11-0.46 mmol/d | 0.277 |
| Sulphate | 96.06 | 7-47 mmol/d | 18.000 |
| Phosphate | 94.97 | 20-50 mmol/day | 23.33 |

Human urine contains about 80% of the total nitrogen load and 45% of phosphorus load in wastewater, so separating urine at the source would improve wastewater management (Wilsenach and van Loosdrecht 2003). To make wastewater management more efficient, it is possible that separation of urine sources could significantly improve wastewater quality and save energy use and investment costs for receiving wastewater treatment plants (Wilsenach and Van Loosdrecht 2003). In addition to this, human urine collected in separation systems is easier to use directly as a hygienic fertilizer (Kirchmann and Pettersson 1995). When urine mixes with faeces, this mixture is much more difficult to handle hygienically (Heinonen-Tanski et al. 2007).

When talking about the hygiene of using urine. Urine is not sterile, but urine of a healthy person contains only a small amount of pathogens (Karak and Bhattacharyya 2011). Pathogens such as *Schistosoma haematobium, Salmonella typhi, Salmonella paratyphi and Leptospira interrogans* (Feachem 1983). Urine contamination is mainly due to faecal contamination (Höglund et al. 1998). Therefore, before using urine as a fertilizer, it is recommended to store it for at least 6 months at a temperature above 22 °C (WHO 2006). In a study related to urine storage, it has been shown that *E. coli* or *Salmonella spp*. are not present after 5 days of storage, and pathogens are significantly reduced after 21 days, and no viruses were detected in urine after 50 days of storage (Vinnerås et al. 2003).

> “If you store urine long enough, about a month or so the pH and ammonia concentration will increase to the point that it becomes self-sterilising (Ulrich 2023). So that means that most of the pathogens in the urine will be destroyed, and that urine can safely be used as a nutrient source (Ulrich 2023). All that is needed for this type of transformation is time (Ulrich 2023)”.

Nitrogen loss occurs when urine is stored (Lv et al. 2020). Nitrogen loss during storage can be minimized by minimizing temperature and avoiding aeration above the liquid level in storage tanks (Höglund et al. 1998). However, high pH, high temperature, concentrated form of urine and long storage times are favorable for hygiene reasons (Höglund et al. 1998). The risks of using urine as a fertilizer are related to the pharmaceutical residues it contains due to their ecotoxicological potential and relatively high concentrations (Patel 2020). Pharmaceutical residues in different urine samples vary widely from absolute zero to several hundreds of micrograms per liter (Winker et al. 2008) and concentrations can even exceed a few milligrams per liter in medicated patients (Bischel et al. 2015).

In order to safely use urine as fertilizer, it must be separated from the source. Urine can be separated from the source with different types of toilets and urinals (Figure 2), the most used of which are waterless male urinals and urine diverting toilets (Rossi et al. 2009). Dilution of urine occurs slightly when flushing in a bypass toilet (Maurer et al. 2006). EcoSan toilets have become more common in developing countries (WaterAid 2014). The EcoSan toilet is a closed system that does not need water (WaterAid 2014). It enables a toilet in places where there is little water or where the groundwater is high (WaterAid 2014). The toilet is based on the principle of nutrient recovery and excrement recycling to create a valuable resource for agriculture (WaterAid 2014). The entire pit of the EcoSan toilet is closed and sealed (WaterAid 2014).

> “During nine months, the faeces are completely composted into organic manure and can be used on farms. When the first pit of the EcoSan toilet is closed, users can switch to using the second pit (WaterAid 2014)”.

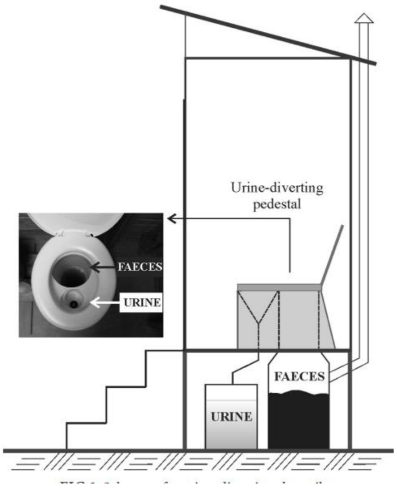

Figure 2. Example of Urine-diverting toilet. (Magri et al. 2013)

In a summary, the use of urine as a fertilizer few things should be considered. Larger application volumes of human urine can increase the salinity and high electrical conductivity of treated soils (Karak and Bhattacharyya 2011). The urine of a healthy person is usually free of pathogens (Karak and Bhattacharyya 2011). When handling urine, it is important to ensure that it does not get mixed with faeces (Karak and Bhattacharyya 2011). When applying urine on plants, it should be noted that urine should not be applied directly to the part of the plant to be repaired and plants must not be fertilized within a month before harvest (Karak and Bhattacharyya 2011). In addition, the spreading of urine should be considered at the right time. Spreading urine at the wrong time or unevenly on the field can cause considerable crop damage (Heinonen-Tanski et al. 2007). From the point of view of hygiene, urine must be stored in a closed space container 6 months before application (WHO 2006). However, if good phosphorus removal is to be ensured, the technology still needs to be improved in terms of quality and the amount of product formed so that it can be used as an economically viable way to recover phosphorus (Le Corre et al. 2009). In addition, hand washing and hand protection when handling urine is important (Karak and Bhattacharyya 2011).

Source-separated urine has been found to be safe and effective fertilizer for many crops and vegetables and has been studied in many different countries. In the (Table 4) below, five studies conducted in Africa related to the use of human urine as fertilizer are briefly presented.

TABLE 4. Studies on the use of urine as a fertilizer for different plants in different parts of Africa.

| Country | Product | Reference |
|---|---|---|
| Burkina Faso | Okra | (Sangare et al. 2014) |
| Ghana | Cabbage | (Amoah et al. 2017) |
| Nigeria | Okra | (Akpan-Idiok et al. 2012) |
| South Africa | Carrot, Maize and Beetroot | (Mnkeni et al. 2008) |
| Zimbabwe | Maize | (Guzha et al. 2005) |

Sangare et al. 2014, studied the use of urine and toilet compost as fertilizer for branch growth in Kamboinsin, Ouagadougou, Burkina Faso in 2013. The study tested seven different treatments: control without fertilizer, chemical fertilizer, urine alone, toilet compost alone, a mixture of 75% urine + 25% toilet compost, 50% urine + 50% toilet compost and 25% urine + 75% toilet compost (Sangare et al. 2014). The study found that the treatment (75% urine + 25% toilet compost) was more suitable in terms of plant height and yield (Sangare et al. 2014). The worst result in the study was obtained with 100% urine treatment compared to other treatments, suggesting possible toxic effects from direct contamination of plants, affecting the final yield. In addition, the study also found that soil chemical properties increased in pH and salinity significantly with urine alone (Sangare et al. 2014). In contrast, applying urine with toilet compost reduced soil acidification and salinization, indicating that applying both urine and toilet compost effectively improves soil chemical properties (Sangare et al. 2014).

Amoah et al. 2017, studied the effectiveness of human urine and other organic matter as fertilizers for growing cabbage in the Dzorwulu district of Accra, the capital of Ghana. The aim of the study was to evaluate the effect of urine fertilizer on cabbage growth, yield, nutrient intake and nitrogen use, efficiency and soil chemical properties (Amoah et al. 2017). In addition, the economic potential of using urine and other organic fertilizers was briefly analyzed (Amoah et al. 2017). Four different treatments were used in the study: urine alone, urine combined with dewatered faecal sludge, urine combined with poultry faeces, and a combination of nitrogen, phosphorus and potassium with poultry faeces (Amoah et al. 2017). The study was also conducted in different seasons in order to take into account the effect of seasons on crop growth (Amoah et al. 2017). The study found no significant differences between treatments in cabbage head weight, total yield and marketable yield (Amoah et al. 2017). However, the combination of nitrogen, phosphorus and potassium and poultry manure resulted in significantly higher non-marketable yields compared to other treatments (Amoah et al. 2017). The time of year had an effect: the focus of cabbage and saleable crops was in the dry rather than the rainy season (Amoah et al. 2017).

Akpan-Idiok et al 2012, studied the use of human urine as a fertilizer for okra cultivation in communities in Cross River State, Nigeria. The study compared the effects of different application rates of urine (10,000, 15,000 and 20,000 l/ha) with conventional nitrogen, phosphorus and potassium inorganic fertilizer in okra cultivation (Akpan-Idiok et al. 2012) 400 kg/ha. The study found that treatment with both urine and inorganic fertilizers significantly increased nutrient uptake by okra plants compared to a control group that received no fertilizer (Akpan-Idiok et al. 2012). A study found that human urine effectively improves nutrient uptake in nutrient-poor soil (Akpan-Idiok et al. 2012). Furthermore, the application of 20,000 l/ha of human urine significantly improved the growth and yield characteristics of okra plants more than standard nitrogen, phosphorus and potassium fertilization (Akpan-Idiok et al. 2012). It was also found that a urine application of 15,000 L/ha produced similar results to inorganic fertilizer (Akpan-Idiok et al. 2012). As a result of the study, it was concluded that human urine, when used in appropriate amounts, can significantly improve the growth and yield of okra, which may provide a cost-effective and sustainable alternative to chemical fertilizers in areas with poor soil fertility (Akpan-Idiok et al. 2012).

Mnkeni et al. 2008, studied the fertilization potential of human urine compared to urea, focusing on its effects on yield and soil health in carrot, beet and maize cultivation in the Eastern Cape of South Africa in 2004. The aim of the study was to identify the optimal nitrogen application rates for each type (carrot, beet and maize) and to assess possible risks related to soil salinity and sodium accumulation (Mnkeni et al. 2008). The study was conducted in a controlled tunnel house environment (Mnkeni et al. 2008). The results of the study showed that the dry matter yield of both corn and tomato increased with higher amounts of nitrogen, up to 200 kg Nha–1 (Mnkeni et al. 2008). The yield increases above this Leveled rate, which could be the optimal application rate for these crops (Mnkeni et al. 2008). Beetroot further increased the dry matter yield of roots and leaves in urine up to 800 kg N ha–1. Carrot reached peak yield at a much lower rate of 50 kg/ha N ha–1 (Mnkeni et al. 2008). The study found that the application of urine led to an increase in the electrical conductivity of the soil (Mnkeni et al. 2008). In addition, leaf tissue sodium concentrations in both maize and tomatoes increased with increasing urine application rates, suggesting possible sodium accumulation in crops (Mnkeni et al. 2008). The study showed that human urine is still an effective source of nitrogen for various crops (Mnkeni et al. 2008). When using urine, however, the amount of use should be taken into account, excessive use of urine can cause soil salinization and sodium accumulation (Mnkeni et al. 2008).

Guzha et al. 2005, studied the use of human urine as a fertilizer for maize production and water productivity in 2003-2004 in Marondera District, Zimbabwe. The study involved six volunteer farmers with four 100 $m^2$ plots, each with a different treatment: control, commercial fertilizer treatment, urine plot alone, and face and urine plot (Guzha et al. 2005). As a result of the study, it was found that the use of human nutrition together with organic fertilizers significantly improved the water productivity in the production of rain maize (Guzha et al. 2005). In particular, the treatment increased water productivity by more than 10%, meaning more maize yield was obtained per unit of water used (Guzha et al. 2005). This result is particularly significant in areas where water scarcity and efficiency are concerns for agricultural sustainability (Guzha et al. 2005). Furthermore, research shows that the use of human urine in combination with organic fertilizer can improve both maize yield and water productivity in challenging agricultural environments (Guzha et al. 2005).

## 1.4 Objectives and purpose of the research

The aim of this master thesis is to produce information on the presence of selected antibiotics and antiretrovirals in groundwater, surface water, wastewater and urine separated from dry toilet in Lusaka, Zambia. As well as considering the possibilities of using the urine separated from the dry toilet as fertilizer. According to a survey by the Global Toilet Association of Finland, interest in using urine as fertilizer can be found in various parts of Zambia. One of the problems with the safe use of urine is the presence of pharmaceutical substances, so urine samples are examined for the concentrations of antibiotics and antiretrovirals. Antibiotics and antiretrovirals were chosen as a pharmaceutical to be investigated because of their wide use and prevalence in Zambia. This research is done together with the Finnish Global Dry Toilet Association (Huussi ry), NECOS and the University of Jyväskylä in the academic year 2022–2023 in Zambia. The research of this work was part of a long-term collaboration between different activities and has included several thesis and projects that aim to develop sanitation and identify and solve its problems, as well as develop risk reduction techniques and increase awareness.

The research questions are:

1. What are the pharmaceutical concentrations in the wastewater, surface water and groundwater in Lusaka area?
2. What are the pharmaceutical concentrations in the urine separated from the source?
3. What is the attitude towards using urine as fertilizer?

## 1.5 Research methods

The focus of the research was the presence of selected pharmaceuticals antibiotics and antiretrovirals, in wastewater, surface water, groundwater and urine, and the case study is limited only to the Lusaka region and especially to the peri-urban area of Madimba. The research was conducted as both qualitative and quantitative research. The qualitative research includes a literature review on the presence of pharmaceuticals in the environment and the use of urine as fertilizer. In addition, different studies on the presence of pharmaceutical substances in urine and water bodies, and use of urine as a fertilizer used for the literature review. Qualitative research includes questions about people's attitudes and opinions on the use of urine as fertilizer. The survey conducted by the Global Dry Toilet Association of Finland is used as a source for this qualitative study. The analysis of the survey is part of this master's thesis work.

The quantitative research included taking samples and analysing the concentrations of selected pharmaceutical substances from them. Samples are from surface and wastewater and source-separated urine. Groundwater samples were taken from the study area in Madimba and a private person's well near the Chunga wastewater treatment plant, surface water samples were taken from the Chunga wastewater treatment plant stream, wastewater samples from the Manchinchi wastewater treatment plant and Chunga wastewater treatment plant and source-separated urine samples from the Madimba area.

The results of the quantitative study are compared to the 2018 master thesis of Johanna Myllyniemi Maldonado of the University of Jyväskylä, Presence of selected pharmaceuticals in groundwater, surface water, wastewater and urine separated from the source in the suburbs of Lusaka, Zambia, which investigated the presence of pharmaceuticals in Lusaka.

The study was conducted in Lusaka, Zambia between September 2022 and March 2023. It included several field visits to the project areas, analysing samples at the University of Zambia and University of Jyväskylä laboratories, participating in the Sanitation Summit meeting, the WTA meeting, and interviewing locals and those working in the sanitation sector, as well as communicating with supervisors at the Finnish Global Dry Toilet association of Finland and at the University of Jyväskylä.

## 1.6 Significance of the study

More specifically, the result of the study reports the presence of selected pharmaceutical substances in the urine used as fertilizer. Based on which, it can be concluded whether the use of urine separated from toilets as fertilizer is safe. The information obtained from the study may help people in the area to consider using urine as a fertilizer in Madimba peri-urban area. In addition, the study complements other studies on the presence of pharmaceutical substances in groundwater, surface water and wastewater in especially in Africa but also in other countries.

## 1.7 Zambia as a reseach context

The chapter talks about Zambia in general and the issues related to doing this research, i.e. sanitation in Zambia, HIV in Zambia and introduces the project peri-urban area Madimba in Lusaka, Zambia. Zambia is a landlocked country (Figure 3) in Southern Africa with a population of 18.9 million (Hobson 2024). Zambia's population is growing rapidly by up to 2.9% per year (Hobson 2024). The rapid population is believed to cause pressure in the number of jobs and in the health care sector (Hobson 2024).

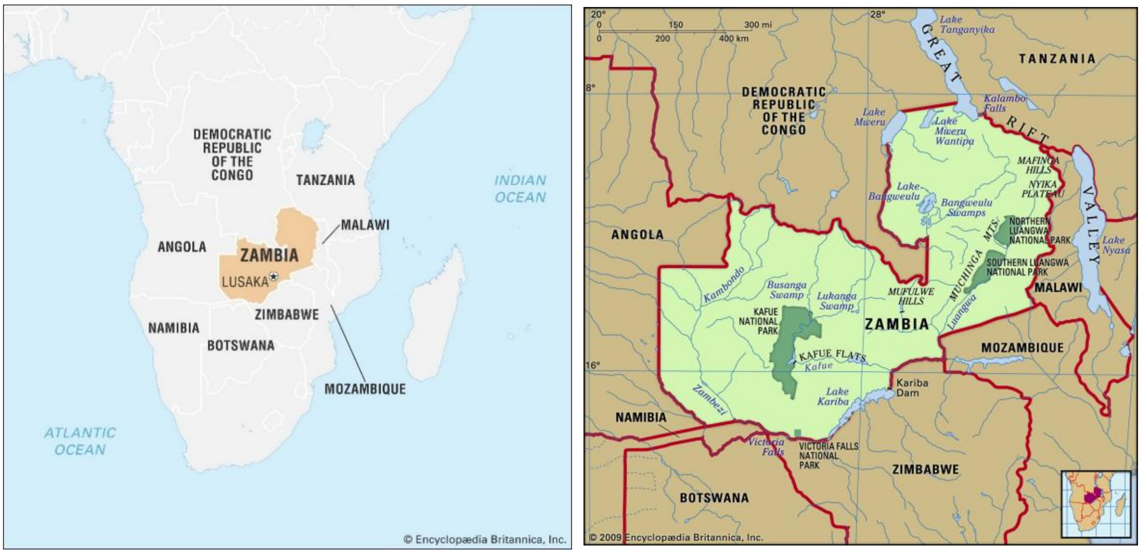

Figure 3. Location of Zambia and its capital city Lusaka (Hobson 2024).

### 1.7.1 Sanitation in Zambia

The information on the presence of sanitation in Zambia is based on the Demographic and Health Survey conducted in 2018 in this study. Zambia Statistics Agency (Formerly Central Statistical Office), Ministry of Health, University Teaching Hospital Virology Laboratory and The DHS Program ICF have participated in the research. Demographic and health surveys have also been conducted in 1992, 1996, 2001–02, 2007 and 2013–14. The study will also be commissioned in 2023-2024.

When talking about sanitation, must understand different toilet facilities terms. Improved toilet facilities are toilets that flush water and waste into a piped sewer system, septic tank, pit latrine, or an unknown destination (Zambia Demographic and Health Survey 2018). Improved Sanitation facilities include flush/pour, ventilated improved pit (VIP) latrine, pit latrine with slab and composting toilet (Zambia Demographic and Health Survey 2018). Basic Sanitation services are improved facilities that are private and not shared with other households (Zambia Demographic and Health Survey 2018). Limited Sanitation services are improved facilities but shared by other households (Zambia Demographic and Health Survey 2018). Unimproved Sanitation facilities include flush/pour flush not to sewer/septic tank/pit latrine, pit latrine without slab/open pit and hanging toilet/hanging latrine (Zambia Demographic and Health Survey 2018). Definition of open defecation is the disposal of human faeces in fields, forests, bushes, open bodies of water, beaches, or other open spaces (Zambia Demographic and Health Survey 2018).

Based on the research, 54.4% of households have access to improved sanitation facility, 77.7% in urban areas and 37.2% in rural areas (Zambia Demographic and Health Survey 2018). The most common sanitation facility in urban areas and rural areas is pit latrine with slab (Zambia Demographic and Health Survey 2018). Based on the study, 35.6% of households have access to unimproved sanitation services, 20.8% in urban areas and 46.5% in rural areas (Zambia Population and Health Survey 2018). The most common sanitary facility in cities and rural areas is a pit toilet without tiles/pits (Zambia Demographic and Health Survey 2018). About 10.0% of households still defecate openly (1.4% in urban areas and 16.2% in rural areas) (Zambia Population and Health Survey 2018).

By province, improved Sanitation facility is most common in Lusaka (80.0%) and Copperbelt (76.7%) (Zambia Demographic and Health Survey 2018). Considerably the worst access to improved sanitation services are people who live in the western province, only 6.2% have access to improved sanitation services (Zambia Demographic and Health Survey 2018). The most common unimproved sanitation facility is North Western (49.2%) and Luapula (46.8%) (Zambia Demographic and Health Survey 2018). Open defecation is most common in Western (50.0%) and Southern (20.4%) (Zambia Demographic and Health Survey 2018).

When sanitation is distributed based on wealth, the lowest class 25.8% use improved sanitation facility, 48.1% use unimproved sanitation facility and 26.1% use open defecation (Zambia Demographic and Health Survey 2018). Second class 35.3% use improved sanitation facility, 51.4% use unimproved sanitation facility and 13.3% use open defecation (Zambia Demographic and Health Survey 2018). Middle class 42.1% use improved sanitation facility 49.5% unimproved sanitation facility and 8.4% use open defecation (Zambia Demographic and Health Survey 2018). Fourth class 72.7% use improved sanitation facility, 26.2% use unimproved sanitation facility and 1.2% use open defecation (Zambia Demographic and Health Survey 2018). Highest class 94.3% use improved sanitation facility, 5.6% use unimproved sanitation facility and 0.1% use open defecation (Zambia Demographic and Health Survey 2018).

### 1.7.2 Water supply in Zambia

The information on the presence of water supply in Zambia is based on the Demographic and Health Survey conducted in 2018 In the study, water sources were divided into two groups: improved water source and unimproved source (Zambia Demographic and Health Survey 2018).

> "Improved sources are piped into dwelling/yard/plot, piped to neighbour, public tap/standpipe, tube well or borehole, protected dug well, protected spring, rainwater, tanker truck/cart with small tank and bottled water (Zambia Demographic and Health Survey 2018)".

Unimproved sources are unprotected dug well, unprotected spring and surface water (Zambia Demographic and Health Survey 2018).

A total of 12,831 households responded to the survey, of which 5,441 were in the urban area and 7,390 were in the rural area (Zambia Demographic and Health Survey 2018). Based on the research, 72.3% of households use an improved water drinking source as their water source (Zambia Demographic and Health Survey 2018). Improved water drinking sources are more common in urban (91.8%) than rural areas (58.0%) (Zambia Demographic and Health Survey 2018). The most common waster source in urban areas is piped into dwelling/yard/plot and in rural areas is tube well or borehole (Zambia Demographic and Health Survey 2018).

### 1.7.3 HIV in Zambia

As a term, HIV is the human immunodeficiency virus (WHO 2023). The HIV virus has two main subtypes: HIV-1 and HIV-2 (Whiteside 2008). HIV-1 is the most common type of HIV and causes most infections, while HIV-2 is relatively rare and less contagious (Whiteside 2008). It is estimated that approximately 40 million people in Africa are estimated to be living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) (WHO 2023). The virus is most transmitted through sexual contact (CDC 2024). The next most important transmission route is mother-to-child transmission, where children are exposed to the virus during birth or breastfeeding (Whiteside 2008). It is also possible to get infected with blood or blood products (CDC 2024).

The information on the presence of HIV in Zambia is based on the Demographic and Health Survey conducted in 2018. As part of the study, the respondents were given HIV tests (Zambia Demographic and Health Survey 2018). Two methods were used for the testing: rapid diagnostic testing and collection of dried blood spot samples (Zambia Demographic and Health Survey 2018). A total of 23,767 people participated in the testing, of which 12,817 were women and 12,817 were men. The women were aged 15-49, the men 15-59 (Zambia Demographic and Health Survey 2018).

All in all, 11.1% of the people who took part in the survey were HIV positive (Zambia Demographic and Health Survey 2018). Of the women who took part in the survey, 14.2% were HIV positive, the most infections were in the 45–49 age group (Zambia Demographic and Health Survey 2018). Of the men who took part in the survey, 7.5% were HIV positive, the age group 50–59 was the most infected (Zambia Demographic and Health Survey 2018). The lowest number of infections occurs in the age group of 15–19 years, 2.6% of women and 1.2% of men are HIV positive (Zambia Demographic and Health Survey 2018).

Based on the study, the prevalence of HIV is twice as high in urban areas as in rural areas (15.9% vs. 7.1%) (Zambia Demographic and Health Survey 2018). HIV prevalence is highest in the Copperbelt (15.4%) and Lusaka (15.1%) (Zambia Demographic and Health Survey 2018). The least infections occur (Figure 4) in Muchinga (5.4%) and Northern (5.6%) (Zambia Demographic and Health Survey 2018). Infections occur throughout Zambia. (Zambia Demographic and Health Survey 2018).

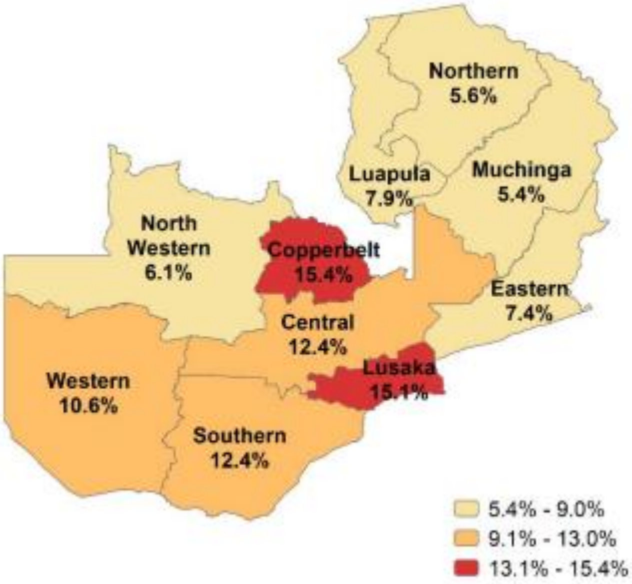

Figure 4. HIV prevalence in percentages across Zambia in 2018 (Zambia Demographic and Health Survey, 2018.)

### 1.7.4 Government of Zambia guidelines for Treatment and Prevention of HIV

There are approximately 1.3 million HIV/AIDS patients in Zambia, of which an estimated 89-98% are receiving antiretroviral therapy (UNAIDS 2023). In 2020, Government of Zambia published Zambian Consolidated Guidelines (Table 5) for the prevention and treatment of HIV infection (ZCG 2022). These guidelines contribute to efforts to reduce new HIV infections and HIV deaths in Zambia (ZCG 2022). The intervention and clinical practice guided by these guidelines will drive Zambia to fight the HIV epidemic (ZCG 2022). According to the guidelines, the primary ART for adults in Zambia to treat the HIV virus are tenofovir disoproxil fumarate, lamivudine or emtricitabine and dolutegravir (ZCG 2022). For pregnant and breastfeeding mothers, the primary pharmaceuticals for HIV treatment are TDF (tenofovir disoproxil fumarate) + XTC (lamivudine or emtricitabine) + DTG (dolutegravir) (ZCG 2022). The guidelines outline the use of antibiotics. The primary antibiotics to be used would be rifampicin, rifabutin and bedaquiline (ZCG 2022).

TABLE 5. Guidelines for the use of antiretrovirals in HIV medication in Zambia from the year 2020 issued by the Zambian government (ZCG 2022).

| Group | | 1 st ART | Alternative ART |
|---|---|---|---|
| Pregnant & Breastfeeding women | All | TDF + XTC (Lamivudine or Emtricitabine)+ DTG | TDF + XTC + EFV<br>ABC + 3TC+ DTG |
| Children (0-2 weeks) | All | ZDV + 3TC + NVP | AZT + 3TC+ RAL |
| Children (2 weeks to < 5 years old) | < 20 kg | ABC + 3TC+LPV | ZDV + 3TC+ LPV<br>ZDV + 3TC+ RAL |
| | 20 – 24.9 kg | ABC + 3TC + DTG | ZDV + 3TC+ RAL<br>ABC + 3TC+ RAL |
| | 25 kg | TAF + 3TC + DTG | ABC + 3TC+ DTG |
| | 30 kg | TAF + 3TC + DTG | TDF + 3TC+ DTG |
| Children co-infected with TB | < 20 kg | ABC + 3TC +RAL OR ABC + 3TC+AZT | ZDV + 3TC+ EFV |
| | 20-29.9 kg | ABC + 3TC + DTG | ABC+3TC+LPV<br>ABC+3TC+EFV<br>ABC+3TC+RAL |
| | 30 kg | TDF + 3TC + DTG | BDL |
| Adolescents (10 to <19 years old) weighing > 30 kg<br>Adults | All | TDF (or TAF) + XTC + DTC | TDF (or TAF) + XTC + EFV<br>ABC + 3TC + DTG |

## 1.8 Global Dry Toilet Association of Finland

The master's thesis was done together with The Global Dry Toilet Association of Finland, which is a non-governmental organization that has been in operation for 20 years. The Global Dry Toilet Association of Finland's main office was located in Tampere, but there are projects improving sanitation all over Africa, for example in Ghana, Zambia & Tanzania (GDTF 2022a). The organization's goal is to improve sanitation around the world and make dry toilets a central part of sustainable development (GDTF 2022a). In addition, the association's activities include sharing information about good sanitation and especially dry toilets (GDTF 2022a).

The association has been improving sanitation in various parts of Zambia and solving sanitation-related problems since 2006 (GDTF 2022b). The key goal is for Zambia to achieve 100% sanitation coverage by 2030 (GDTF 2022b). Now, only about 26% of Zambians have access to basic sanitation (GDTF 2022b). The project is carried out together with the local associations Green Living moment (GLM), Network for Environmental Concerns and Solutions (NECOS), Livingstone Green Iniative (LGI) and UIP (GDTF 2022b).

## 1.9 Madimba

One of the Global Dry Toilet of Finland project areas and the research area of this master's thesis is Madimba, where a sanitation improvement project was started in 2008 (Kawanga and Piirilä 2015). The area is a 1.2 square kilometre area about 10 km west of the centre of Lusaka (Figure 5). More than 3,000 people in more than 500 households live in Madimba, 53% of the area's population are women and 47% are men (Kawanga and Piirilä 2015).

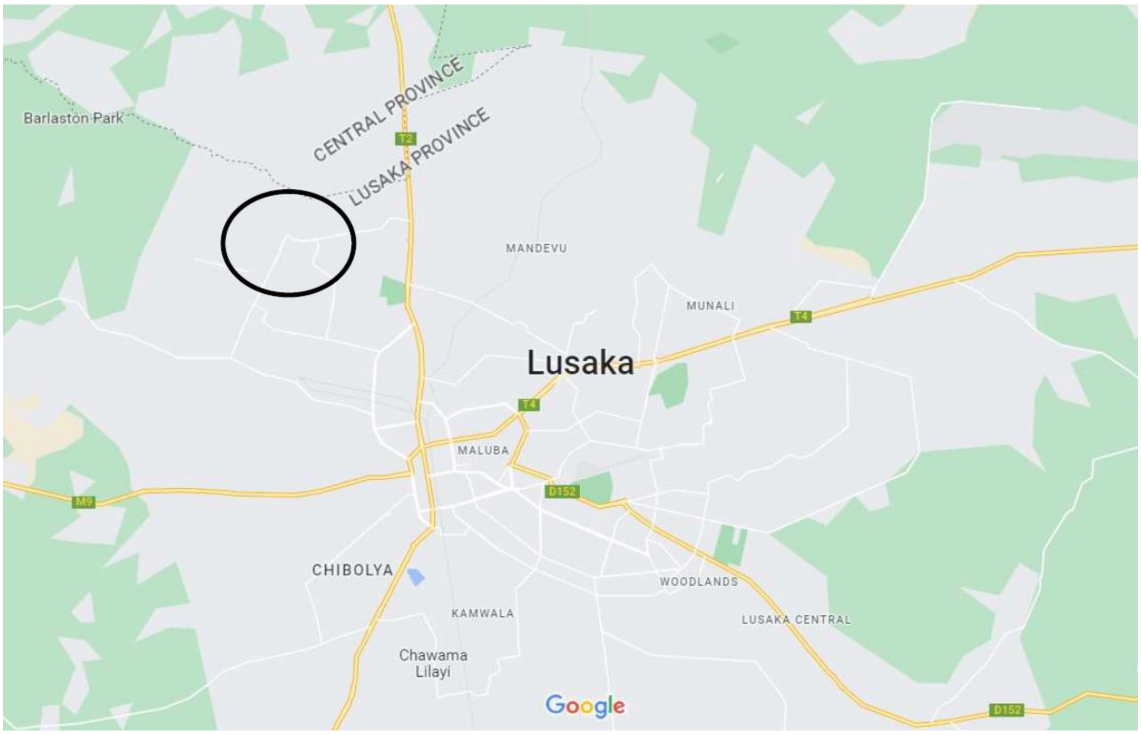

Figure 5. Study area location of Madimba in Lusaka District. Made with Google Maps 2024.

There is no safe drinking water or sewage system in the Madimba area (Kawanga and Piirilä 2015). There is no drainage in the area, as a result of which during the rainy season, water irrigates yards and roads (Kawanga and Piirilä 2015). Another challenge in the area is that the groundwater level is close to the surface, which leads to a situation where pit latrines easily contaminate groundwater (Kawanga and Piirilä 2015). Poor sanitation is the main cause of human infection in the peri-urban areas of Zambia, and Madimba is no exception (Kawanga and Piirilä 2015). However, sanitation in the Madimba area has been improved. The following chapter presents dry toilet projects carried out in the Madimba area.

### 1.9.1 Projects to improve sanitation in Madimba

The Global Dry Toilet Association of Finland and the Zambian NGO Network for Environmental Concerns and Solutions have worked together to improve sanitation in Zambia and Madimba since 2008 (Kawanga and Piirilä 2015). Zambia is one of the countries where the lack of adequate sanitation services or clean and safe water is worrying (Kawanga and Piirilä 2015). Investments made by the Zambian government have mainly focused on the water sector, leaving less attention to sanitation (Kawanga and Piirilä 2015). Despite the fact that many Zambians lack adequate sanitation (Kawanga and Piirilä 2015). Insufficient sanitation can cause e.g. health problems, loss of income, difficulties and humility (Kawanga and Piirilä 2015).

Pit latrines, bush facilities, and "flying toilets" (plastic bags used for defecation and thrown into the environment) are the most common sanitation facilities in peri-urban areas, like Madimba area (Kawanga and Piirilä 2015). These sanitary facilities often have a number of problems, such as odours, collapse and filling of pit toilets (Kawanga and Piirilä 2015). The aim of the dry sanitation projects carried out in Madimba has been to renew the suburban community of Madimba into an eco-village model, improve the residents' livelihood, provide hygiene education, improve access to clean water and create sustainable sanitation solutions (Kawanga and Piirilä 2015). In addition, pit latrines have been built during the projects, old pit latrines have been repaired and improved to make them safer and more hygienic, and water kiosks have been built to produce clean drinking water (Kawanga and Piirilä 2015).

Not all projects done in Madimba are limited to water and sanitation. In Madimba, waste management has also been developed and backyard gardening has been promoted (Kawanga and Piirilä 2015). The care of the garden has been promoted by growing more vegetables that can withstand the dry season (Kawanga and Piirilä 2015). This increases household food security and improves the nutritional status of households (Kawanga and Piirilä 2015).

A tractor was introduced to improve waste management from households (Kawanga and Piirilä 2015). The tractor enables household solid waste to be transported directly to the city's landfill (Kawanga and Piirilä 2015). Waste has previously been buried in the soil (Kawanga and Piirilä 2015). In addition, the goal of the projects has been to change people's attitude towards recycling and waste reuse (Kawanga and Piirilä 2015). Biowaste composting has also been increased (Kawanga and Piirilä 2015). The composting product has been used in growing vegetables (Kawanga and Piirilä 2015).

The projects have also created jobs in the area (Kawanga and Piirilä 2015). The projects have resulted in the creation of Madimba Sustainable Sanitation Enterprise, a company that is trained to build dry toilets and provide toilet maintenance and emptying services to households that are unable or unwilling to use compost and/or urine from their dry toilets in their own gardens (Kawanga and Piirilä 2015). Not all dry toilet owners want or are able to use the end products of the dry toilet in their own garden (Kawanga and Piirilä 2015). Surplus end products will be utilized in the NECOS test facility established near the project area (Kawanga and Piirilä 2015). The purpose of the pilot farm is to increase the knowledge of people in the Madimba area about dry latrine fertilizers and also to reduce the stigma against the use of human manure that still seems to exist among some members of the community (Kawanga and Piirilä 2015). Various vegetables have also been grown in the experimental area (Kawanga and Piirilä 2015).

As a result of the projects, the Madimba Women's Group, which improves the status of women, has also been born (Kawanga and Piirilä 2015). The group's goal is to strengthen the women living in Madimba, build capacity among them and increase food security in the area (Kawanga and Piirilä 2015). The group collects funds e.g. baking and selling pastries and samosas and making door mats (Kawanga and Piirilä 2015). In addition, NECOS implemented the Urban Greening project, where more than 1,000 trees were planted both in schools and in the community (Kawanga and Piirilä 2015).

In addition, the Zambian association NECOS plans to try to commercialize the use of urine as fertilizer. Their goal is to start selling urine collected from Madimba area in Lusaka (Kawanga 2023). Urine is collected from the area's dry toilets (Kawanga 2023). After the urine has been collected, it is stored at about one month before use (Kawanga 2023). Urine will be sold in 2.5 l & 10 l & 20 l canisters on the internet, in markets and as direct sales to customers (Kawanga 2023). The main targets are small farmers and the public sector, but it is also possible to buy fertilizer for home use (Kawanga 2023).

Urine acts as fertilizer when 1/3 of water is added to it, i.e., for example 20 l of urine requires 60 l of water (Kawanga 2023). Prepared fertilizer is added to the soil before the start of growth, 2–3 weeks after the start of cultivation and for the last time about a month after the start of cultivation (Kawanga 2023). Fertilizer sales are believed to have a good market in Zambia in the future due to the high price of chemical fertilizers and the ecological nature of urine fertilizer (Kawanga 2023).

# 2 MATERIALS AND METHODS

## 2.1 Sampling plan

A total of three surface water samples were collected from the study area, two groundwater samples were collected from the Madimba area and one from near the Chunga wastewater treatment plant. Five wastewater samples were collected, two from the Chunga wastewater treatment plant and three from the Manchianchi wastewater treatment plant. Two surface water samples were collected from the Chunga River. Ten urine samples were collected from the Madimba area.

### 2.1.1 Groundwater samples

A total of three 500 ml duplicate groundwater samples were taken for the study. One of the samples was taken in March 2022 near the Chunga wastewater treatment plant. Two groundwater samples were taken in March 2023 from the Madimba area. All groundwater samples were taken from private people's wells. In some places, groundwater is used for drinking and in some places only for washing. The samples were poured into new plastic bottles with a volume of 500 ml and closed with a cap. After collection, the samples were immediately transported in a cooler box to the laboratory of the University of Zambia for overnight storage in the refrigerator. The analysis of the samples started the next day.

### 2.1.2 Surface and wastewater samples

A total of five duplicate samples of wastewater were collected in November 2022 and March 2023 from Manchianchi and Chunga wastewater treatment plants. Manchianchi wastewater treatment plant collected 500 mL duplicate samples from influent, effluent, and faecal sludge management (Figure 6). Chunga wastewater treatment plant collected 500 mL duplicate samples from influent and effluent. In addition, duplicate samples of 500 mL were taken from the upper and down part of the Chunga River. The Chunga River is the discharge point for wastewater from the Chunga wastewater treatment plant. The samples were poured into new plastic bottles with a volume of 500 mL and closed with a cap. After collection, the samples were immediately transported in a cooler box to the laboratory of the University of Zambia for overnight storage in the refrigerator. The analysis of the samples started the next day.

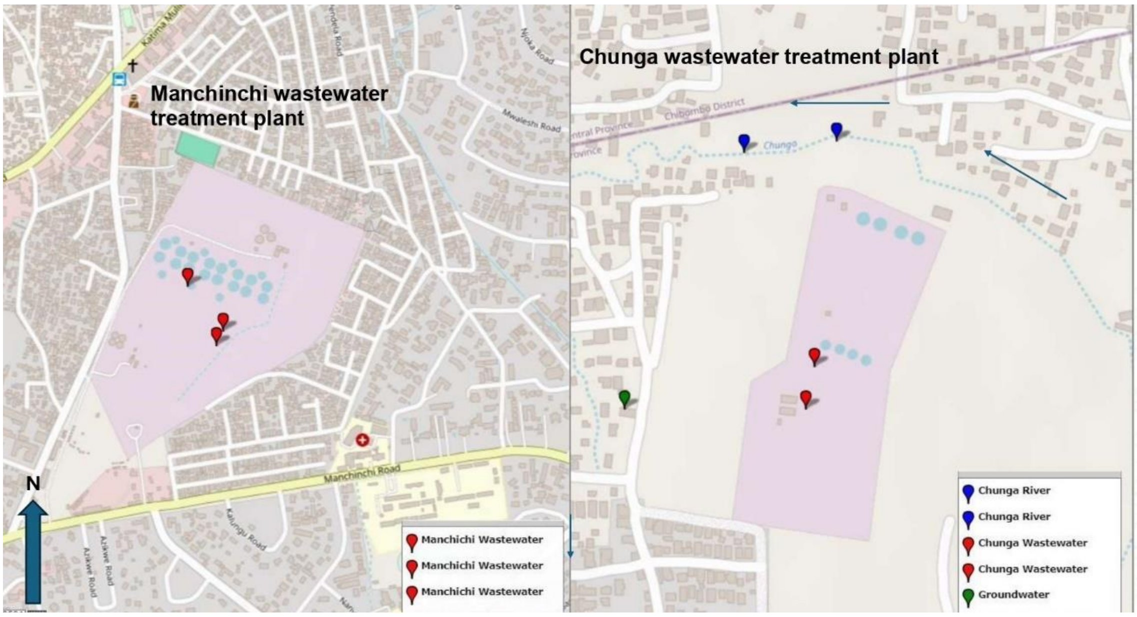

Figure 6. Location of surface water samples and wastewater samples on the map. Map made with www.gpsvisualizer.com.

### 2.1.3 Source-separated urine samples

In March 2023, a total of seven duplicate urine samples were collected from Madimba, Lusaka region (Figure 7). The samples were collected from the urine diverting dry toilets in the study area, which were located in private people's yards and some of the toilets were shared between two different households. Some of the urine had been in the containers for several months and some was fresher. One duplicate urine sample was taken from urine stored for several years, which is to be sold as fertilizer in the future.

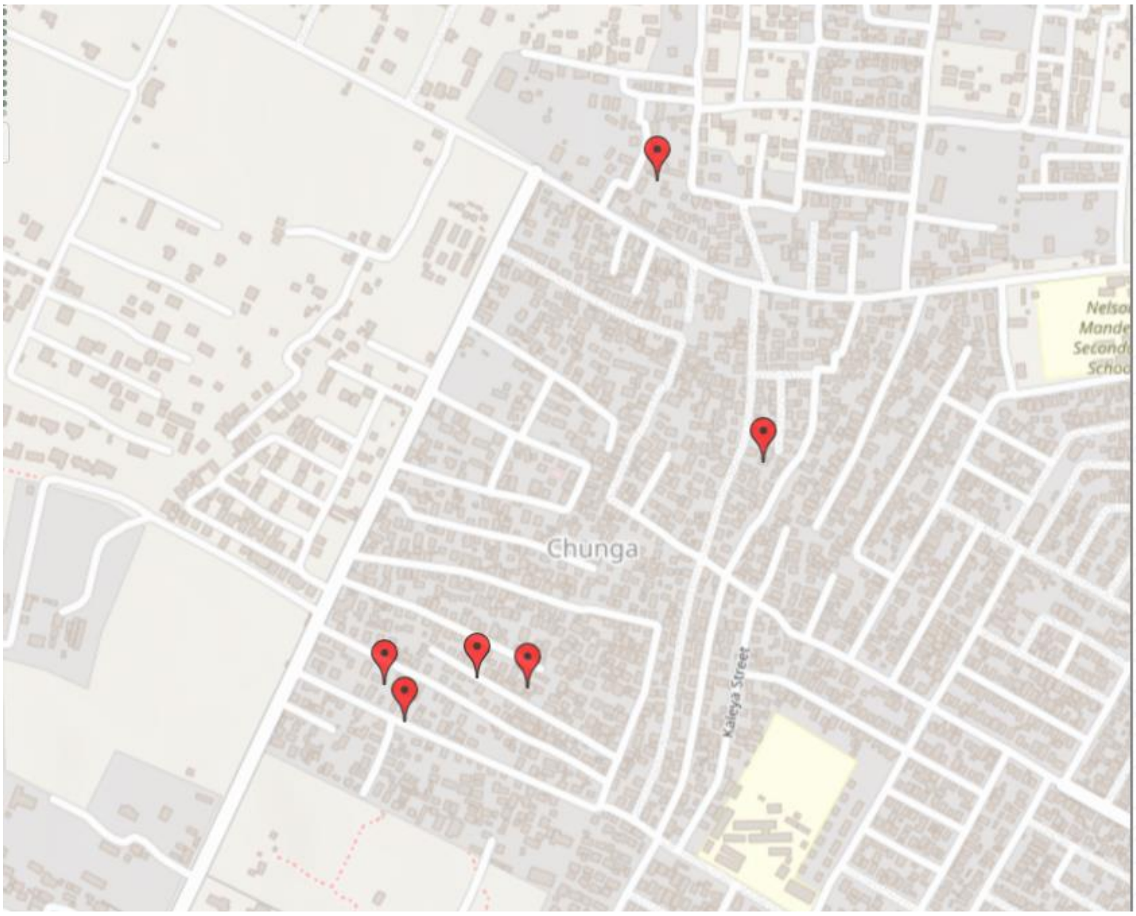

Figure 7. Locations of urine samples on the map in the Madimba peri-urban area. Map made with www.gpsvisualizer.com.

The samples were poured from the urine containers into new plastic bottles with a volume of 500 mL and closed with a cap (Figure 8). After collection, the samples were immediately transported in a cooler box to the laboratory of the University of Zambia for overnight storage in the refrigerator. The analysis of the samples started the next day. NECOS helped in taking the samples.

Figure 8. Urine and surface water samples in the University of Zambia laboratory before analysis.

## 2.2 Analyse

In this study, solid-phase extraction was used for sample preparation and liquid chromatography mass spectrometry was used for sample analysis. Solid-phase extraction was performed at the University of Zambia laboratory in November 2022 and March 2023 and Liquid chromatography-mass was performed at the University of Jyväskylä laboratory in January 2023 and April 2023 by PhD Pius Kairigo.

### 2.2.1 Selected pharmaceuticals

This work investigated the presence of 9 different antibiotics and 5 antiretrovirals. Antibiotics were amoxicillin, trimethoprim, oxytetracycline, ofloxacin, tetracycline, ciprofloxacin, sulfamethoxazole, sulfamethoxypyrazine and sulfadoxine. Antiretrovirals were valacyclovir, acyclovir, lamivudine, zidovudine and nevirapine. The table below presents the below shows the medicinal substances investigated in this study and their chemical properties (Table 6).

TABLE 6. The table below shows the medicinal substances investigated in this study and their chemical properties. (Source: https://pubchem.ncbi.nlm.nih.gov (Accessed on 15.9.2024)

| | CAS reference | Molecular weight (g/mol) | Water solubility (mg/mL) | Log Kow |
|---|---|---|---|---|
| Acyclovir | 59277-89-3 | 225.20 | 1.2–1.6 | -1.56 |
| Amoxicillin | 26787-78-0 | 365.4 | 4.0 | 0.87 |
| Ciprofloxacin | 85721-33-1 | 331.34 | <1 | 0.28 |
| Erythromycin | 114-07-8 | 733.9 | 2 | 3.06 |
| Lamivudine | 134678-17-4 | 229.26 | 3.0 | -9.54 |
| Nevirapine | 129618-40-2 | 266.30 | 0.399 | 3.89 |
| Ofloxacin | 82419-36-1 | 361.4 | 28.3 | -0.39 |
| Oxytetracycline | 79-57-2 | 460.4 | 0.6 | -0.90 |
| Rifampicin | 13292-46-1 | 822.9 | 1400 | 4.24 |
| Sulfadoxine | 2447-57-6 | 310.33 | 2.96e-01 | |
| Sulfamethoxazole | 723-46-6 | 253.28 | 0.038 | 0.89 |
| Sulfamethoxypyrazine | 152-47-6 | 322.34 | 0.042 | |
| Tetracycline | 60-54-8 | 444.4 | 231 | -1.37 |
| Trimethoprim | 738-70-5 | 290.32 | 0.4 | 0.91 |
| Valacyclovir | 124832-27-5 | 324.34 | 174 | -0.3 |
| Zidovudine | 30516-87-1 | 267.24 | 10 | 0.05 |

### 2.2.2 Sample preparation with SPE in University of Zambia

The water and urine samples were transported to the laboratory of the University of Jyväskylä for analysis. The samples were pre-treated in the University of Zambia laboratory using the solid phase extraction technique (SPE). The solid phase extraction technique is used to extract pharmaceuticals of interest from filtered urine (Ngumba et al. 2016).

The samples were brought to the University of Zambia laboratory the day before to settle overnight to facilitate filtration. Samples usually contain a large amount of particles that can interfere with pharmaceutical extraction and subsequent analysis. These particles removed by filtering water samples. The samples vacuum filtered using two glass microfibers of different sizes placed side by side to remove the micro particles. Glass mix filters include 47 mm 2.7 µm GF/D and 0.7 µm GF/F. Some of the water samples had to be filtered several times to prevent clogging.

Before loading the samples into the Waters Oasis HLB Extraction Cartridges, they were first prepared by conditioning, using 3 mL of 100% methanol, followed by the addition of 3 mL of ultra purified water. Methanol is used to wash the cartridge and to selectively remove possible impurities from the solid substrate. After preparation, approximately 240-300 mL of wastewater samples, 1000 mL groundwater, 1000 mL surface water and 100-200 mL urine samples were loaded into the HLB Extraction Cartridges. A water suction pump was used for filtration and extraction (Figure 9).

Figure 9. Filtering the urine sample using a suction pump in the laboratory of the University of Zambia.

### 2.2.3 Sample analysing with LC-MS/MSS in University of Jyväskylä

The samples were stored in the refrigerator in HLB cartridges and in a sealed bag. The finished HLB cartridges were transported in two batches within a few weeks of sampling to the laboratory of the University of Jyväskylä for final analysis. The analysis was done together with PhD Pius Kairigo. Identification and quantification of pharmaceutical concentrations was performed by liquid chromatography-tandem mass spectrometry LCMS/MS. Wastewater samples and one groundwater sample were analyzed in December 2022. In April 2023, two groundwater samples and all urine samples were analyzed. The analysis used in this study was based on the methods described in Ngumba et al. (2016) article.

When the samples have been transported at the laboratory of the University of Jyväskylä, the analysis of the samples began with the elution of the pharmaceutical substances from the HLB cartridges with a 50:50% methanol acetonitrile solution. The samples were preconcentrated under a stream of nitrogen at 40 °C and dissolved to 1 mL in vials using an elution solvent (95:5 water:acetonitrile). The sample was then analysed using a liquid chromatography-tandem mass spectrometer LC-MS/MS. The obtained chromatograms and fragmentation patterns were used for qualitative and quantitative analysis of selected pharmaceutical compounds. Liquid chromatography was performed on a Waters Alliance 2795 (Milford, MA, USA).

The compounds were separated on a reversed phase Waters XBridgeTM 3.5 µm, 2.1 × 100 mm and 3.5 µm, 2.1 × 10 mm guard columns) C18 column (Milford, MA, USA). The acetonitrile gradient was kept at 20% for the first 2 min, then increased linearly to 100% in 3 min. Acetonitrite was then reduced to 20% in 5 minutes and held there for 2 minutes. A Micromass Quattro Mass (Micromass, Manchester, UK) spectrometer analysis was performed in positive electrospray ionization (ESI+) mode and the spectrometer operated in multiple reaction monitoring (MRM) with a dwell time and channel-to-channel delay of 200 ms.

## 2.3 Survey: Sanitation situation in Zambia

The sanitation situation in Zambia were analysed based on a survey conducted by the Finnish Global Dry Toilet Association with its partner organisations. The study was conducted by Green Living Moment (GLM), Network for Environmental Concerns and Solutions (NECOS), Livingstone Green Initiative (LGI) and Ukadzipalile Integrated Project (UIP). The study areas were Nchelenge, Chibombo, Livingstone, Kazangula, Ndola, Machenje, Luanshya, Mpongwe, Kundalumwanshya, Mwanza and Isoka (Figure 10). The areas located in very different parts of Zambia, so the analysis of the study gives a comprehensive picture of the sanitation situation in the peri-urban areas. It was supposed to receive 20 responses from each region, but only 16 responses were received from Ndola and 18 from Mpongwedld. So, a total of 214 responses were received to the survey. In this work, the analysis was not done regionally, but an average has been formed from the responses of all regions to describe the situation in general.

The questions of the survey were mainly related to the sanitation situation of people, knowledge about sanitation, opinions about the current sanitation situation, water use, waste management, using urine as fertilizer and wishes regarding sanitation. The survey contained a total of 47 questions (Appendix 1). In this analysis, only part of the questions will be reviewed, focusing on the sanitation situation of the persons and the use of urine as fertilizer.

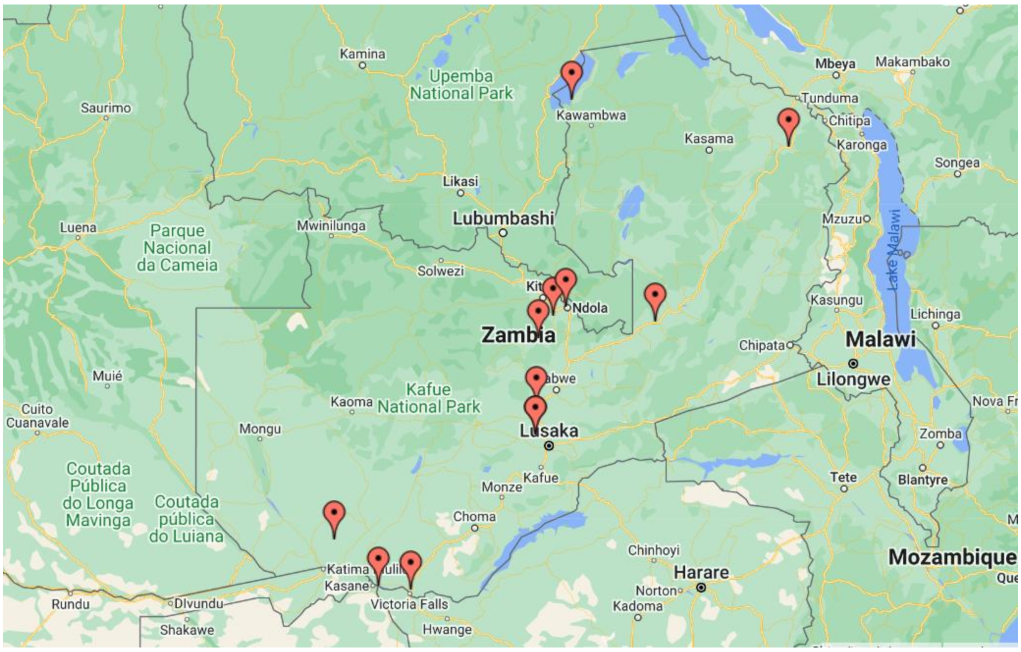

Figure 10. The locations of the villages interviewed in the survey on the map of Zambia. Map made with www.gpsvisualizer.com.

# 3 RESULTS

## 3.1 Groundwater samples

Two types of pharmaceuticals, including 6 antibiotics and 3 antiretrovirals were selected as the target substances.

TABLE 6. Concentration of pharmaceuticals in Lusaka groundwater samples (ng/L)

| Pharmaceutical | Groundwater samples<br>Chunga | <br>Madimba 1 | <br>Madimba 2 |
|---|---|---|---|
| Ciprofloxacin | 9 | BDL | 116.2 ± 12.4 |
| Erythromycin | 30 ± 1* | 35.7 ± 2.0 | 38.3 ± 2.0 |
| Lamivudine | 5 | 121.2 ± 2.9 | 4.4 ± 0.2 |
| Nevirapine | 151 ± 7 | 37.7 ± 0.2 | 124.8 ± 1.2 |
| Sulfadoxine | 10.1 ± 1 | 2.9 | 39.7 ± 2.3 |
| Sulfamethoxazole | 10 | 49.5 ± 0.4 | 26.7 ± 1.1 |
| Tetracycline | BDL | BDL | BDL |
| Trimethoprim | 2 ± 1 | 1.9 ± 0.3 | BDL |
| Zidovudine | 6 ± 1 | 1.4 | 3.6 ± 0.5 |

*Mean ±SD
BDL: below detection limit

Three groundwater samples were taken. Two of the samples were taken from the Madimba and one from a private residence near the Chunga wastewater treatment plant. Madimba 1 sample groundwater is used by residents as drinking water, but Madimba 2 sample water is not used as drinking water.

The concentrations of the pharmaceuticals in the samples taken from Madimba were mainly higher than the concentrations of the pharmaceuticals detected in the groundwater sample taken from Chunga (Table 6). Regarding lamivudine, the significantly highest concentration was observed in Madimba 1 sample at 121 ng/L. The concentration of Madimba 2 sample was 4.4 ng/L and Chunga sample was 5 ng/L. Trimethoprim concentrations in both samples are almost the same. Chunga sample 2 ng/L and Madima 1 sample 1.9 ng/L. Trimethoprim was not detected at all in the Madimba 2 sample. Zidovudine concentrations were in the same range in all samples. Chunga sample 6 ng/L, Madimba 1 sample 1.4 ng/L and Madimba 2 sample 3.6 ng/L. Tetracycline was not detected in any sample. The ciprofloxacin concentration in Chunga sample was 9 ng/L. In the Madimba 2 sample, the concentration was more than ten times higher, 116.2 ng/L. Madimba 1 sample did not detect ciprofloxacin. Sulfamethoxazole concentration in Chunga sample was 10 ng/L. The concentrations of the samples taken from Madimba were higher Madimba 1 sample, 49.5 ng/L and Madimba 2 sample 26.7 ng/L. The concentration of sulfadoxine in Chunga sample was 10 ng/L. In the Madimba 1 sample, the concentration was lowest, 2.9 ng/L and in the Madimba 2 sample the highest 39.7 ng/L. The concentration of nevirapine in Chunga sample was 151 ng/L, which was the highest concentration of all samples. In the Madimba 1 sample the concentration was 33.7 ng/L and in the Madimba 2 sample 124.8 ng/L. The concentration of erythromycin in all samples was almost the same. Chunga sample 30 ng/L, Madimba 1 sample 35.5 ng/L and Madimba 2 sample 38.3 ng/L.

## 3.2 Wastewater samples

Two types of pharmaceuticals, including 9 antibiotics and 6 antiretrovirals were selected as the target substances. Influent and effluent were sampled from both wastewater treatment plants. In addition, a sample FSM was taken from the Manchinchi wastewater treatment plant (Table 7).

TABLE 7. Concentration of pharmaceuticals in Lusaka wastewater samples (ng/L)

| | Chunga & Manchinchi wastewater treatment plant | | | | |
|---|---|---|---|---|---|
| | Influent | Effluent | Influent | Effluent | FSM |
| ACV | BDL | BDL | 31 ± 6 | BDL | 29 ± 1 |
| AMX | BDL | BDL | BDL | BDL | BDL |
| CIP | 312 ± 19 | 83 ± 1* | 213 ± 41 | 188±79 | 68 ± 9 |
| ETC | 1785 ± 146 | 443 ± 27 | 877 | 991 | 2290 |
| 3TC | 2167±45 | 905 ± 29 | 1199±0.033 | 1231 ± 36 | 3170 ± 4 |
| NVP | 246 ± 4 | 103 ± 1 | 73 | 76 | 51 |
| OFL | 38 ± 7 | 8 ± 1 | 46 ± 38 | BDL | 22 ± 6 |
| OTL | BDL | BDL | BDL | BDL | BDL |
| RFP | 213 ± 1 | 24 ± 3 | 385 | 647 | 20 |
| SDX | 828 ± 25 | 230 ± 5 | 227 | 239 | 514 |
| SMX | 721 ± 23 | 358 ± 6 | 411 ± 9 | 573 ± 17 | 2648±0.019 |
| SMP | BDL | BDL | BDL | BDL | BDL |
| TET | BDL | BDL | BDL | BDL | BDL |
| TMP | 1364 ± 25 | 635 ± 9 | 1131 ± 52 | 844 ± 4 | 804 ± 9 |
| VAL | BDL | BDL | BDL | BDL | BDL |
| ZDV | 698 ± 53 | 355 ± 11 | 104 | 1470 ± 23 | 51 ± 5 |

*Mean ±SD; BDL: below detection limit

Of the investigated pharmaceuticals, valacyclovir, oxytetracycline, tetracycline, amoxicillin and sulfamethoxypyrazine were not detected at all in the wastewater of both wastewater plants. Acyclovir was only detected in Manchinchi wastewater treatment plant influent 31 ng/L and feacal slugde management 29 ng/L. Antiretroviral pharmaceuticals commonly used in Zambia, lamivudine, nevirapine and zidovudine, were detected in high concentrations in wastewater. Lamivudine concentration at Chunga wastewater treatment plant was effluent 905 ng/L and influent 2167 ng/L. For the Manchianchi wastewater treatment plant, the concentrations were higher effluent 1231 ng/L, influent 1199 ng/L and faecal sludge management 3170 ng/L. Nevirapine concentrations at the Chunga wastewater treatment plant were effluent 103 ng/L and influent 246 ng/L. At the Manchianchi wastewater treatment plant, the effluent concentration was 76 ng/L, the influent was 73 ng/L and faecal sludge management was 51 ng/L.

Zidovudine concentrations at the Chunga wastewater treatment plant were effluent 355 ng/L and influent 698 ng/L. At the Manchinchi wastewater treatment plant, the effluent concentration was slightly higher at 1470 ng/L and the influent slightly lower at 104 ng/L. Faecal sludge management was 51 ng/L. Ofloxacin was detected in both wastewater treatment plants in low concentrations. Chunga wastewater treatment plant effluent 8 ng/L and influent 38 ng/L. Manchinchi wastewater treatment plant effluent did not detect the pharmaceutical substances in question at all, influent 46 ng/L and faecal sludge management 22 ng/L.

Ciprofloxacin was detected at both wastewater treatment plants. At the Chunga wastewater treatment plant, the concentration was slightly lower at effluent 83 ng/L and influent 312 ng/L. At the Manchinchi wastewater treatment plant, the concentrations were effluent 188 ng/L and influent 213 ng/L, and faecal sludge management 68 ng/L. Trimethoprim concentrations at the Chunga wastewater treatment plant were effluent 635 ng/L and influent 1364 ng/L. At the Manchinchi wastewater treatment plant, the concentrations were at the same level for effluent 844 ng/L, influent 1131 ng/L and faecal sludge management 804 ng/L. Sulfamethoxazole concentrations at the Chunga wastewater treatment plant were effluent 635 ng/L and influent 1364 ng/L. At the Manchinchi wastewater treatment plant, the concentrations were at the same level for effluent 573 ng/L, influent 411 ng/L and faecal sludge management 2648 ng/L.

In Chunga wastewater treatment plant the effluent concentration was 443 ng/L and influent 1785 ng/L. The Manchinchi wastewater treatment plant concentrations were at the same level effluent 991 ng/L, influent 877 ng/L and faecal sludge management 2290 ng/L. Concentration of rifampicin in Chunga wastewater treatment plant influent 24 ng/L and effluent 213 ng/L. Manchinchi wastewater treatment plant concentrations were higher, influent 385 ng/L, effluent 647 ng/L and 20 ng/L. Chunga wastewater treatment plant effluent 230 ng/L and influent 828 ng/L. At the Manchinchi wastewater treatment plant, the concentrations were slightly lower at effluent 239 ng/L and influent 227 ng/L and faecal sludge management at 51 ng/L.

### 3.2.1 Removal rates

The removal rate of wastewater treatment plants was calculated using the equation (1):

$$\frac{Influent - Effluent}{Influent} x100 \tag{1}$$

Where influent is untreated water flowing into the WWTP ready for processing and effluent is wastewater that has been treated in WWTP and which flows out of the WWTP. The removal rate of wastewater treatment plants is shown in Table 8. In addition, the removal of the pharmaceuticals is illustrated in Figure 11.

TABLE 8. Removal rates in WWTPs obtained from total loads of antibiotics in influent and effluent.

| | Removal rates in WWTPs (%) | |
|---|---|---|
| | Chunga WWTP | Manchinchi WWTP |
| Acyclovir | BDL | 100 |
| Ciprofloxacin | 73 | 12 |
| Erythromycin | 75 | -13 |
| Lamivudine | 58 | -3 |
| Nevirapine | 58 | -4 |
| Trimethoprim | 53 | 25 |
| Ofloxacin | 79 | 100 |
| Rifampicin | 89 | -68 |
| Sulfadoxine | 72 | -4 |
| Sulfamethoxazole | 50 | -28 |
| Zidovudine | 50 | -13 |

*Mean ±SD; bdl: below detection limit

The removal rates of pharmaceuticals at the Chunga wastewater treatment plant were more efficient than at the Manchinchi wastewater treatment plant. Chunga wastewater treatment plant removal rates were between 50% to 89% (Table 8). The highest removal rates were rifampicin followed by ofloxacin, erythromycin and ciprofloxacin. The lowest removal rate was sulfamethoxazole and trimethoprim. The removal rates of the wastewater treatment plant in Manchinchi were between 0% to 100%. Acyclovir and ofloxacin concentrations were not detected at all in the effluent. While sulfadoxine, nevirapine, erythromycin, rifampicin, sulfamethoxazole, zidovudine, and lamivudine concentrations were higher in effluent compared to influent. The increases in the concentrations of pharmaceutical substances were between 3% to 68%. The largest increase occurred in rifampicin and only a few percent increase in concentration occurred in lamivudine, sulfadoxine and nevirapine.

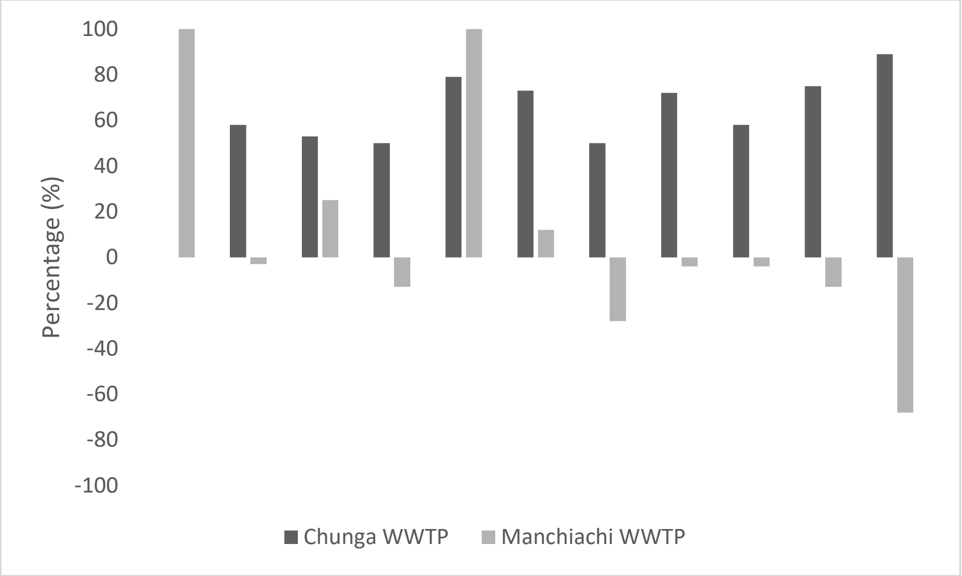

Figure 11. Removal efficiency (%) for pharmaceuticals in the WWTP estimated form total loads of antibiotics in influent and effluent.

> "The fact that some pharmaceuticals still remain in the treated wastewater may suppose a risk for the aquatic environment. Therefore, additional treatments are required to improve the removal of these emerging contaminants, as well as conducting periodically ambitious monitoring campaigns to evaluate the performance of the WWTP and the potential impact of treated water on the aquatic environment" (Bijlsma et al., 2021).

## 3.3 Surface water samples

Two types of pharmaceuticals, including 11 antibiotics and 5 antiretrovirals were selected as the target substances. Chunga River concentrations were significantly lower than in the wastewater samples (Table 9). Wastewater from the Chunga wastewater treatment plant is discharged into the Chunga River. Chunga stream up is a sample taken from the upstream of the wastewater discharge site and Chunga stream down sample is a sample taken from the downstream of the pipe site.

TABLE 9. Concentration of pharmaceuticals in Chunga River samples (ng/L)

| | Chunga River | |
|---|---|---|
| | Stream up | Stream down |
| Acyclovir | BDL | BDL |
| Amoxicillin | BDL | BDL |
| Ciprofloxacin | BDL | 88 ± 3 |
| Erythromycin | 219 | 1021 |
| Lamivudine | 688 ± 2* | 868 ± 22 |
| Nevirapine | 653 | 257 |
| Ofloxacin | BDL | 10 ± 7 |
| Oxytetracycline | BDL | BDL |
| Rifampicin | BDL | 41 |
| Sulfadoxine | 74 | 221 |
| Sulfamethoxazole | 716 ± 4 | 464 ± 9 |
| Sulfamethoxypyrazine | BDL | BDL |
| | BDL | BDL |
| Tetracycline | 101 ± 4 | 621 ± 6 |
| Trimethoprim | BDL | BDL |
| Valacyclovir | BDL | 436 ±17 |
| Zidovudine | | |

*Mean ±SD; BDL: below detection limit

Table (8) shows the presence of pharmaceutical substances in the surface water samples in each sample. No pharmaceutical target substances were detected at the Chunga wastewater treatment plant: valacyclovir, oxytetracycline, tetracycline, amoxicillin and sulfamethoxypyrazine. These pharmaceutical substances were also not detected in the Chunga River. In addition, Chunga River was not detected oxytetracycline. The highest observed antiretroviral concentration was lamivudine stream up 688 ng/L and downstream 868 ng/L. The concentrations of other antiretrovirals were zidovudine stream down 436 ng/L, nevirapine stream up 653 ng/L and stream down 257 ng/L. The concentrations of these antiretrovirals were also high in the wastewater samples. Regarding antibiotics, the highest concentration was found to be erythromycin upstream 621 ng/L and downstream 101 ng/L. The concentrations of other antibiotics were trimethoprim upstream 621 ng/L and downstream 101 ng/L. Ofloxacin upstream 10 ng/L and downstream ofloxacin was not detected at all. Ciprofloxacin up stream was not detected at all and downstream 88 ng/L. Sulfamethoxazole upstream 716 ng/L and downstream 464 ng/L. Rifampicin up stream was not detected at all and downstream 41 ng/L. The concentrations of the antibiotics were also high in the wastewater samples.

## 3.4 Source-separated urine samples

Two types of pharmaceuticals, including 6 antibiotics and 3 antiretrovirals were selected as the target substances.

TABLE 10. Concentration of pharmaceuticals in Lusaka urine samples (ng/L)

| | Urine samples | | | | | | |
|---|---|---|---|---|---|---|---|
| | Urine 1 | Urine 2 | Urine 3 | Urine 4 | Urine 5 | Urine 6 | Old urine |
| CIP | BDL | BDL | 3881.0 ± 1.7 | 94.1 ± 31.0 | BDL | 10.7 | BDL |
| ETC | 1899.0±79.2 | BDL | 224.0 ± 4.2 | BDL | 114.3 ± 1.1 | BDL | BDL |
| 3TC | 23742.0±972.3 | 48211.5 | 75.0 ± 2.8 | 20728.0±28.3 | 153.3±11.7 | BDL | 4405.0±340.8 |
| NVP | 2.0 | 25.5 | BDL | BDL | BDL | BDL | BDL |
| SDX | BDL | 57.5 | 4414.5±112.4 | 53.5 ± 2.8 | 13.5 ± 0.7 | BDL | BDL |
| SMX | 987.3 ± 8.8 | 12.5 | 1741.5 ± 44.5 | 27.3 ± 1.8 | BDL | BDL | BDL |
| TET | BDL | BDL | BDL | 9901.8±850.3 | 890.8±82.4 | BDL | BDL |
| TMP | 32.0 ± 0.7 | BDL | 93.5 ± 3.5 | BDL | 4.3 ± 0.4 | BDL | BDL |
| ZDV | BDL | BDL | BDL | BDL | BDL | BDL | BDL |

*Mean ±SD; BDL: below detection limit

Table (10) shows the presence of pharmaceutical substances in the urine samples in each sample. Table (11) presents the results as a summary of all samples. The occurrence of antiretroviral pharmaceuticals in the source-separated urine varied significantly between the samples. The highest concentrations of pharmaceutical substances were found for lamivudine. The lamivudine was detectable in 86% of the samples. The second highest detection rates were observed for sulfamethoxazole and sulfadoxine. The detection rate of both was 57%. Sulfamethoxazole the highest observed concentration was 1741.5 ng/L and sulfadoxine the highest observed concentration was 4414.5 ng/L. The detection rate for trimethoprim was 43%. The highest concentration was the lowest compared to other pharmaceutical substances, 93.5 ng/L. Ciprofloxacin and eryhromycin also had the same detection rate with trimethoprim. The highest concentrations of these pharmaceuticals were ciprofloxacin 3881.0 ng/L and erythromycin 1899.0 ng/L. Tetracycline and nevirapine had the lowest detection frequency of all. Tetracycline had the second highest concentration at 9901.8 ng/L.

TABLE 11 Detection frequency, concentration rate, median and mean concentrations of pharmaceuticals in urine samples (ng/L)

| Pharmaceutical | Chunga River | | | |
|---|---|---|---|---|
| | Detection frequency (%) | Concentration range | Median concentration | Mean concentration |
| Ciprofloxacin | 43 | BDL-3881.0 | 94 | 1329 |
| Erythromycin | 43 | BDL-1899.0 | 224 | 746 |
| Lamivudine | 86 | BDL-23742.0 | 12567 | 16219 |
| Nevirapine | 29 | BDL-25.5 | 14 | 14 |
| Sulfadoxine | 57 | BDL-4414.5 | 56 | 1135 |
| Sulfamethoxazole | 57 | BDL-1741.5 | 507 | 692 |
| Tetracycline | 29 | BDL-9901.8 | 5396 | 5396 |
| Trimethoprim | 43 | BDL-93.5 | 32 | 43 |
| Zidovudine | 0 | BDL | BDL | BDL |

## 3.5 The results of the survey

At the beginning of the survey, background information was asked: gender, age range and source of income (Figure 12). The background information of the survey showed that slightly more answers were received from women (54%) than from men (44%) and a small part of the respondents did not want to tell their gender (2%). Most of the respondents were between the ages of 41 and 60, less than a third were under the age of 61, and about a third were between the ages of 21 and 40. Most of the respondents earned their living from agriculture. Other livelihoods were, for example, fishing, carving, salary, artist, business and hunting.

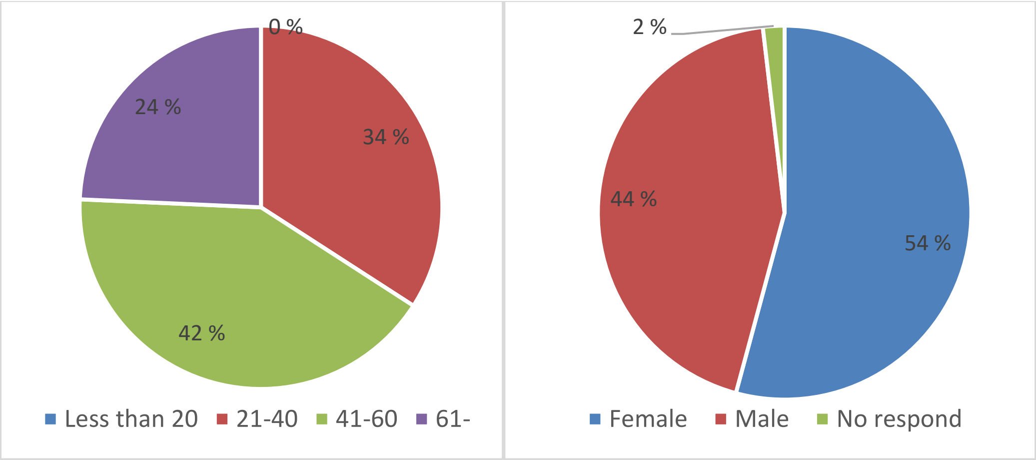

Figure 12. Results to the questions "Age and gender of the respondent"

Although the sanitation situation in Zambia has improved over the years, there are still major gaps. During the survey, 13% of those who responded to the survey answered that they live without a toilet, 80% have a pit toilet and 6% have a flush toilet. Toilets are located far from people's homes (Figure 13). About half of the respondents have a toilet located more than 21 meters from home, 17% 16-20 meters from home, 8% 10-15 meters from home, 10% 6-10 meters from home and 14% 0-5 meters from home. Household toilets are almost new. 77% of the toilets are 1-3 years old, 7% are less than a year old, 11% are 4-6 years old and only 5% of the toilets are over 6 years old.

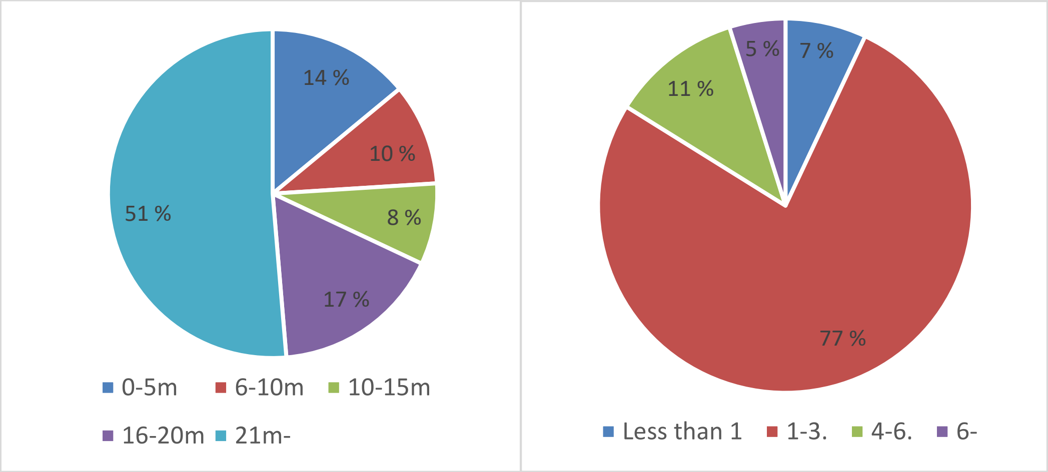

Figure 13. Results to the questions "Toilet distance from home and age of the toilet. "

The questions covered the availability of various hygiene items (Figure 14). The question regarding access to soap received 206 respondents, of which 32% have access to soap always, 41% have access sometimes but not always, 27% have no access to soap. A total of 204 responses were received to the question regarding toilet cleaning materials, 38% always access to toilet cleaning materials, 30% access sometimes but not always and 31% do not access toilet cleaning materials. A total of 191 responses were received to the question regarding access to toilet paper, 32% always have access to toilet paper, 37% have access sometimes but not always and 30% do not have access to toilet cleaning materials. In Zambia, toilet paper is commonly used after using the toilet. Using water after using the toilet is not a common practice. A total of 189 responses were received to the question regarding access to menstrual hygiene, 30% always have access to menstrual hygiene, 40% have access sometimes but not always and 26% have no access to menstrual hygiene.

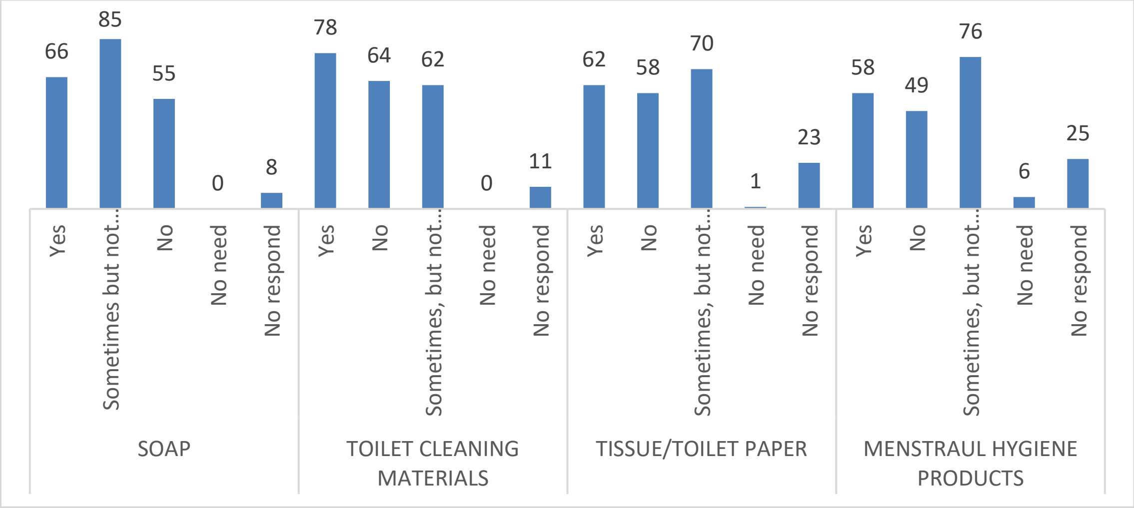

Figure 14. Results to the questions” Do you have access to soap, toilet cleaning materials, toilet paper and menstrual hygiene products”.

The questions about sanitation also addressed the respondents' experiences related to the sanitation situation and how they defined sanitation. Table 12 below summarizes some of the answers to the open question about how they defined sanitation.

TABLE 12. Answers to the open question, how respondents define the term sanitation.

| |
|---|
| “Having toilet, cleaning surrounding”. |
| “Having pit for garbage”. |
| “Cutting grass by yard, keeping surrounding cleaning digging pit”. |
| “Cleanliness of toilet, keeping water clean”. |
| “A way of having safe drinking water and adequate sewer disposal”. |
| “Process of keeping places free from dirt, infection, diseases etc.” |
| “It is the way of keeping and areas free from dirt, infections, and diseases”. |
| “The way we take care of surrounding and the thing we use”. |
| “Not having dirty compound and having access to clean drinking water and toilets”. |
| “Preventing diseases being clean”. |
| “Washing hands after the toilet”. |
| “Very useful to mainfund to impress health”. |
| “Important in every home” |
| “Great importance to the family members” |

Almost half of the respondents (45%) were not satisfied with their current sanitation situation, 18% considered the situation bad, 28% were ok and 13% did not answer the question at all (Figure 15). None of the respondents praised the situation as really good. The biggest reason for dissatisfaction is believed to be the lack of a proper toilet or the condition of the current toilet. Almost all of the respondents (97%) would be ready to improve their sanitation by building a new toilet. The most wanted new toilet is a dry toilet (58%), the second most wanted is a flush toilet (20%) and the third is a pit toilet (6%). However, currently the most commonly used toilet is a pit latrine. The sit-down toilet was more popular among respondents, with 56% preferring a sit-down toilet and 25% a squat toilet, 5% saying both are fine. We did not receive an answer for 14%. Three quarters (85%) of the respondents were ready to contribute to the costs of the toilet building. The most common answers about participating in the costs were: 9% of the respondents were not ready to participate in the costs of building a toilet and 6% we did not get an answer. Sanitation was considered important, 88% of respondents considered good sanitation important, 7% important and 5% somewhat important. None of the respondents said that good sanitation is not important.

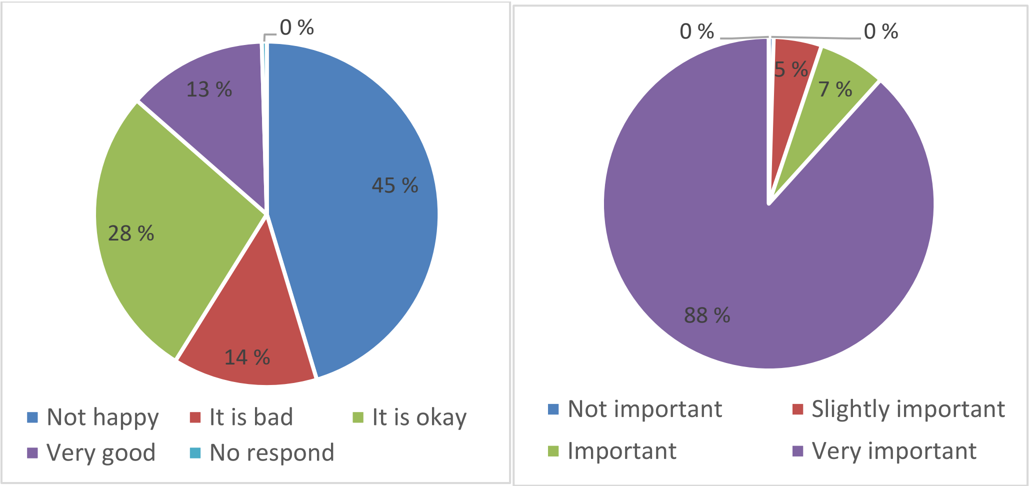

Figure 15. Results to the questions" happiness of current sanitation situation and importance of good sanitation".

The survey also asked about the possibility of people washing their hands (Figure 16). Only slightly more than half of the respondents have the opportunity to wash their hands near the toilet, 42% do not have the opportunity to wash their hands and 2% did not receive an answer to the question. However, 86% of the respondents believe that they wash their hands sufficiently and 14% of the respondents do not believe that they wash their hands sufficiently. In general, people who have the opportunity to wash their hands near a toilet feel that they do not wash their hands sufficiently because there is not always water or soap available at the handwashing station. In addition, some respondents simply said that they were too lazy to wash their hands.

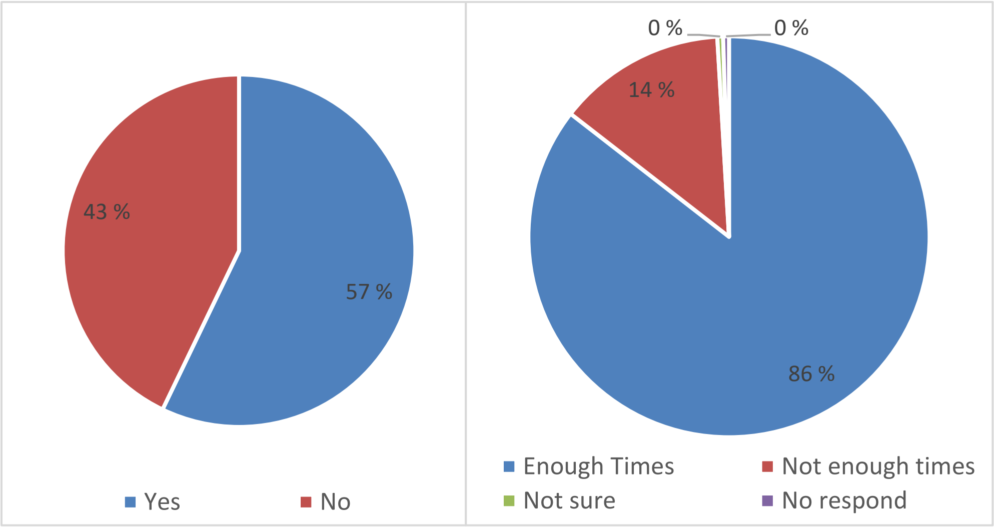

Figure 16. Results to the questions” Any handwashing facility near your toilet and do you wash your hand enough”.

Some of the questions in the survey were related to people's water sources and water use (Figure 17). Residents get drinking and other drinking water from different sources. Common sources of drinking water were wells and rivers. There was variety in homes with water points. 33% of respondents had a water point located 0-100 meters from home, 23% 100-300 meters from home, 9% 301-500 meters from home, and even 10% of respondents have to get their water more than 500 meters away. 40% of respondents use more than 100 l of water per day, 25% 76-100 l, 9% 51-75 l and 8% only 20-50 l per day. 18% we did not get an answer to the question. 53% have problems with drinking water and 46% have no problems and 1% did not answer the survey. Respondents were able to talk about water problems as an open question. People from the same region experienced the problems of their region as equal. In many different areas, drinking water problems were similar. Common problems with drinking water were: the water is not clean, the water must be boiled before use, the water source dries up in the dry season, the distance from the water to the source, and the water source must be shared by many people, so it is not enough. In addition, animals use water.

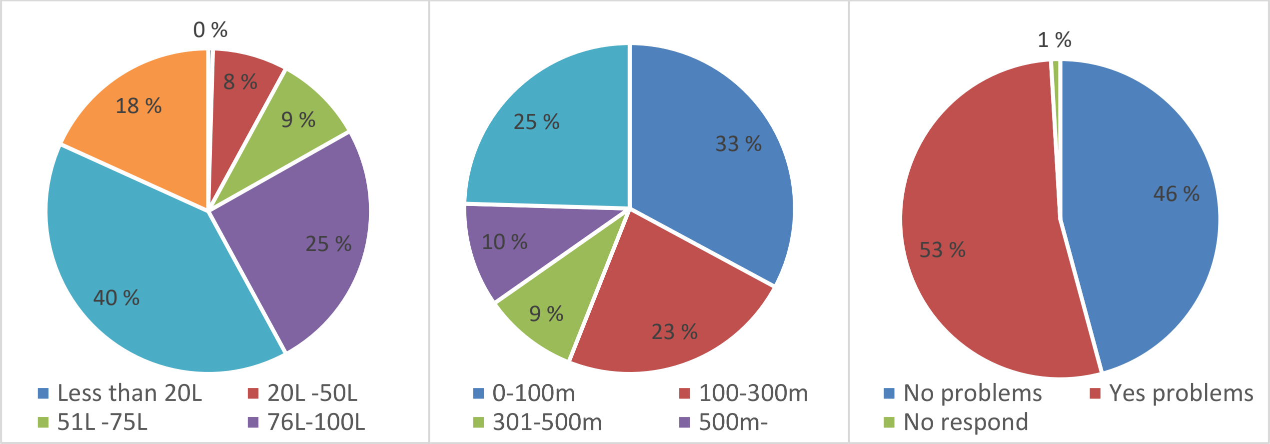

Figure 17. Results to the questions” How much water you use per day, water distance from house and do you have problems with your drinking water”.

The questions at the end of the survey were related to the use of urine as fertilizer. The survey investigated people's interest and attitude towards the use of urine as a free fertilizer. Most of the respondents (69%) were ready to use urine as fertilizer, 20% said they could use urine as fertilizer if they were told more about it, 9% said can they use urine as fertilizer and 2% of respondents did not get an answer. As an open question, the respondents were able to tell the reasons why they would not like to use urine as fertilizer. The most common reasons for not wanting to use urine as fertilizer, according to interviews, are that it is considered dirty, that it is believed to be associated with witchcraft, that it is believed to be harmful to plants, and that there is not enough information about it.

Yes, the response rate increased slightly from 69% to 72% when asked if urine fertilizer could generate income (Figure 18). Maybe response rate stayed the same at 20% and the non-response rate dropped from 9% to 6%, 1% did not answer. Most of the respondents (73%) have their own backyard garden or field for cultivation, 1% did not answer the question. Commonly grown things were corn, peanuts, soybeans, sweet potatoes, and various vegetables.

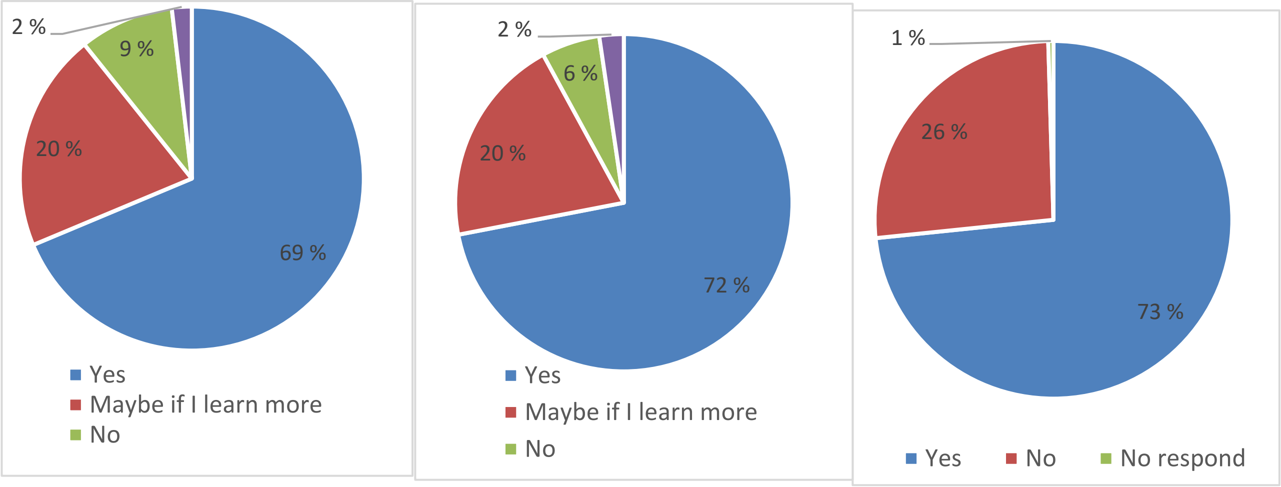

Figure 18. Results to the questions" If you have free fertilizer from urine would you use it in your garden, if you get income from using urine compost, would you use those fertilizers and do you have backyard garden or fields for cultivation.".

At the end of the survey, other comments related to the survey and the topic were freely written (Table 12). There were a lot of answers in the free comment field, and they wanted to tell very similar things. Many comments relate to the importance of water and sanitation. However, the majority of comments asked for new boreholes and toilets. A small number of comments also praised the activities for the benefit of the communities.

TABLE 12. Answers in the open comment field.

| |
|---|
| "We should involve ourselves in water and sanitation issues".<br>" We need clean water and learn more about sanitation".<br>" We are happy to be visited and to talk about issues of sanitation and hygiene + water".<br>" I really need a water borehole for clean and safe drinking water".<br>" Sanitation can improve if the water supply is improved".<br>" I would love to see new boreholes in my community".<br>" Water for drinking and food we eat should be taken care with good hygiene."<br>" Need help with to build borehole as too many people using one bore".<br>" Build more boreholes and water to near people".<br>" Build more toilets".<br>" Too many using one borehole".<br>" Distances from water source very far so require more boreholes".<br>" Water and sanitation are very important". |

# 4 DISCUSSION

To the best of our knowledge, this study provides information on the presence of selected antibiotics and antiretrovirals in aquatic environments and source separated urine in Lusaka, Zambia. In summary, based on the results, all pharmaceuticals commonly used in the treatment of HIV infection were frequently detected in water sources and source separated urine in Lusaka. Overall, lamivudine had the highest concentration among antiretrovirals and tetracycline among antibiotics. The detection of pharmaceuticals in wastewater from wastewater treatment plants indicates that these wastewaters are an important source of pharmaceuticals that cause environmental pollution. The detection of pharmaceutical substances in high concentrations in the urine separated from dry toilets indicates heavy use of these pharmaceuticals.

The removal rates of several pharmaceuticals were negative in the Chunga and Manchinchi wastewater treatment plants. Negative removal rates are common in various wastewater treatment plants worldwide (Kumar et al. 2022). Conjugation of pharmaceuticals has a big impact on the negative removal rate (Kumar et al. 2022). The pharmaceuticals are excreted from the body, often accompanied by small amounts of the original pharmaceutical (Kumar et al. 2022). Studies have shown that the presence of these metabolites can increase the total concentration of pharmaceuticals in wastewater, which in turn negatively affects the removal rates of wastewater treatment plants (Kosma et al. 2010). In addition, wastewater treatment plants face challenges in removing pharmaceuticals from wastewater, as they can bind to other substances and revert to their original form during treatment steps, which in turn increases the total concentration of pharmaceuticals in wastewater (Kumar et al. 2022). Other factors affecting the negative removal rate include biodegradation (Kumar et al. 2022). Poor biodegradation often leads to low removal efficiency (Kumar et al. 2022). In addition, different wastewater treatment methods greatly affect the removal efficiency of pharmaceutical compounds (Kumar et al. 2022). Only a small sample is taken from the wastewater in question, which does not reveal the total amount of pharmaceuticals contained in the wastewater.

## 4.1 Comprising to previous results

In the summer of 2016, Johanna Myllyniemi Maldonado studied the presence of pharmaceuticals, especially antibiotics, in Madimba, Lusaka. For the research, 26 groundwater samples, 5 samples from wastewater and surface water and 10 urine samples were collected. As a result of the study, pharmaceutical substances were detected in groundwater samples in relatively low concentrations. The maximum concentration of amoxicillin was 880 ng/L. Sulfamethoxazole was detected in more than 42% and nevirapine in more than 38% of the samples. The concentrations in surface and wastewater were significantly higher than in groundwater. The antibiotic sulfamethoxazole was measured at 11,800 ng/L in surface water and 33,300 ng/L in wastewater. The antiretroviral lamivudine was detected at 49700 ng/L in surface water and 232920 ng/L in wastewater. Trimethoprim, lamivudine and sulfamethoxazole were found at the highest concentrations of 12800 µg/L, 10010 µg/L and 7740 µg/L in source-separated urine samples (Myllyniemi Maldonado 2018). The results of this work compared with the results of this previous work (Table 13).

Table 13. comparing surface water results (ng/L). (Myllyniemi Maldonado 2018)

| | Up stream | Down stream | River A | River B |
|---|---|---|---|---|
| AMX | BDL | BDL | 2500 ± 660 | 34100 ± 440 |
| CIP | BDL* | 88 ± 3 | 400 ± 90 | 540 ± 70 |
| 3TC | 688 ± 2 | 868 ± 22 | 49700 ± 4000 | 42630 ± 3660 |
| NVP | 653 | 257 | 210 ± 30 | 220 ± 30 |
| SMX | 716 ± 4 | 464 ± 9 | 11800 ± 1200 | 7810 ± 740 |
| TET | BDL | BDL | 2200 ± 700 | 4220 ± 740 |
| ZDV | BDL | 436 ± 17 | 1280 ± 400 | 9670 ± 1290 |

*BDL: below detection limit

The samples of both studies were taken from the Chunga River. The samples in this study were up steam and downstream. The samples in Myllyniemi Maldonado's research were named as River A and River B. River A sample was taken under a road bridge, towards upper reaches from the WWTP (Myllyniemi Maldonado 2018). River B was taken two meters from the WWTP effluent discharge point (Myllyniemi Maldonado 2018). Samples were collected in July 2016 (Myllyniemi Maldonado 2018). In our study downstream samples were closer to Chunga wastewater treatment plant discharge point.

Amoxicillin was not detected at all in the samples taken in our master's thesis in 2022 from Chunga upstream and downstream. In Myllyniemi Maldonado's study, amoxicillin concentrations were high, River A 2500 ng/L and River B 34 100 ng/L. Ciprofloxacin was found in our study only in the downstream sample at 88 ng/L. In Myllyniemi Maldonado's study, the concentrations were higher in River A sample 400 ng/L and River B sample 540 ng/L. Sulfamethoxazole concentration in the samples of our study was upstream

716 ng/L and downstream 464 ng/L. Concentrations in Myllyniemi Maldonado's concentrations are ten times higher River A sample 11 800 ng/L and River B sample 7810 ng/L. Tetracycline was not detected in the upstream and downstream samples. In Myllyniemi-Maldonado's study, tetracycline concentrations were high in River A sample 2200 ng/L and River B sample 4220 ng/L. The observed lamivudine concentrations in this work were 688 ng/upstream and 868 ng/L downstream. The concentrations found in Myllyniemi Maldonado's study were significantly higher. River A sample 49 700 ng/L and River B sample 42 630 ng/L. In contrast to other results, nevirapine concentrations were higher in this work compared to the results of Myllyniemi-Maldonado's study. Nevirapine concentrations in our study were 653 ng/L up stream sample and 257 ng/L downstream sample. The results of Myllyniemi Maldonado's study were 210 ng/L in River A sample and 220 ng/L in River B sample. However, there is not such a big difference in the concentrations compared to the concentrations of other pharmaceutical substances. Zidovudine was found in our study only in the downstream sample at 436 ng/L. In Myllyniemi Maldonado's study, the concentrations were River A sample 1280 ng/L and River B sample 9670 ng/L.

From Myllyniemi Maldonado's research, the highest concentrations in the Chunga River surface water samples were lamivudine followed by sulfamethoxazole and zidovudine. The lowest concentrations were nevirapine and ciprofloxacin. Compared to the results of our study, Myllyniemi Maldonado's concentrations were significantly higher. However, the highest concentrations in our study are the same pharmaceutical substances. The highest concentrations found are sulfamethoxazole and lamivudine. The significant difference to the results of Myllyniemi Maldonado's study is that in our study the nevirapine concentration was higher than in Myllyniemi Maldonado's study.

TABLE 14. Comparing urine samples. (µg/L)

| | Our study | | | Myllyniemi Maldonado's study | | |
|---|---|---|---|---|---|---|
| | Detection frequency (%) | Concentration range | Median concentration | Detection frequency (%) | Concentration range | Median concentration |
| CIP | 43 | BDL-3.8 | 0.0941 | 90 | BDL-660 | 5.2 |
| ETC | 43 | BDL-1.9 | 0.224 | ND | ND | ND |
| 3TC | 86 | BDL-23.8 | 2.374 | 100 | 19-10010 | 19.6 |
| NVP | 29 | BDL-0.03 | 0.01375 | 10 | BDL-5 | 5 |
| SDX | 57 | BDL-4.42 | 0.0555 | ND | ND | ND |
| SMX | 57 | BDL-1.74 | 0.507 | 40 | BDL-7740 | 1662.4 |
| TET | 29 | BDL-9.9 | 5.3955 | 40 | BDL-2.8 | 0.9 |
| TMP | 43 | BDL-0.094 | 0.032 | 100 | 0.7-12800 | 4.9 |
| ZDV | 0 | BDL* | BDL | 0 | BDL | BDL |

*BDL: below detection limit

The results of the urine samples are presented in concentration µg/L due to the higher concentrations of their results. The urine samples for both studies were taken from dry toilets in the Madimba peri-urban area (Table 15). The concentrations of pharmaceutical substances in the samples from Myllyniemi-Maldonado's study were higher compared to the results of our study. Table 14. only compares the concentrations of the pharmaceutical substances that have been investigated in both studies.

The maximum concentration for lamivudine was many times higher in Myllyniemi-Maldonado's study 10 010 µg/L compared to 23.8 µg/L in our study. However, the median concentration value 19.6 µg/L of Myllyniemi-Maldonado's research was close to the maximum concentration of this research. The presence of lamivudine in the samples was high in both studies. In our study, 86% and in Myllyniemi-Maldonado's study, 100% in all samples.

The concentration of trimethoprim in our study was 0.094 µg/L. Correspondingly, in Myllyniemi-Maldonado's support, the maximum concentration was 12 800 µg/L and median concentration was 4.9 µg/L. In our study, trimethoprim was detected in only about half of the samples. In Myllyniemi-Maldonado's research, trimethoprim was found in all samples.

Tetracycline was found in low concentrations in both studies. The concentration found in our study was 9.9 µg/L. In Myllyniemi-Maldonado's study, the concentration was 2.8 µg/L. Ciprofloxacin concentration in our study was 3.8 µg/L. In Myllyniemi-Maldonado's study, the highest concentration was 660 µg/L. However, the media concentration was significantly lower at 5.2 µg/L.

Sulfamethoxazole concentration in our study was 1.74 µg/L. In Myllyniemi-Maldonado's study, the concentration was 7740 ug/. Sulfadoxine concentration in our study was 4.42 µg/L. Myllyniemi-Maldonado's study did not detect the pharmaceutical in question at all. The concentration of nevirapine found in our study was 0.03 µg/L. The concentration found in Myllyniemi-Maldonado's study was 5 µg/L. The prevalence was slightly lower in Myllyniemi-Maldonado's samples, 10% compared to 29%. The concentration of erythromycin in our study was 1.9 µg/L. Myllyniemi-Maldonado's study did not detect this pharmaceutical.

TABLE 15. Comparing groundwater samples (ng/L) to samples taken in 2016 and samples taken in 2022

|  | Our study | Myllyniemi-Maldonado's study |
|---|---|---|
| Pharmaceutical | Concentration range | Concentration range |
| Ciprofloxacin | BDL-3881.0 | BDL-150 |
| Lamivudine | BDL-20728.0 | BDL |
| Nevirapine | BDL-25.5 | BDL-410 |
| Sulfamethoxazole | BDL-1741.5 | NDL-660 |
| Tetracycline | BDL-9901.8 | BDL |
| Trimethoprim | BDL-93.5 | BDL-140 |
| Zidovudine | BDL* | BDL |

BDL: below detection limit

The groundwater samples of both studies were taken from the Madimba peri-urban area. The concentrations of the samples from Myllyniemi-Maldonado's study were lower compared to the samples of this study. Lamivudine maximum concentration in this study was up to 20728 ng/L. On the other hand, Myllyniemi-Maldonado's study did not detect the pharmaceutical in question at all. The highest concentration of trimetroprim was slightly higher in this study at 93.5 ng/L compared to Myllyniemi-Maldonado's study at 140 ng/L. Zidovudine was not found in samples from either study. In this study, Tetracycline was detected at a high concentration of 9901.8 ng/L. Correspondingly, this was not found in Myllyniemi-Maldonado's research. The highest ciprofloxacin concentration in this study was 3381 ng/L. The highest concentration found in Myllyniemi-Maldonado's samples was 150 ng/L. Sulfamethaxazole concentration in this study was 1741 ng/L. The concentration observed in Myllyniemi-Maldonado's study was 660 ng/L. The highest nevirapine concentration in this study was 25.5 ng/L. The highest concentration of this antiretroviral was exceptionally higher in Myllyniemi-Maldonado's study, 410 ng/L.

The results of this study compared with at the same level studies presented in the introduction in other African countries. The concentrations of pharmaceuticals found in the Chunga upstream and surface water sample were same level compared to other same level studies. For example, 3TC concentrations were 868 ng/L in Lusaka, Zambia, 200 ng/L in South Africa and 5428 ng/L in Nairobi, Kenya (Wood, et al. 2015; Ngumba, et al. 2016). The influent and effluent results of the Chunga wastewater treatment plant were same level compared to other same level studies. For example, the 3TC concentration in wastewater influent Lusaka, Zambia was 2167 ng/L. The highest 3TC concentration observed in Nairobi, Kenya was 3985 ng/L (Ngumba, et al. 2016). The highest concentration lamivudine observed in Pretoria, South Africa was 1001 ng/L (Mhuka, et al. 2020).

### 4.1.1 Reasons for differences

Pharmaceutical concentrations are determined by several factors, such as the source and timing of contamination, wastewater treatment plant technology, operation and removal efficiency, agricultural and veterinary practices, sensitivity of the receiving environment, and exposure history.

Based on Myllyniemi Maldoano's research, pharmaceutical concentrations have been higher in wastewater, surface and groundwater and source separated urine samples. There are many reasons for differences in results, but the most common possible reasons for differences in results are listed below. The biggest reason for the difference can be considered the time of year.

> "Zambia has a predominantly subtropical climate characterized by three distinct seasons: the hot and dry season (mid-August to mid-November), the wet rainy season (mid-November to April), and the cool dry season (mid-May to August) (Word Bank 2021)".

At the time of Myllyniemi Maldoano's research (May-July), Zambia has had a cold and dry season. In addition, the rainy season has just ended. At the time of our study, the water samples were taken in November and the urine samples in February, the dry season was ending, and the rainy season was beginning in Zambia. In 2022, the average monthly rainfall has been 1034.15 mm, and in 2016 the corresponding figure was 897.08 mm (World Bank Climate Change Knowledge Portal 2024). The amount of rain affects the amount of water flows and the dissolution of pharmaceutical substances in the water.

In addition, there could have been other reasons for the differences in the results. Although the samples were taken from the same wastewater treatment plants. Changes may occur in the operation of wastewater treatment plants over the years. The cleaning techniques of wastewater treatment plants may have changed. As wastewater treatment plants age, it is also possible that their cleaning efficiency decreases. In addition, the Manchinchi wastewater treatment plant was not operational during this study.

In the urine separated from dry toilets, the concentrations of pharmaceutical substances have been at the same levels. However, the samples are not taken from exactly the same households. In addition, the use of antibiotics is not continuous, but they are used only for a certain period to treat a certain disease. The concentration of antibiotics in pharmaceuticals also depends entirely on the current level of economic health.

Sampling and the success of laboratory tests also have an impact on the results of the work. Factors affecting sampling include, for example, sampling technique, sample size and time of sampling. In Myllyniemi Maldoano's study, the size of the urine sample was 125 mL, and in this study, it was 500 mL. Laboratory work can always involve technical issues, e.g. in measurements that have an impact on the final result.

## 4.2 Use of urine as a fertilizer

The purpose of the research was to investigate the presence of selected pharmaceutical substances in urine separated from dry toilets. High concentrations of different antiretrovirals and antibiotics were found in urine samples collected from dry toilets in the Madimba peri-urban area. Based on these findings, the use of urine as a fertilizer for directly edible plants cannot be recommended.

Agriculture is very common in the population of the survey areas, 63% of respondents have crops in their home yard, 31% have no crops of any kind and 6% did not answer the question. The most common crops are vegetables, corn, peanuts and sugarcane. Of those who responded to the survey, 69% were interested in using urine as a fertilizer on farmland, 20% could possibly use it if they gained more awareness about it, and only 9% were completely negative about it. The most common reasons for negativity were the belief that urine is dirty and disgusting. If it were possible to get an income from the use of urine as fertilizer, the percentage of those interested increased by a few percent to 72 %.

Based on the survey, it can be thought that there is interest in using urine as a fertilizer. The problem with using urine fertilizer is the pharmaceutical content it contains. NECOS still had urine left in the canister from the same sampling time when Johanna Myllyniemi Maldoano’s research samples were taken in 2016. So, the sample has been stored for 6 years. In this study, this urine was analysed. Compared to samples from Johanna Myllyniemi Maldoano's study. Only the lamivudine concentration has remained. The concentrations of all other analysed pharmaceutical substances have fallen below the detection limit. based on this finding, it can be concluded that the storage of urine could have a possible effect on the concentrations of pharmaceutical substances in the urine.

# 5 CONCLUSION

In this study, all examined urine samples separated from dry toilets contained high concentrations of antibiotics and antiretrovirals. So based on this research, using urine as a fertilizer is not recommended for use on edible plants or without pre-treatment. The wastewater samples, surface water samples, and groundwater samples also contained high concentrations of pharmaceuticals, which indicates the high use of antibiotics and antiretrovirals.

From a hygienic point of view, storage and use require special care. The World Health Organization recommending storing urine for 6 months before use can reduce pathogens and help achieve a pH of 9 increased and increased. (Pathy, Ray and Paramasivan, 2021). The long storage of urine makes possible fertilizer use difficult due to its demanding storage facilities. In this study, it was not possible to investigate more deeply the changes in the concentration of pharmaceutical substances when the storage time of urine was extended. The study only examined the matter from one sample, which would give some chance that, with the exception of one, the pharmaceutical substances would no longer appear in the urine after a long storage period. However, without information on the sample's original pharmaceutical concentrations and based on one sample, the matter cannot be stated as completely reliable.

Despite everything, technically viable, economically viable and socially acceptable ways to use urine as fertilizer must be developed. The use of urine as a fertilizer reduces the dependence on non-renewable phosphorus sources and further protects the water ecosystem from the effects of eutrophication. The recovery of phosphorus and nitrogen from urine could change the perception of wastewater pollution and could be especially the estimated resources of the phosphorus and nitrogen industry in the agricultural fertilizer sector.

Survey as a summary of the research it can be stated that although the sanitation situation in Zambia has improved over the years. Based on analyse of the collected data the sanitation situation in the areas where the research was conducted is still poor. Up to 13% of respondents live without a proper toilet. The areas comprise mainly peri-urban areas of Zambia. The drinking water situation in many areas is also bad. Proper water sources are not available. In many places, even if a water source is found, the water quality is poor. Poor water quality causes illnesses in people. The study asked about the use of urine as fertilizer. The result of the survey shows that the attitude towards the use of urine as a fertilizer has potential. For those who still doubt the use of urine, they would need more information on the matter. The use of urine as fertilizer would have good opportunities in the region.

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Kokkola 12.11.2024
Pinja Laukkanen

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# APPENDIX 1.

Sanitation project Baseline study – Questionnaire

Global Dry Toilet Association of Finland – ZDSCP/Phase 2

## 1. Basic information:

1)Area: 2) Age:

3) Gender of answer: 4) Number of household occupant:

5) Main source of income in the family:

## 2. Current level of toilets

6) Your current toilet is:

Indoor ☐ Outdoor ☐ How far from your home___m

Flushing toilet with soakaway ☐

Flushing toilet with septic tank ☐

Flushing toilet connected to public sewer ☐

Pit latrine ☐

Other type, what_____________ ☐

We do not have a toilet ☐

7) How many people use same toilet than you?

8) Is there someone who is responsible for taking care of the toilet?

9) How long have you been using the same toilet?

10) Have you improved your sanitation/toilet within past 2 years, if so how?

## 3. Hygiene, knowledge and attitudes

11) Please continue the sentence: Sanitation is

12) What kind of different toilet solutions do you know?

13) Explain with your own words what is dry toilet?

14) How the sanitation issues are handled currently in your area/village?

Each family have their toilet ☐ Yes ☐ No ☐ Don't know

Some practise open defecation ☐ Yes ☐ No ☐ Don't know

We have been cleared Open defecation free (ODF) ☐ Yes ☐ No ☐ Don't know

15) How happy are you with the current sanitation situation? (on the scale 1-4, 1=not happy, it is very poor and should be fixed immediately 2= It is kind of bad but we manage to do with it, 3= It is okay, some improvements should be done, but they are not urgent, 4= It is very good and we are happy about it)

16) How important a good sanitation/toilet is to you personally? (on the scale 1-4, 1=not relevant/important, 2=slightly important, 3=important, but there are other things which are even more important, 4=very important, it is a must to have a proper toilet, and I couldn't cope without)

17)If you think of your family members, do you think a good sanitation/toilet is a) less b) as important c) more important to them than you, Explain why?

18) Are there any family members with special needs concerning the sanitation (concerning e.g. accessibility, usability etc.)? If yes, explain how?

19) Does your family have access to following sanitation/WASH products:

Soap: Yes ☐ Sometimes, but not always ☐ No ☐ No need ☐
Toilet cleaning products: Yes ☐ Sometimes, but not always ☐No ☐No need ☐
Tissue/Toilet paper: Yes ☐ Sometimes, but not always ☐ No ☐ No need ☐
Menstrual hygiene products: Yes ☐ Sometimes, but not always ☐ No ☐
No need☐
if yes, define the products used: Yes ☐ Sometimes, but not always ☐ No ☐
No need ☐

20) How do diseases spread from excreta to people?

21) Have you or your family members had any of the following diseases during past 12 months:

Cholera ☐ How many times:

Diarrhoea ☐ How many times:

Malaria ☐ How many times:

Skin infection ☐ How many times:

Intestinal worms ☐ How many times:

Other hygiene related diseases ☐ How many times:

22) What kind of believes are linked to excreta and urine?

23) What kind of characteristics do you think good toilet includes (odourless, safety.)?

24) What kind of bad characteristics you do not want into a toilet?

25) Do you like more to be squatting or sit on toilet seat while relieving yourself?

26) Do you have hand washing possibility within/near your toilet?
Yes ☐ No ☐

27) When do you wash your hands normally during the day? Mention some examples.

28)Do you think you wash your hands often enough? If not, what are the limitations? How the situation could be improved?

29) What kind of information have you received about hygiene and sanitation lately? Where from?

30) Would you need more information on hygiene and sanitation? If yes, what kind?

## 4. Water supply

31) Where do you get your water from?

Household tap, water from private well/borehole ☐
Household tap, public water service ☐
Communal tap ☐ Distance from the house_________m
Open well ☐ Distance from the house_________m

Other, what:

32) How much water do you use (litres/day) and how much you pay for it?

33) Who is responsible for water supply to your home?

34) Is there any toilet nearby your water source (less than 30 m)?

35) What are common problems that you have with drinking water?

## 5. Waste management, recycling and climate change

36) Where do you dispose your solid/household waste of?

37) Do you have a backyard garden or fields for cultivation? Yes ☐, what crops do you grow? No ☐

38) Do you know what composting is? Yes ☐ Maybe, but I'm not sure ☐
No ☐

39) If you could get free fertilizer from the urine and compost excreta, would you be ready to use it in your gardens/fields? Yes ☐ Maybe, If I learn more ☐
No ☐

40) If you would like to get income from utilising the urine and compost, would it encourage you to use those fertilizers? Yes ☐
Maybe, If I learn more ☐ No ☐

41)Have you seen any effects of climate change in your area? Yes, describe what kind of? ☐ No, haven't noticed anything but I am aware of what climate change mean ☐ I don't know what it means ☐

42) Is there any connection between climate change and sanitation/WASH? Yes, for example ☐ No, I can't think of any relations ☐ Maybe, and I definitely would like to learn more ☐

## 4. Capacity, Construction and planning?

43) Currently, would you need to improve your sanitation by building up toilet Yes ☐ No ☐
If yes, What type of toilet would your prefer?

44) Estimate, how much would you be ready to contribute for toilet improvement?

45) Are you aware of how to build a) pit latrine Yes ☐ No ☐
b) dry toilet Yes ☐ No ☐

46) How do you participate in WASH planning/activities in your community?

47) What else you want to say about water or sanitation issues? Free word