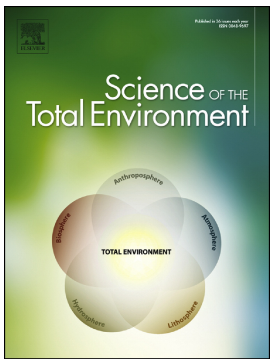


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# Mass Loading, Distribution, and Removal of Antibiotics and Antiretroviral Drugs in Selected Wastewater Treatment Plants in Kenya

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## ABSTRACT

The discharge of active pharmaceutical ingredients (APIs) into the aquatic environment from wastewater effluents is a concern in many countries. Although many studies have been conducted to evaluate the APIs removal efficiencies and emissions to the environment in wastewater treatment plants (WWTPs), most of these studies considered the aqueous and sludge phases, disregarding the suspended particulate matter (SPM) phase. To try to understand the role of the SPM, the occurrence of five most common antibiotics and three antiretroviral drugs (ARVDs) commonly used in Kenya were investigated in this study. APIs partitioning and mass loading in influents and effluents of three different WWTPs: trickling filters, stabilization ponds, and decentralised fecal sludge system, were evaluated. API concentration levels ranging from <LOQ (limit of quantification) to 92 $\mu gL^{-1}$ and <LOQ to 82.2 $mgkg^{-1}$ were observed in aqueous samples and solid samples respectively, with SPM accounting for most of the higher concentrations. The use of the aqueous phase alone for determination of removal efficiencies

showed underestimations of API removal as compared to when solid phases are also considered. Negative removal efficiencies were observed, depending on the compound and the type of WWTP. The negative removals were associated with deconjugation of metabolites, aggregated accumulation of APIs in the WWTPs, as well as unaccounted hydraulic retention time during sampling. Compound characteristics, environmental factors, and WWTPs operation influenced WWTPs removal efficiencies. Wastewater stabilization ponds had the poorest removals efficiencies with an average of -322%. High total mass loads into the WWTPs influent and effluent of 22,729 and 22,385 mg $day^{-1}$ 1000 $PE^{-1}$ were observed respectively. The results aims at aiding scientists and engineers in planning and designing of WWTPs. Findings also aim at aiding policy-making on pharmaceutical drug use and recommend proper wastewater management practices to manage the high mass loading observed in the WWTPs.

**Keywords**

Pharmaceuticals, environmental emission, sludge, suspended particulate matter, negative removal efficiencies.

## Introduction

With the continuous use of pharmaceutical compounds globally, residual pharmaceutical active ingredients (APIs) in the different compartments of the hydrological cycles have been reported (aus der Beek et al., 2016; Madikizela, Ncube, & Chimuka, 2020; Tran, Reinhard, & Gin, 2018; Wilkinson et al., 2019). As a result of the high prevalence and effect to environmental microorganisms and aquatic life, the concerns of APIs as emerging contaminants in the

environment increases (Carvalho & Santos, 2016; Guo, Selby & Boxall, 2016; Tran, Chen, Reinhard, Mao, & Yew-Hoong Gin, 2016; Zhou, Wu, Zhang, Zhao, & Zhao, 2016).

Municipal wastewater treatment plants (WWTPs) have been identified as major sources of APIs in the environment, with several published studies showing varying concentrations of APIs released into the environment from WWTPs (Vieno, Tuhkanen & Kronberg, 2005; Verlicchi, Al Aukidy & Zambello, 2012; Tewari, Jindal, Kho, Eo & Choi, 2013) . APIs generally end up in the municipal wastewater stream through urine and fecal matter. It's estimated that about 30-90% of an administered dose is excreted in urine and fecal matter as an active ingredient in unchanged compounds or as active metabolites (Kümmerer, 2009; Kwon, et al., 2011; Kosma et al., 2014). The amount of API absorbed and metabolized in the body vary based on the physicochemical characteristics of individual APIs such as solubility (S), Henry's coefficients (H), dissociation constants (pKa) and the octanol-water partition coefficient ($K_{ow}$) (Table 1). Existing conventional wastewater treatment plants, however, are not able to fully eliminate APIs from wastewater, resulting in environmental contamination (Verlicchi et al., 2012; Kosma et al., 2014; Luo et al., 2014). A range from -148 % to 100% removal efficiencies of micropollutants in WWTPs has been reported depending on the nature of the compound and WWTPs technology and operating conditions (Yunlong Luo et al., 2014; Matamoros, Rodríguez, & Albaigés, 2016; Nam, Jo, Yoon, & Zoh, 2014; Verlicchi et al., 2012). API removal is explained by three primary mechanisms: volatilization, biodegradation, and sorption. The removal of APIs by these three mechanisms is influenced by different factors including the physicochemical properties of the compound, environmental, and operational conditions (Wang & Wang, 2016). Through adsorption, the presence of APIs in sludge and suspended particulate matter (SPM) can introduce a source of contamination to the environment. SPM in WWTPs effluent can result in

contaminated sediment deposition in surface water resources, while the use of sludge as fertilizer or for soil amendment purposes reintroduces contaminants in the environment (Barbosa, Moreira, Ribeiro, Pereira, & Silva, 2016). Hence, understanding the occurrence, fate, and removal of APIs by different WWTPs is paramount to enable proper control and management of APIs in the environment.

Globally, the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) is still a major health concern. Approximately 37.9 million people are living with HIV/AIDS worldwide, of which 25.7 million are in the African region. In 2018, 1.6 million people were said to be living with HIV/AIDS in Kenya, of which 68 % are on antiretroviral therapy (UNAIDS, 2019). Pharmaceutical regiments used in the fight against HIV/AIDS pandemic and other opportunistic infections in developing countries have contributed to the increased consumption of antibiotics and antiretrovirals (ARVD's). As a result of the increased consumption, residual antibiotics, and antiretroviral drug compounds are observed in the hydrological cycle. Although the data is scanty within the African continent, residual antibiotics and ARVD's in WWTPs and the environment have been reported (Abafe et al., 2018; Ncube et al., 2018; Ngumba et al., 2016; Wood et al., 2015). In this study wastewater influent and effluent, suspended particulate matter (SPM) and sludge samples from three wastewater treatment plants in Kenya, were sampled and analyzed. The prevalence and phase distribution of the selected antibiotics and ARVD's in the aqueous, SPM, and sludge was determined, and mass loadings calculated.

## 2. *Materials and methods*

### 2.1 Study area and sample collection

Sample collection was done in three WWTPs located in two Kenyan counties; Machakos and Nyeri. WWTP-1 (latitude: -0.4261, longitude: 36.9807) and WWTP-2 (latitude: -0.3975, longitude: 37.0190) are located in Nyeri county, on the slopes of Mount Kenya, approximately 200 Km north of Nairobi capital city (Figure 1). The two Nyeri WWTPs serve about 23.3 % of the Nyeri central population (≈32,710), based on the 2019 census data (KNBS, 2019), with the rest of the population utilizing decentralized systems. WWTP-1 is a trickling filter system with primary and secondary clarifiers. The tertiary treatment utilizes three maturation ponds for water polishing. The maturation ponds have a retention period of 21 days, with fish as biological indicators. WWTP-2 utilizes wastewater stabilization ponds with screening and grit removal, anaerobic, facultative, and maturation ponds for the treatment processes. WWTP-1 and WWTP-2 are currently operating at half their design capacity based on dry weather flow (Table 2) and their final effluent is discharged into Chania River and Sagana River respectively. The average daily wastewater flow of the WWTPs was obtained from documented operational data at approximately 3000 $m^3 day^{-1}$ and 1000 $m^3 day^{-1}$ for WWTP-1 and WWTP-2 respectively

WWTP -3 is a decentralized treatment facility (DTF), situated 80 km southeast of Nairobi at latitude: -1.5367 and longitude: 37.2507, and caters for dry and wet fecal sludge brought by vacuum tankers (Dubois, 2017). The facility utilizes a combination of physical and biological treatment methods with a receiving bay/balancing tank, settling tank, anaerobic baffled reactor, and vertical flow constructed wetlands stages. The treatment of the wastewater was done in batches with a maximum volume of six medium vacuum tankers per day (22 $m^3 day^{-1}$). Effluent from the DTF discharges into River Mitheu. The population served by the three WWTPs and

their design characteristics are shown in Table 2. Although sludge production varies based on composition, the volume of incoming wastewater and type of treatment process, WWTP-1 and 2 sludge production was estimated based on 0.94 kg of sludge produced for every 3.78 $m^3$ of wastewater, which is recommended for a typical wastewater treatment plants with primary and secondary treatments (Metcalf & Eddy. Inc, 2003). Turovskiy & Mathai (2005) also documents solid production rates in WWTPs ranging from 0.2 to 0.3 kg/m3, with an average rate of 0.24 kg/m3 for typical wastewater treatment, similar to that estimated by Metcalf & Eddy (2003). These typical values have been utilized in simplified models to estimate sludge production for WWTPs lacking documented historic data (Seiple, Coleman, & Skaggs, 2017). For WWTP-3, the sludge produced was estimated based on 5% total solids content in sewage sludge and a 35% sludge reduction efficiency for the anaerobic digestion (Ferrentino, Langone, Merzari, Tramonte, & Andreottola, 2016; Mei, Narihiro, Nobu, Kuroda, & Liu, 2016; Kruger, 2002).

Influent and effluent wastewater, as well as sludge in the three WWTPs, were sampled in the dry month of September 2019. From each of the sampling points, hourly grab samples were collected over a period of 8 hours (n=8) and pooled together into a 1 L composite sample. Wet sludge composite samples were also collected from the sedimentation tanks of the WWTP-1 and 3, while sludge from the anaerobic pond was collected from WWTP-2. The composite samples were transported to the laboratory in a cool box and refrigerated at $4^oC$ until further processing. Influent and effluent wastewater temperature, pH, and conductivity were measured in situ**,** and results are presented in Table S1 of supplementary information. In the Jomo Kenyatta University of Agriculture and Technology environmental laboratory, the total suspended solids (TSS) was determined by filtering a known volume of wastewater and drying in the oven for 24 hours at

100$^{o}$C (Table S1). Suspended particulate matter (SPM) for pharmaceutical analysis was determined by tandem vacuum filtration of 200 ml of the aqueous sample through Whatman GF/D (1.5 µm) and GF/F (0.7µm) microfilters. The filters were air-dried at room temperature and weighed.

### *2.2 Chemicals and standards*

The pharmaceutical standards and their corresponding isotopically labeled internal standards were of >99% purity and were purchased from commercial vendors (Kairigo, Ngumba, Sundberg, Gachanja, & Tuhkanen, 2020). Individual 1000 mgL$^{-1}$ standards stock solutions were prepared by dissolving them in methanol apart from CIP which was dissolved in ultrapure water. The standards were then diluted with 1:1 (v/v) methanol/ultrapure water to a pooled 10mgL$^{-1}$ mixed standard and stored at +4 °C in amber vials.

### *2.3* Sample cleanup and pre-concentration

The sample concentrations for five antibiotics: sulfamethoxazole (SMX), trimethoprim (TMP), amoxicillin (AMO), ciprofloxacin (CIP) and norfloxacin (NOR) and three antiretrovirals: lamivudine (3TC), nevirapine (NVP) and zidovudine (ZDV) were measured in wastewater, sludge, and SPM. Wastewater samples were processed and extracted on a solid-phase extraction Oasis TM HLB cartridge (6 cc, 200 mg, Waters, Milford, USA) according to the published Environmental Protection Agency (EPA) Method 1694 (USEPA, 2007). The wastewater samples were first filtered, through 47 mm GF/D (2.7 µm) and GF/F (0.7 µm) glass microfiber filters (Whatman, Maidstone, England). After the filtration, the sample pH was adjusted to 9 with aqueous $NH_4OH$ to enhance the recovery of the analytes. Oasis HLB cartridges were

preconditioned with 6 mL methanol followed by 6 mL ultrapure water and replicate 200 mL samples were then loaded using a vacuum manifold at a flow rate of approximately 10 mL per minute. After loading the cartridges were dried in vacuum for 5 min, washed with 5 mL of 2 % methanol solution in 5 % aqueous $NH_4OH$, and then dried for a further 10 min. The analytes were eluted with 3 mL acetonitrile/methanol/acetic acid (50:50:2 v/v). The solvent was then evaporated in a stream of nitrogen at 40 °C, reconstituted to 1 mL with acetonitrile/water (20:80 v/v), and then filtered through a 0.2 μm cellulose acetate syringe filter before injection into an LC–MS/MS system.

The sludge and suspended particulate matter (SPM) samples were analyzed by the ultrasonication method described by Subedi et al. (2013) with some modifications. 1g of dried sediment was weighed into a 50 mL VWR® centrifuge tube and spiked with 40 µL of 10 $mgL^{-1}$ mixture of isotopically labeled internal standards and allowed to equilibrate for ~30 minutes at room temperature. Then, 6 mL of extracting solvent (methanol: water, 80:20) was added to the mixture and vortexed for one minute. The mixture was sonicated for 20 min (VWR USC 1200TH). Extracts were centrifuged at 4500 rpm (SANYO HARRIER18/80, UK) for 10 min and the supernatant collected in a 15 mL glass tube. The extraction protocol was repeated with 6 mL of 100% methanol and extracts combined into the 15ml tube. This was evaporated under a stream of nitrogen to approximately 1 mL and reconstituted to 10 mL using milli-Q water. The reconstituted sample cleanup was done similarly to the wastewater samples. The amount of APIs in the filtered SPM was extracted as described above for solid samples and results reported based on the SPM weight.

### *2.4* Instrumental Analysis

The internal standard method was used in the quantification of all the target compounds except AMO, which were quantified using a matrix-matched calibration method as described by Kairigo et al., (2020) and Ngumba et al., (2016). For quality control and quality assurance (QA/QC), matrix-matched spiked samples were used to evaluate the recovery, while procedural blanks were simultaneously analyzed with the extracted samples to assess possible sources of contamination. Eight point calibration curves were prepared for each analyte and are presented in Figure S1 of the supplementary data. APIs were analyzed using a Quattro micro tandem mass spectrometer interfaced with a waters alliance 2975 liquid chromatographic system (LC, Milford, MA, USA). A 3.5 µm x 2.1 mm x 100 mm Xbridge™ $C_{18}$ reversed-phase column, fitted with a 2.1mm x 5mm Vanguard® pre-column was used for separation. Gradient elution method was used, with formic acid (0.1%) in water and 100 % acetonitrile as the mobile phase. Multiple reaction monitoring (MRM) in positive ion mode was used for the determination of the analytes. The multi-residue method for trace-level analysis of antibiotics and antiretroviral drugs published by Ngumba et al. (2016) was used without modification.

### *2.5 API Mass Loading, Removal Efficiency and Emission to Environment*

Mass loading of the pharmaceuticals in wastewater influent (i), effluent (e) and sludge (s) were calculated based on Equations 1 and 2, assuming same flow rates of the influent and effluent. (Lin et al., 2018):

$$Mass\ loading\ (M)\ in\ influent/effluent\left(\frac{g}{d}\right) = Q\sum[\frac{C_{aq}}{10^6} + (\frac{C_{spm}x\ TSS}{10^9})] \quad (1)$$

$$Sludge\ API\ Daily\ mass\ flux\ (Ms)\ \left(\frac{\mathrm{g}}{\mathrm{d}}\right) = \frac{C_s x SP_{stp}}{10^6} \quad (2)$$

where:

Q = wastewater flow rate in the WWTP ($m^3\ d^{-1}$) (Assuming $Q_{influent} = Q_{effluent}$)

$C_{aq}$ = aqueous API concentration in influent /effluent ($\mu gL^{-1}$)

$C_{spm}$ = SPM API concentration in influent/effluent ($\mu gkg^{-1}$)

TSS = total suspended solids in the influent/effluent ($mgL^{-1}$)

$C_s$ = concentration of individual pharmaceutical compound in the sludge ($\mu gkg^{-1}$)

$SP_{stp}$ = daily sludge production ($kgd^{-1}$)

The daily load of individual compounds into the WWTPs normalized by the population equivalent (PE) and the daily emission into receiving environment per 1000 inhabitants were estimated using Equations 3 and 4 (Subedi, Balakrishna, Joshua & Kannan, 2017a).

$$Mass\ load\ in \frac{WWTP}{1000} inhibitants = \frac{M_i\ X10^3}{P_{stp}}\ X\ 10^3 \quad (3)$$

$$\frac{Emissions}{1000} inhibitants = \frac{(M_e + M_s)\ X10^3}{P_{stp}}\ X\ 10^3 \quad (4)$$

Where:

$P_{stp}$ = population served by the WWTP

$M_i$ = influent API Daily mass flux ($gd^{-1}$)

$M_e$ = effluent API Daily mass flux ($gd^{-1}$)

$M_s$ = sludge API Daily mass flux ($gd^{-1}$)

To evaluate the removal efficiencies of the WWTP, the following two equations were used considering the aqueous phase and the mass loadings.

$$R_{\text{aqueous}}\ \% = \frac{C_I - C_e}{C_i}\ x\ 100 \quad (5)$$

$$R_{\text{overall}}\ \% = \frac{(C_i + C_{Tssi}) - (C_e + C_s + C_{Tsse})}{C_i + C_{Tssi}}\ x\ 100 = \left(\frac{M_{loss}}{M_i}\right) x\ 100 \quad (6)$$

Where:

$C_i$ = API concentration in influent ($\mu gL^{-1}$)

$C_e$ = API concentration in effluent ($\mu gL^{-1}$)

$C_s$ = concentration of API in the sludge ($\mu gkg^{-1}$)

$TSS_i$ = total suspended solids in the influent ($mgL^{-1}$)

$TSS_e$ = total suspended solids in the effluent ($mgL^{-1}$)

The removal of the APIs from the WWTPs by other removal mechanisms, other than adsorption, was also estimated using Equation 7.

$$Mass\ loss = M_i - (M_e + M_s) \quad (7)$$

Where:

$M_i$ = influent API daily mass flux ($gd^{-1}$)

$M_e$ = effluent API daily mass flux ($gd^{-1}$)

$M_s$ = sludge API daily mass flux ($gd^{-1}$)

## 3.0 Results and Discussion

### 3.1 LCMS/MS analysis

From the calibration curves (Figure S1), the regression coefficients ($r^2$) for all the target APIs were ≥ 0.99. The limit of detection (LOD) and limit of quantification (LOQ), were derived from the calibration plots and were defined as 3.3 and 10 times the standard deviation of the spiked blank samples recoveries respectively (Şengül, 2016). The LOD and LOQ of the target APIs in wastewater and sludge samples ranged between 0.1 to 1.9 $ngL^{-1}$ and are presented in Table S2 of the supplementary data. The percentage recovery of quality control samples ranged between 84

% and 113 % while detection frequencies of the APIs in the samples ranged between 53 % to 100 %. The concentration of the APIs in SPM and sludge are reported on a dry-weight basis.

### 3.2 Occurrence and partitioning of APIs in the wastewater treatment plant.

#### a) Occurrence in the aqueous phase

The occurrence of the target compounds in the influent and effluent samples of the three WWTPs varied greatly across the samples as shown in Figure 2 and supplementary data Table S3. All antibiotics and antiretrovirals were detected in all the sampling sites, apart from AMO which was detected below LOQ in the influent and effluent of all the WWTPs. 3TC had the highest measured environmental concentration across all sites, with WWTP-3 recording 1463.5 $\mu gL^{-1}$ in its influent and at 847.1 $\mu gL^{-1}$ in the effluent. In WWTP-1 and WWTP-2, 3TC concentrations of 76.0 $\mu gL^{-1}$ and 53.7 $\mu gL^{-1}$ were observed in the influents respectively.

In the WWTPs influents, NVP had the lowest concentration across WWTP-1 and 2 (1.3 $\mu gL^{-1}$ and 0.7 $\mu gL^{-1}$), while the highest concentrations were observed in WWTP-3 at 47.3 $\mu gL^{-1}$. Significant decreases in NVP concentrations were however observed in effluents of WWTP-1(0.9 $\mu gL^{-1}$) and WWTP-3 (9.5 $\mu gL^{-1}$), while WWTP-2 showed an increase in the effluent concentrations (2.38 $\mu gL^{-1}$). Similar results of NVP decreasing concentrations in the effluent have been documented by Schoeman, Dlamini, & Okonkwo (2017) who reported influent and effluent NVP concentrations of 2.1 $\mu gL^{-1}$ and 0.35 $\mu gL^{-1}$. However, several studies have also documented increased NVP concentrations in WWTPs effluent (K'oreje et al., 2016; Prasse, Schlüsener, Schulz, & Ternes, 2010). K'oreje recorded influent and effluent concentrations of

850 $ngL^{-1}$ and 1000 $ngL^{-1}$ and attributed the increase to the de-conjugation of the NVP hydroxylated metabolites within the WWTP.

Prevalence of 3TC in the WWTPs influent was observed to be similar to other documented studies in Kenya, apart from WWTP-2, which exceeded concentrations observed in existing literature (Funke, Prasse, & Ternes, 2016; K'oreje et al., 2016; Elijah Ngumba, Kosunen, Gachanja, & Tuhkanen, 2016b). Funke, Prasse & Ternes (2016), K'oreje et al. (2016) and Ngumba et al. (2016), observed 3TC concentrations of 60.7 $\mu gL^{-1}$, 31.1 $\mu gL^{-1}$ and 5.4 $\mu gL^{-1}$ respectively in influents of WWTPs in Kenya. The high 3TC concentrations are associated with high drug consumption in the study region and the potential persistence of the drug in the environment (Vaňková, 2010; Bouazza et al., 2014). According to UNAIDS (2019), Kenya was estimated to have 1.6 million people living with HIV by 2018, with 68 % of the people living with HIV being on antiretroviral therapy (ART) treatment. Machakos and Nyeri counties have 80% and 61% HIV adults and 90% and 84% HIV kids respectively, under ART coverage. Lamivudine (3TC) is one of the commonly used drugs in first-line highly active antiretroviral therapy (NACC, 2018; Wamalwa et al., 2007).

TMP and SMX had high influent concentrations (24.6 $\mu gL^{-1}$ and 22.5 $\mu gL^{-1}$) in WWTP-1 and high effluent concentrations (15.8 $\mu gL^{-1}$ and 94.2$\mu gL^{-1}$) in WWTP-3 respectively. TMP-SMX is a synergistic antimicrobial combination used against a wide range of bacterial infections and has been applied in the treatment of tuberculosis (Kronbichler et al., 2018; Vilchèze & Jacobs, 2012). For TMX and SMX compounds, the adsorption process is expected to be the main route of removal from WWTPs, based on their hydrophobic nature, low biodegradability, and lower

solubilities. However, higher effluent concentrations of the two compounds were observed, particularly SMX in WWTP-3, which increased from 9.1 $\mu gL^{-1}$ to 94.2 $\mu gL^{-1}$. This increase may be attributed to SMX characteristics, resuspension of SMX from accumulated sludge as well as to deconjugation and retransformation of the metabolites, $N_4$-acetylSMX (Ac-SMX) and SMX-$N_1$-glucuronide (SMX-Glu), into parent compound (Li, Niu, Yao, Yang, & Lu, 2019b; Polesel, Andersen, Trapp, & Plósz, 2016; Radke, Lauwigi, Heinkele, Mürdter, & Letzel, 2009). A column study by Yang, et al. (2011) assessed the desorption of sulfonamides including SMX from sterilized activated sludge, and concluded that in the absence of biodegradation, the partition of sulfonamides is reversible. Yang, et al. (2011) showed a decline by desorption from an initial SMX concentration of 2.9 $\mu g\ g^{-1}$ in the activated sludge to an SMX residue of 0.4 $\mu g\ g^{-1}$. However, sorption to solids is expected to be significant for APIs with Log $K_{ow}$ greater than 4.0, for which APIs in this study were below, hence the aspect of resuspension by desorption is concluded to play a minor role (Das et al., 2017; Radjenović, Petrović, & Barceló, 2009; Thompson, Zhang, & Zhang, 2011). Nguyen et al. (2018) also documented complete biotransformation of (Ac-SMX) and (SMX-Glu) in 6 hours of an aerobic reactor, with SMX concentration increasing within the first 4-6 hours. These findings greatly support the increase in SMX concentration by deconjugation and transformation of its metabolites.

The hydraulic loading rates (HLR), sludge retention time (SRT) and hydraulic retention time (HRT), which are design parameters in wastewater treatment plants, have been observed to play a significant role in the removal of SMX from wastewater, with their influence being documented for anaerobic and wetland systems (Azimi, Hassani, Darzi, & Borghei, 2017; Christofilopoulos et al., 2019; Hatoum et al., 2019; Koh et al., 2008; Sochacki et al., 2018).

Christofilopoulos et al. (2019) showed significant differences in the removal of SMX with a change in the HRT from a vertical constructed wetland, with HRT of 1 day showing negative removal. Although anaerobic processes have been observed to significantly reduce the concentrations of SMX in wastewater, in short term operations, SMX is almost non-biodegradable (Alvarino, Suarez, Lema, & Omil, 2014; Carballa, Omil, Alder, & Lema, 2006; Chang, Chao, Yeh, Kuo, & Yang, 2019; Falås et al., 2016; Gartiser, Urich, Alexy, & Kümmerer, 2007). In an anoxic batch non-bioaugmented reactor test, no net removal of SMX was observed overall but SMX concentrations were observed to have increased in the first 6 hours simultaneously with deconjugation of Ac-SMX and SMX-Glu conjugates. SMX concentrations also remained constant thereafter for 16 hours (Nguyen et al., 2018). Compared to WWTP-1 and WWTP-2, WWTP-3, using both an anaerobic baffled reactor (ABR) and vertical wetland system, had the lowest HRT of 6 days, with a minimum HRT of 12 hours in the ABR. As a result of the lower HRT, SMX accumulation and persistence, coupled with deconjugation of its conjugated metabolites may explain the significantly high effluent concentrations observed in WWTP-3.

***b)*** **Occurrence in the Suspended Particulate Matter (SPM) phase**

Suspended particulate matter (SPM) showed high measured concentrations ranging from 11 to 82267 $\mu g kg^{-1}$ as shown in Table 3. All target compounds were found adsorbed onto the SPM of the influent and effluent of all the WWTPs, apart from AMO. This observation identifies SPM as the major transfer route of the antibiotics and antiretrovirals into and out of the WWTPs. Compared to the influent, the effluent SPM showed higher API concentrations in all the WWTPs. WWTP-1, utilizing the trickling filter system, showed an increase in TMP, CIP, and

SMX concentrations in the effluent SPM by twice as much as the influent, while WWTP-3 had SMX and NOR increasing by 4 and 5 times in effluent SPM. On average, the total API concentrations in WWTP-1 and WWTP- 3 increased from 6276 $\mu gkg^{-1}$ and 10,731 $\mu gkg^{-1}$in the influent to 7563 $\mu gkg^{-1}$ and 27,149 $\mu gkg^{-1}$ in the effluent. WWTP-2 utilizing wastewater stabilization ponds, showed a decline in the average API concentrations with 8552 $\mu gkg^{-1}$ and 8484 $\mu gkg^{-1}$ in the influent and effluent respectively. However, in WWTP-2, observations of increased individual API concentrations in the effluent SPM compared to the influent are observed. All the API compounds in the SPM effluent, other than NOR, increased by a factor range of 1-5 of the influent concentration. TMP in WWTP-2 was however observed to increase in the effluent by a factor of 12. TMP is an amphoteric molecule and weak base, with a pKa value of 7.12 (Andrade, Rocha-Filho, Cass, & Fatibello-Filho, 2009). At pKa < pH of wastewater (pH 8.5), the non-ionized species of the compound increases. This, in turn, increases the hydrophobic interaction and more TMP is adsorbed.

The prevalence of the antibiotics in the SPM phase, of both influent and effluent, followed the order NOR>CIP>SMX>TMP>AMO while 3TC>ZDV>NVP for the antiretrovirals, based on average concentrations in the three WWTPs. The adsorption behavior of a compound to solids can be described by their Octanol-Water partition coefficients ($K_{OW}$), with Log Kow< 2.5 compounds exhibiting low adsorption potential (Coimbra, Calisto, Ferreira, Esteves & Otero, 2019; Golet, Xifra, Siegrist, Alder, & Giger, 2003; Li et al., 2019). It is generally expected that compounds with larger Log $K_{ow}$ values and low water solubilities, such as TMP and SMX, will be more likely present in high concentrations the solid phases of the wastewater. In this study, however, the more hydrophilic compounds i.e NOR, CIP, and 3TC with Log $K_{ow}$ values of -1.03,

-0.28, and -1.4 respectively, were observed to be the most prevalent in the SPM. NOR and CIP are fluoroquinolone antibiotic agents, which despite their negative Log $K_{ow,}$ have zwitterionic characteristics at pKa ranges of 6.1 and 8.7 (Table 1). With the pH levels of raw wastewater in WWTPs ranging between 7.0 and 8.5, the presence of the zwitterions results to sludge adsorption of NOR and CIP by hydrophobic and electrostatic interactions (Golet et al., 2003; Lindberg et al., 2006; Nowara, Burhenne, & Spiteller, 1997). 3TC is a weak base and is protonated at pKa 4.3, hence in the wastewater it is mainly predominant in cationic form, resulting in increased adsorption by electrostatic interactions. In WWTP- 3, 3TC high influent concentration in the aqueous phase may also be attributed to the higher concentrations of 3TC in the suspended solids. Other factors that may influence the adsorptive processes include; cation exchange capacity, surface area, and organic matter content of the sludge (Yunhe Luo et al., 2019; Tambosi, Yamanaka, José, De Fátima Peralta Muniz Moreira, & Schröder, 2010).

***c)*** **Occurrence of APIs in the Sludge**

The target APIs in the sludge ranged between <LOQ to 31555 $\mu gkg^{-1}$ as illustrated by Figure 3. The antibiotics NOR and CIP had the highest measured concentrations across all WWTPs, while 3TC was the most prevalent antiretroviral drugs in all the WWTPs. 3TC concentration of 31,555 $\mu gkg^{-1}$, was observed in WWTP- 3. AMO was observed as the lowest API concentration in all the WWTPs ranging from <LOQ-69.4 $\mu gkg^{-1}$. WWTP-2 had the highest average API concentration in sludge with 4260 $\mu gkg^{-1}$, compared to WWTP-1 and 2 with 520 $\mu gkg^{-1}$ and 1112 $\mu gkg^{-1}$ respectively. Among the three WWTPs, WWTP-2 had the highest concentrations for all of the individual API compounds, apart from 3TC and ZDV which were highest in

WWTP-3. This high 3TC and ZDV concentrations in WWTP-3 was also observed in the aqueous phase and can be attributed to high HIV drug consumption in the Machakos region.

In all WWTPs, the concentrations of API in the sludge are lower than the SPM phase. However, NOR, CIP and 3TC prevalence in sludge were also similar to that observed in the SPM phases, attributing to their zwitterions and high influent concentrations. Previous studies have also shown CIP and NOR zwitterions to have high sorption in sludge, up to 70-90% (Guerra, Kim, Shah, Alaee, & Smyth, 2014; Verlicchi et al., 2012). The concentrations of NOR and CIP from this study are comparable to a study by Lindberg et al. (2006)**,** who observed ranges of NOR at 1400-4200 and CIP at 2,200 – 4,000 $\mu gkg^{-1}$ in primary and secondary wastewater clarifiers. Although AMO was observed to be lacking in the aqueous and SPM phases of the WWTPs, AMO was detected in WWTP-1 and 3 at 0.5 $\mu gkg^{-1}$ and 69.4 $\mu gkg^{-1}$, respectively. The presence of AMO in sludge agrees with its hydrophobic nature with a log $K_{ow}$ of 0.87, hence adsorbing to sludge.

From this study, the accumulation of sludge in WWTPs is observed to play a critical role in the increased concentrations observed in the effluent. With the high sludge retention time in the stabilization ponds, before sludge is removed from the ponds, the accumulation of non-biodegradable micropollutants occurs. This accumulated sludge acts as a source of contamination for the effluent water, when sludge is mineralized or resuspended under turbulence or increased flow (Chow, Ruzaiman, Rashid, & Chong, 2019; Moreno, 2004; Nemerow, 2007). The accumulation of contaminants in the stabilization ponds sludge can result in the ponds acting as

breeding and propagation sites for antimicrobial resistance (Jury, Vancov, Stuetz, & Khan, 2010; Pazda, Kumirska, Stepnowski, & Mulkiewicz, 2019).

### 3.3 Mass loading of APIs in the WWTPs

The daily mass load (DML) of individual API compound into the WWTPs normalized by the population equivalent and the daily emission into receiving environment per 1000 inhabitants were estimated based on Equations 3 and 4 and results shown in Figure 4. The total daily mass loading of all the targeted APIs getting into the wastewater treatment facility was 11,813 mg $day^{-1}$ 1000 $PE^{-1}$, 22,729 mg $day^{-1}$ 1000 $PE^{-1}$, and 234 mg $day^{-1}$ 1000 $PE^{-1}$ for WWTPs 1, 2, and 3 respectively. DML of NOR into WWTP-1 and WWTP-2 was 6,858 mg $day^{-1}$ 1000 $PE^{-1}$ and 16,800 mg $day^{-1}$ 1000 $PE^{-1}$ respectively. CIP had daily mass load of 3,010 mg $day^{-1}$ 1000 $PE^{-1}$ and 1,786 mg $day^{-1}$ 1000 $PE^{-1}$ in WWTP-1 and WWTP-2 respectively. All other antibiotics compounds had mass loads ranging from 6.7 mg $day^{-1}$ 1000 $PE^{-1}$ to 3,010 mg $day^{-1}$ 1000 $PE^{-1}$. For the antiretroviral drugs, ZDV had the highest loads in WWTP-I and WWTP-2 with 966 mg $day^{-1}$ 1000 $PE^{-1}$ and 1,686 mg $day^{-1}$ 1000 $PE^{-1}$ respectively while 3TC in WWTP-3 was the highest with a value of 97.5 mg $day^{-1}$ 1000 $PE^{-1}$. The mass loadings into WWTP-3 was observed to be significantly low with a range of 6.7 - 97.5 mg $day^{-1}$ 1000 $PE^{-1}$, compared to the other two WWTPs.

K'oreje et al. (2018) report mass fluxes of wastewater stabilization ponds in Kenya's Nzoia Basin, of 7,220 g $day^{-1}$ for 9 antibiotics and 5,660 g $day^{-1}$ for 4 antiretrovirals, which is the highest value in the reviewed literature. Compared to the study by K'oreje et al. (2018), this study findings report lower mass fluxes of 2.34 - 352 g $day^{-1}$ for the studied APIs in the three

WWTPs influents (Table S4). However, the mass fluxes were higher than the finding from Kimosop et al. (2016) who records 0.08 – 3.03 g $day^{-1}$ of five antibiotics in hospital wastewater lagoon systems. Mass fluxes ranging from 1.12 - 308 g $day^{-1}$ and 1.22 - 43.5 g $day^{-1}$ for the 4 antibiotics and 3 antiretrovirals respectively are observed in this study. Mass flux of SMX into the WWTPs was also lower than those recorded by Gao, et al., (2012), while WWTP-1 and WWTP-2 exceeded the discharge mass flux of 8.1±2.6 g $day^{-1}$. Compared to other studies, significantly higher individual APIs normalized mass loadings were observed (Table S5). It is important to note that the samples were collected during the dry season, hence, during the wet season, with high hydraulic loads, dilution can significantly lower the daily mass loadings (Paxéus, Bester, & El-taliawy, 2016).

The total daily mass loading observed were relatively higher or similar to results from other studies reported (Yuan et al., 2015; Subedi et al., 2017; Huang et al., 2018; Lin et al., 2018). Total DML of 2800 mg $day^{-1}$ 1000 $PE^{-1}$ was reported in China (Lin et al., 2018), while Li et al. (2019) reported mass loads of individual APIs ranging from 3.1 to 51,080 mg $day^{-1}$ in aqueous and SPM phases. In southern India, total mass loads of 4970 mg $day^{-1}$ 1000 $PE^{-1}$ were reported although over 75% of the load was contributed by stimulants (Subedi et al., 2017). However, a study by K'oreje et al. (2016), in 3 WWTPs in Nairobi and Kisumu, Kenya, records significantly higher loadings compared to this study. K'oreje et al. (2016), records mass loading of 40,000-122,000 mg $day^{-1}$ 1000 $PE^{-1}$ into WWTPs and 14,000 mg $day^{-1}$ 1000 $PE^{-1}$ as environmental emissions. The high mass loading observed in this study and by K'oreje indicates a high consumption of antibiotics and antiretroviral drugs, and the potential for high health and environmental risks of APIs consumption in Kenya.

### 3.4 Removal efficiency and mass balance

API removal efficiencies in the WWTPs were evaluated based on Equation 5 and 6 and the results are presented in Figure 5. To account for the total removal ($R_{overall}$ %) of the pharmaceuticals in the WWTP, environmental emission via aqueous and the suspended phase was calculated. Removal efficiencies of individual API are provided in the supplemental data in Table S6. $R_{overall}$ % TMP and $R_{overall}$ % SMX for WWTP- 2 and 3 not shown in the graph.

API removal in WWTP-1 was characterized by positive removals, while API removal in WWTP-2 and 3 were characterized by negative removal efficiencies in the majority of the target compound albeit with varying magnitudes. The average negative removal rates of all the APIs in WWTP 2 and 3 were -40.4% and -322 % and -90.1% and -135 % in the aqueous and overall combined phases respectively. WWTP-1 showed positive average removal rates of 40.9% in the aqueous phase and 69.2 % overall, considering the aqueous and SPM phases. In WWTP-2 and 3, negative removals ranging from -20.6 % to -148.0 % were observed for antibiotics TMP and CIP in the aqueous phase. CIP, however, showed positive overall removal of 60.8 % in WWTP-3, indicating that removal by solid adsorption plays a critical role in its fate. TMP in WWTP-2 however, showed -1072 %, attributed to its high increases in SPM. SMX showed 97 % and -939 % removals in the aqueous phase, and negative overall removals of -343 % and -419 % in WWTP- 2 and 3 respectively. WWTP-1 and 3 showed positive aqueous removals of antiretroviral drugs ranging from 27.5 % to 97.4 %, apart from ZDV which showed negative removals of -56.6 % in WWTP-1. Positive overall removals were also observed for all ARVDs, apart from 3TC with -131 %. ZDV in WWTP-1 was also observed to have positive removals of

88.4%. Overall removals of WWTP- 3 were however lower compared to WWTP-1, with average removals of -33.9.% and 70.5% respectively. In WWTP-2, aqueous and overall negative removals as high as –229 % and -395 % are observed for the ARVDs respectively. In WTTP-2, although positive removals of 88.9 % are observed for 3TC in the aqueous phase, negative removals of -395 % are observed when solids are considered. In contrast, NVP showed negative aqueous removals of -229 % but showed positive overall removals of 1.3 %.

Negative removal efficiencies for APIs in WWTPs has been reported in existing literature studies (Li, Zhang, Xu, & Fang, 2009; Haddad, Baginska, & Kümmerer, 2015; Polesel et al., 2016; Thiebault, Boussafir, & Le Milbeau, 2017; Kenneth Otieno K'oreje et al., 2018; Kairigo et al., 2020). Negative removals of as high as –450 % have been reported for pharmaceutical compounds (Guerra et al., 2014). SMX high concentrations were comparable to a study by Angeles et al. (2020), which indicates negative removal of SMX, with the compound not being detected in the influent but 50 $ngL^{-1}$ being observed in the effluent. Prabhasankar et al. (2016) also record negative removals of TMP and SMX with higher effluent concentrations of 3 times the influent in the summer season, indicating an influence of season. The negative removal rates can be attributed to signal suppression due to the strong matrix effects in raw water and deconjugation and abiotic retransformation of metabolites and transformational products in WWTPs (Li, Niu, Yao, Yang, & Lu, 2019; Polesel, Andersen, Rasmus, Trapp, & Plósz, 2020). Microbial deconjugation within the WWTP of the acetylated and glucuronide conjugates of SMX, accounting to about 75 % of the administered dose excreted in urine, have been reported (Barbosa et al., 2016). With effluents having 2-5 times greater SPM concentrations than

influents, the negative removals are also attributed to resuspension of SMX from accumulated sludge in the WWTPs.

The APIs removal can also be attributed to varying physicochemical characteristics of the target compounds. Hydrophobicity and hydrophilicity of the compounds described by Log $K_{ow}$ values, hydraulic and sludge retention times, and other temporal dynamics influence the fate of APIs in the WWTP. The observed aqueous and overall removals of 3TC and NVP can be explained by their hydrophobic natures as well as influent concentrations. NVP is highly hydrophobic with Log $K_{ow}$ values of 9.86, hence its tendency to adsorb to solids explains its positive overall removal efficiencies. Similarly, 3TC is also hydrophobic and is adsorbed to the solid phases, explaining its positive removals in the aqueous phase. Its high concentrations in the WWTP, however, increases the concentrations of the solids significantly, resulting in negative removals overall. TMP and SMX, also hydrophobic compounds, and are said to be significantly influenced by the water matrix, including the organic matter content (Ji et al., 2016; Martínez-Costa et al., 2018). Based on this study, the removal efficiencies can be associated with the influence of sludge retention times in the WWTPs. WWTP- 1, a trickling filter system, had most of its sludge in the primary and secondary clarifiers removed after 2-3 hours. This, in turn, prevents high API accumulations in the sludge, reducing resuspension and mineralization, explaining its positive aqueous and overall removals. WWTP-2, with the highest sludge retention time, had the highest concentration increases in the effluents than the influents. Sludge accumulation over some time results in high tendencies of sludge resuspension and mineralization, which may be the greatest contributors to these APIs increases in effluent concentrations. The aging of sludge has also been shown to influence the adsorptive capacities. Increasing sludge age has been associated with

reduced adsorption and consequently increased pharmaceutical concentrations (Jacobsen, Nyholm, Pedersen, & PrebenØstfeldt, 1993; Kipopoulou, Zouboulis, Samara, & Kouimtzis, 2004). However, negative removals in the WWTPs can be attributed to the lack of consideration of the hydraulic retention times and mixing effect during sampling (Blair, Nikolaus, Hedman, Klaper, & Grundl, 2015; Majewsky et al., 2011). Wastewater effluent samples undergo flow equalization during its resident time in the WWTPs, compared to influent samples which vary in flow and concentration daily, which consequently can result in biased and erroneous removal efficiencies (Majewsky et al., 2011).

A comparative analysis of the mass losses of the specific APIs in WWTPs is shown in Figure 6. Percentage removal in the aqueous and solid phase (SPM and sludge) and loss attributed majorly to degradation processes were evaluated. It has been shown experimentally that compounds with Log Kd values < 2.4 L $Kg^{-1}$, removal by sorption in municipal sewage treatment plant is negligible (Joss et al., 2005). The major pathways of removal include biodegradation, photolysis, and sorption to SPM, algae, and sludge (Petrovic et al., 2009). Due to APIs low values of Henrys constant ($K_H$) removal by volatilization is negligible. In WWTP 1, almost 50 % of the mass of NOR, 3TC, and ZDV, was eliminated through sorption to solids. TMP, CIP, SMX and NVP showed low removal (19.4 % to 31.5%) by degradation processes. In WWTP-2 most of the API mass load was eliminated in the aqueous phase by degradation, with approximately >40 % degradation loss being observed in most of the compounds. Sludge adsorption in WWTP-2 was observed to be significantly low, apart from NOR and NVP, which showed high adsorption rates of about 50 %. Most of the pharmaceuticals compounds entering the WWTP-2 were being reintroduced back, by either mineralization, deconjugation of metabolites or resuspension of sludge, and released as effluent (aqueous + SPM) into the environment. WWTP-3 had most of

the APIs removed by degradation process with a range of (20-60%), compared to adsorption to sludge, where CIP and NVP showed highest sludge removals of 40-50%.

### 3.5 API Environment Emissions

Data on API emission to the environment per 1000 inhabitants was estimated based on Equation 4 and the results illustrated in Figure 4. The antibiotics CIP and NOR had the highest emission values of 1,476 mg $day^{-1}$ 1000 $PE^{-1}$ and 604 mg $day^{-1}$ 1000 $PE^{-1}$ respectively at WWTP-1. The antiretroviral 3TC was highly emitted at WWTP-3 with values of 247 mg $day^{-1}$ 1000 $PE^{-1}$, while other APIs concentrations ranged between 10.0 to 282 mg $day^{-1}$ 1000 $PE^{-1}$. WWTP-2 had the highest emission rates with a total mass load of 22,385 mg $day^{-1}$ 1000 $PE^{-1}$, with individual API mass emission loads ranging from 192 mg $day^{-1}$ 1000 $PE^{-1}$ to 3,862 mg $day^{-1}$ 1000 $PE^{-1}$. WWTP-1 and WWTP-3 had total mass emission loads of 2,554 mg $day^{-1}$ 1000 $PE^{-1}$ and 691 mg $day^{-1}$ 1000 $PE^{-1}$. Compared to the total mass loads of the studied APIs into the WWTPs at 11,813 mg $day^{-1}$ 1000 $PE^{-1}$, 22,729 mg $day^{-1}$ 1000 $PE^{-1}$, and 234 mg $day^{-1}$ 1000 $PE^{-1}$ respectively for WWTP 1-3, lower total mass emission loads were observed in WWTP-1 and WWTP-2. WWTP-1 showed a great reduction in the total API mass loads, while WWTP-3 showed an increase in its emission total mass loadings. This increase in WWTP-3 can be attributed to the batch operation system, poor removal efficiencies and longer sludge accumulation periods in the WWTP. Sludge accumulation also best explains the low reduction rates in WWTP-2, utilizing stabilization ponds, by increasing the sludge concentrations over time. Generally, antibiotics were observed to have higher emissions to the environment as compared to the antiretrovirals. This study observation, however, can be a result of the higher number of antibiotics drugs studied compared to ARVDs. Mass loading and environmental emission information can be used to describe the

consumption patterns of the studied area. However, the experimental data does not always tally with the theoretical calculations, although they may occur in the same level of magnitude (Thiebault et al., 2017).

## 4. Conclusions

Data on the occurrence, partitioning, removal efficiencies, and mass loading of APIs in selected WWTPs is presented here. It has been shown that large amounts of API exist in hydrological cycles of the selected study sites. Data presented here also show that the SPM constitutes a phase of significant interest which is normally overlooked in environmental loading analyses of micropollutants. In literature, most removal efficiency studies are based on the aqueous concentrations in the influent and effluents which might lead to underestimation of the amount of APIs emitted to the environment. The negative removal efficiencies in the WWTPs also point out the complex processes that occur within WWTPs. More studies on the removal pathways especially for complex pharmaceutical mixtures is recommended. The use of decentralized treatment plants to treat raw sewage, in areas lacking sewer networks system, can help mitigate high loading and eventual contamination of surface waters with APIs in raw sewage. From this study, it is evident that high antibiotics and antiretroviral consumptions can result in subsequent high mass loading in WWTPs. Municipal WWTPs, however, cannot effectively handle the emerging organic micropollutants, releasing significant amounts in the environment. This, in turn, increases environmental risks such as the development of antibiotics resistance, which is a threat to global health.

WWTPs are identified as point sources of APIs in the environment, with influent loadings, environmental factors, and operational parameters influencing their removal. However, in order to evaluate realistic WWTP removal efficiencies, it is important to consider the suspended solids phase and the influence of hydraulic retention times, which has mainly been overlooked. Findings reported here are important for WWTP design and operations experts. Policies on drug use and wastewater management also need to be put in place to manage the high loading observed in the WWTPs. Further studies on the environmental effect and drug resistance of antibiotics and antiretrovirals in WWTPs effluent receiving surface water is recommended.

**Conflict of interest**

The authors declare no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found attached.

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**CRediT Author Statement**

**Cecilia Muriuki:** Conceptualization, Methodology, Data curation, Writing- Original draft preparation, Visualization. **Pius Kairigo:** Conceptualization, Methodology, Data curation, Writing- Original draft preparation, visualization. **Patrick Home:** Writing- reviewing and editing, Supervision. **Elijah Ngumba**: Funding acquisition, Methodology, Writing- reviewing and editing, Supervision**. James Raude:** Funding acquisition, Writing- Reviewing and editing, Supervision. **Anthony Gachanja:** Funding acquisition, Resources; Writing - review & editing, Supervision. **Tuula Tuhkanen:** Funding acquisition, Resources; Writing - review & editing, supervision.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**Figure 1: The map of Kenya and the Study Wastewater Treatment plants in Nyeri and Machakos.**
**Figure 2: Concentration of Selected Antibiotics and Antiretrovirals in Aqueous Phase of WWTP Influent and Effluent**
**Figure 3: Prevalence of antibiotic and antiretroviral drugs in the sludge phase of WWTP 1-3.**
**Figure 4: API daily mass loads in mg/day/ 1000 inhabitants.**
**Figure 5: Aqueous Phase and Overall Removal Efficiency of API's in the WWTP's**
**Figure 6: Percentage mass loss in WWTP 1-3 in the aqueous and solid phases.**

**Table 1: Physico-chemical characteristics of studied antibiotics and ARVD's**

| Chemical | Mol mass (g/mol) | Physico-chemical properties and biodegradability kinetics of selected PPCPs | | | | |
|---|---|---|---|---|---|---|
| | | S | H | pKa | Log $K_{ow}$ | $k_{biol}$ |
| **Sulfamethoxazole** | 253.3 | 610 | $2.69 \times 10^{-11}$ | 1.6-5.7 | 0.89 | 0.19-0.2 |
| **Trimethoprim** | 290.3 | 400 | $9.89 \times 10^{-13}$ | 7.12 | 0.91 | 0.05- 0.09 |
| **Ciprofloxacin** | 331.3 | $3 \times 10^{4}$ | - | 6.1- 8.6 | -0.28 | 0.55 |
| **Norfloxacin** | 319.3 | $1.8 \times 10^{5}$ | $6.8 \times 10^{-13}$ | 6.3- 8.7 | -1.03 | 0.01 - 0.3 |
| **Amoxicillin** | 365.4 | $3.4 \times 10^{3}$ | $2.49 \times 10^{-21}$ | 3.2 | 0.87 | 1.33 |
| **Lamivudine** | 229.3 | $7.0 \times 10^{4}$ | - | 4.3 | -1.4 | - |
| **Nevirapine** | 266.3 | $7.0 \times 10^{-4}$ | $3.3 \times 10^{-17}$ | 2.8 | 2.5 | 0.03[b] |
| **Zidovudine** | 267.2 | $2.0 \times 10^{4}$ | - | 9.86 | 0.05 | 0.04 |

(S, solubility in water(mg $l^{-1}$); H, Henry coefficient(1g $m^{-3}$ air/1g $m^{-3}$ wastewater); pKa, Dissociation constant; $K_{ow}$, octanol-water partition coefficient; $k_{biol}$, pseudo first-order degradation constant($1g^{-1}$SS $day^{-1}$) : Sources: (PubChem Database, 2019)

**Table 2: Study WWTPs Population, Design and Operation Data**

| Treatment plant | WWTP 1 | WWTP 2 | WWTP 3 |
|---|---|---|---|
| Type of system | Trickling filter + STP | Stabilization ponds (STP) | Decentralized Treatment Facility (DTF) |
| Population served (%)* | 23.3% | | 13.1% |
| Population served (No.)* | ~29,774 | ~2,935 | 22,350 |
| Design Capacity (Based on dry weather flow) | 6000 $m^3$/day | 2000 $m^3$/day | 22 $m^3$/day of wet sludge |
| Current Average discharge | 3000 $m^3$/day | 1000 $m^3$/day | 22 $m^3$/day |
| Retention time | >21 days | 90 days | 6 days |
| Estimated sludge production (Kg/day) | 746.0 | 248.7 | 286 |

| | | | |
|---|---|---|---|
| Sludge retention Time | 1-2 hours | Anaerobic ponds- 3.5 days,<br>Other ponds- 4 years | 2 weeks |

*Population served is based on KNBS (2019) Kenya census data and the report by Ngugi et al.(2013).

**Table 3: Concentration of Antibiotics and Antiretroviral Drugs in Suspended Particulate Matter of WWTP 1-3**

| API | WWTP 1 | | WWTP 2 | | WWTP 3 | |
|---|---|---|---|---|---|---|
| | Influent SPM ($\mu gkg^{-1}$) | Effluent SPM ($\mu gkg^{-1}$) | Influent SPM ($\mu gkg^{-1}$) | Effluent SPM ($\mu gkg^{-1}$) | Influent SPM ($\mu gkg^{-1}$) | Effluent SPM ($\mu gkg^{-1}$) |
| NOR | 25,536 (529) | 12,617 (712) | 44,317 (3347) | 11(0.35) | 16,057(1230) | 82,267(559) |
| TMP | 754 (85) | 1567 (419) | 148(179) | 1817 (328) | 2,193 (273) | 3,080 (845) |
| CIP | 11,204(928) | 31,117(349) | 4,701(103) | 15,852(598) | 13,383(1171) | 5,017 (344) |
| SMX | 1,030 (356) | 2,085(510) | 2,198(493) | 10,286 (711) | 4,725(114) | 23,448 (1959) |
| AMO | N.D | N.D | N.D | N.D | N.D | N.D |
| 3TC | 1,248 (288) | 1,131(315) | 1,838(290) | 9,701(525) | 30,761(1514) | 69,681(5824) |
| ZDV | 3,596 (322) | 2,415(505) | 4,447(481) | 19,464(610) | 4,202(301) | 3,336(119) |
| NVP | 563 (54) | 2,006(133) | 2,212(199) | 2,269(161) | 3,795(176) | 3,214(146) |

Concentration in ng/L (sd) (n=3) ; N.D- Not detected

## Highlights

- Partitioning of APIs into aqueous, sludge and suspended particulate matter (SPM) was investigated.
- High mass loading of antibiotics and antiretrovirals in Kenyan WWTPs.
- High API concentrations in suspended particulate matter (82.3 mg $kg^{-1}$) and sludge (31.6 mg $kg^{-1}$) observed.
- Overlooking the solid phases (SPM and sludge) when evaluating removal efficiency of WWTPs underestimates the results.
- Average negative removal efficiency of up to -322 % observed when SPM was considered.

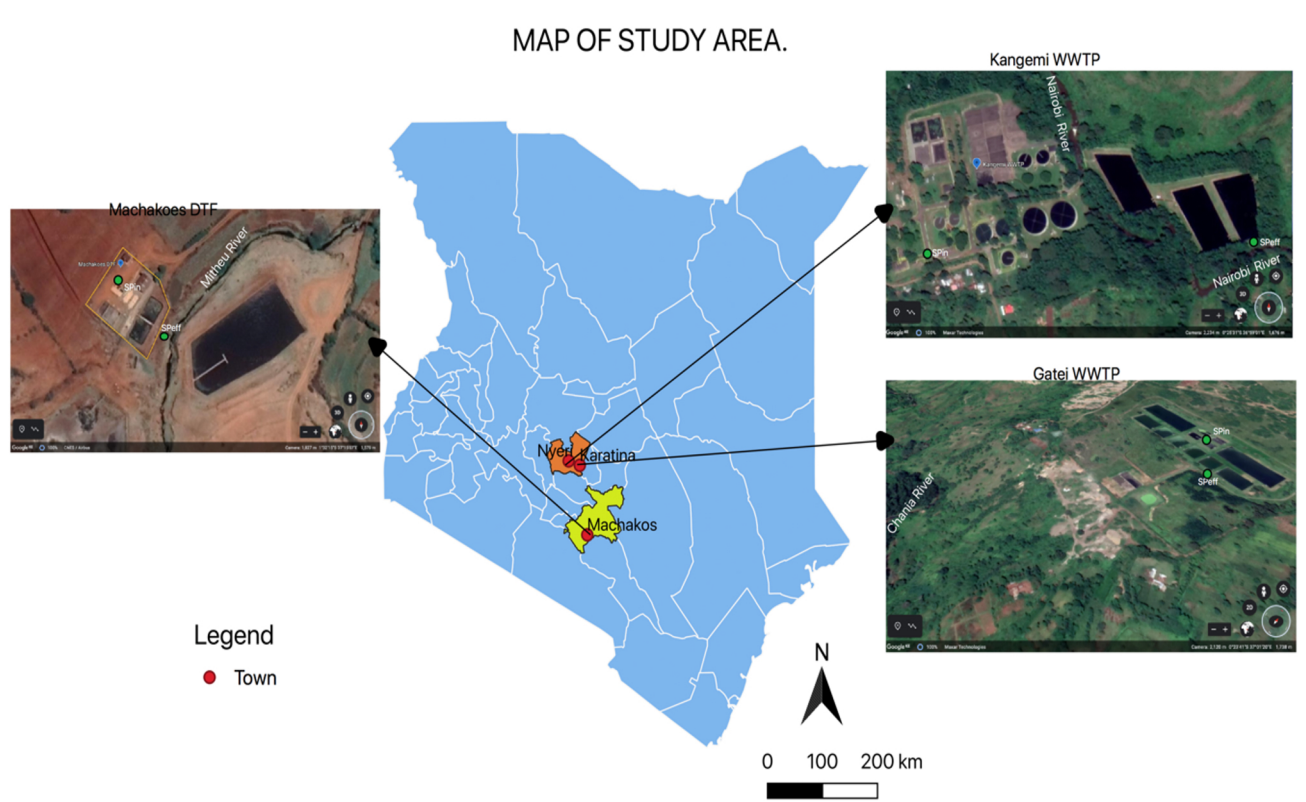

MAP OF STUDY AREA.
Kangemi WWTP
Nairobi River
SPin
SPeff
Nairobi River
Machakoes DTF
Mitheu River
SPin
SPeff
Gatei WWTP
SPin
SPeff
Chania River
Nyeri
Karatina
Machakos
Legend
Town
N
0 100 200 km

Figure 1

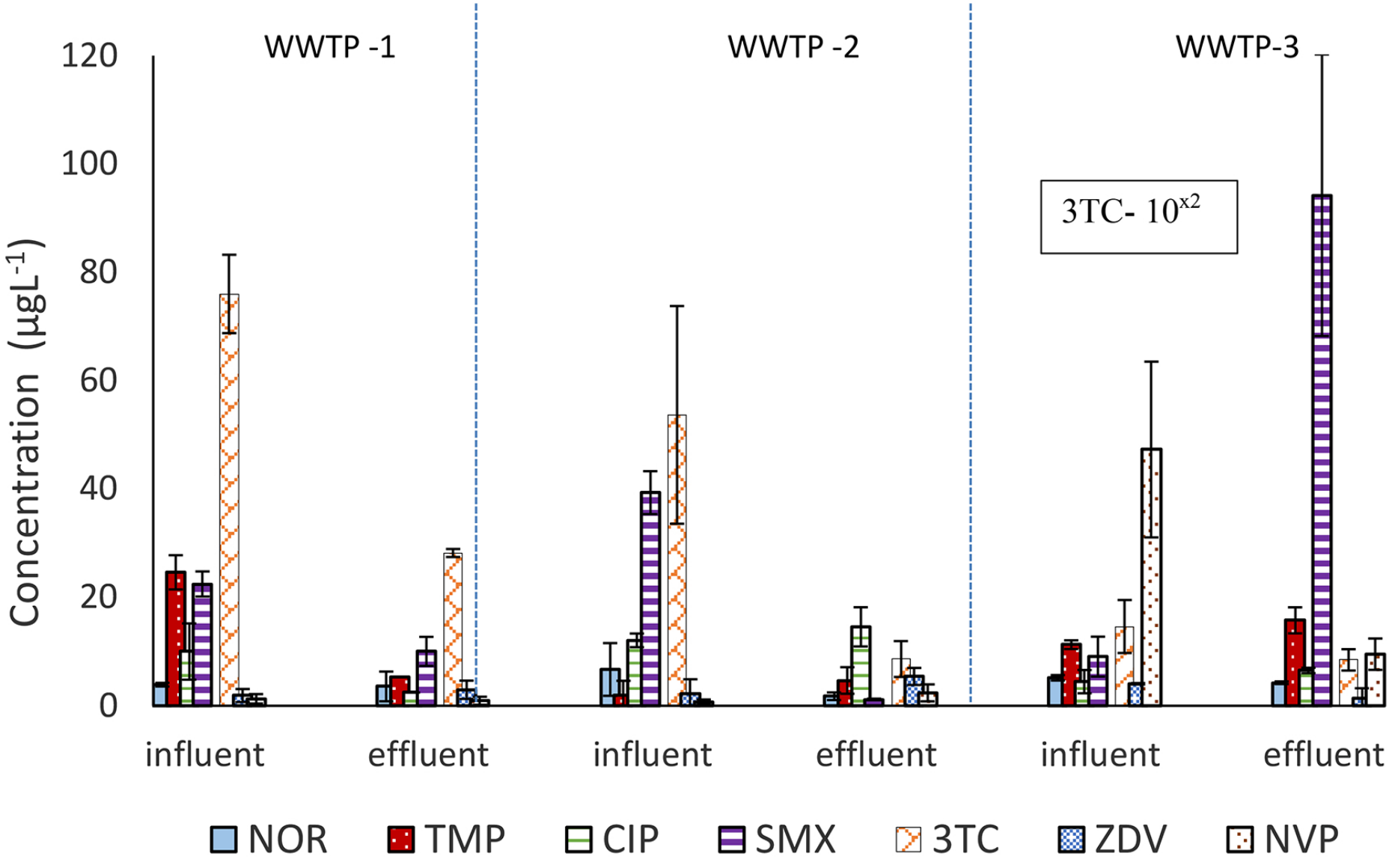

WWTP -1
WWTP -2
WWTP-3
120
100
80
60
40
20
0
Concentration (µgL-1)
3TC- 10x2
influent
effluent
influent
effluent
influent
effluent
NOR
TMP
CIP
SMX
3TC
ZDV
NVP

Figure 2

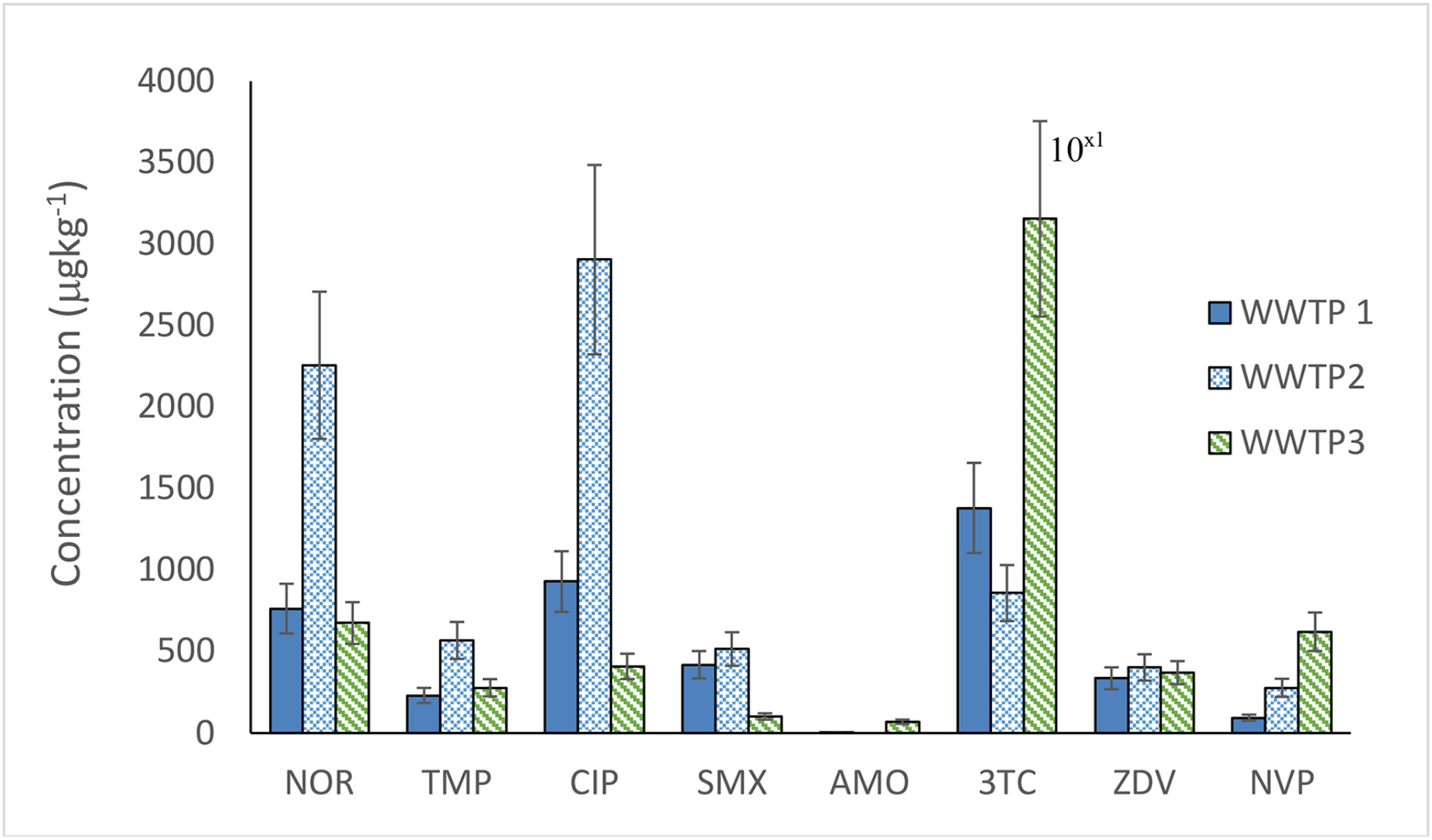

4000
3500
3000
2500
2000
1500
1000
500
0
Concentration (µgkg-1)
10x1
WWTP 1
WWTP2
WWTP3
NOR
TMP
CIP
SMX
AMO
3TC
ZDV
NVP

Figure 3

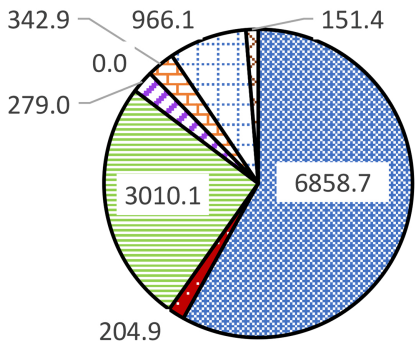
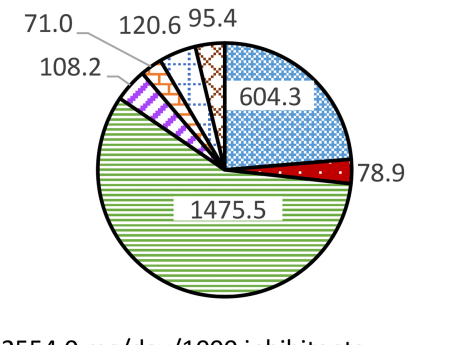
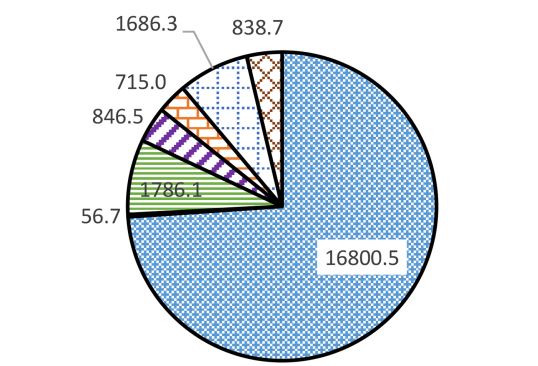
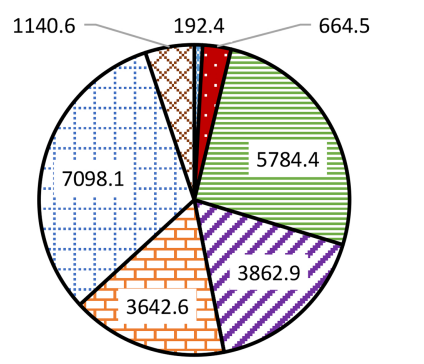
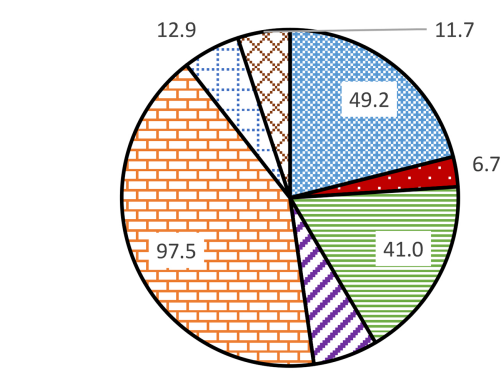
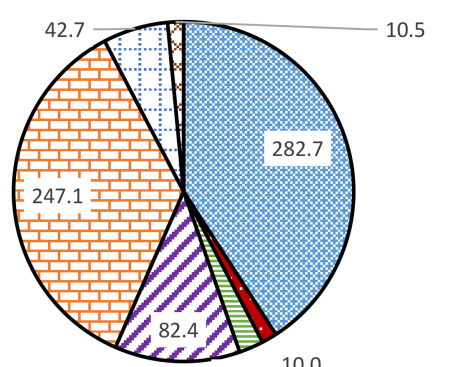
| | Mass loading in WWTPs (mg/day/1000 inhabitants) | Environmental Emission (mg/day/1000 inhabitants) |
|---|---|---|
| WWTP 1 |  |  |
| WWTP 2 |  |  |
| WWTP 3 |  |  |

Figure 4

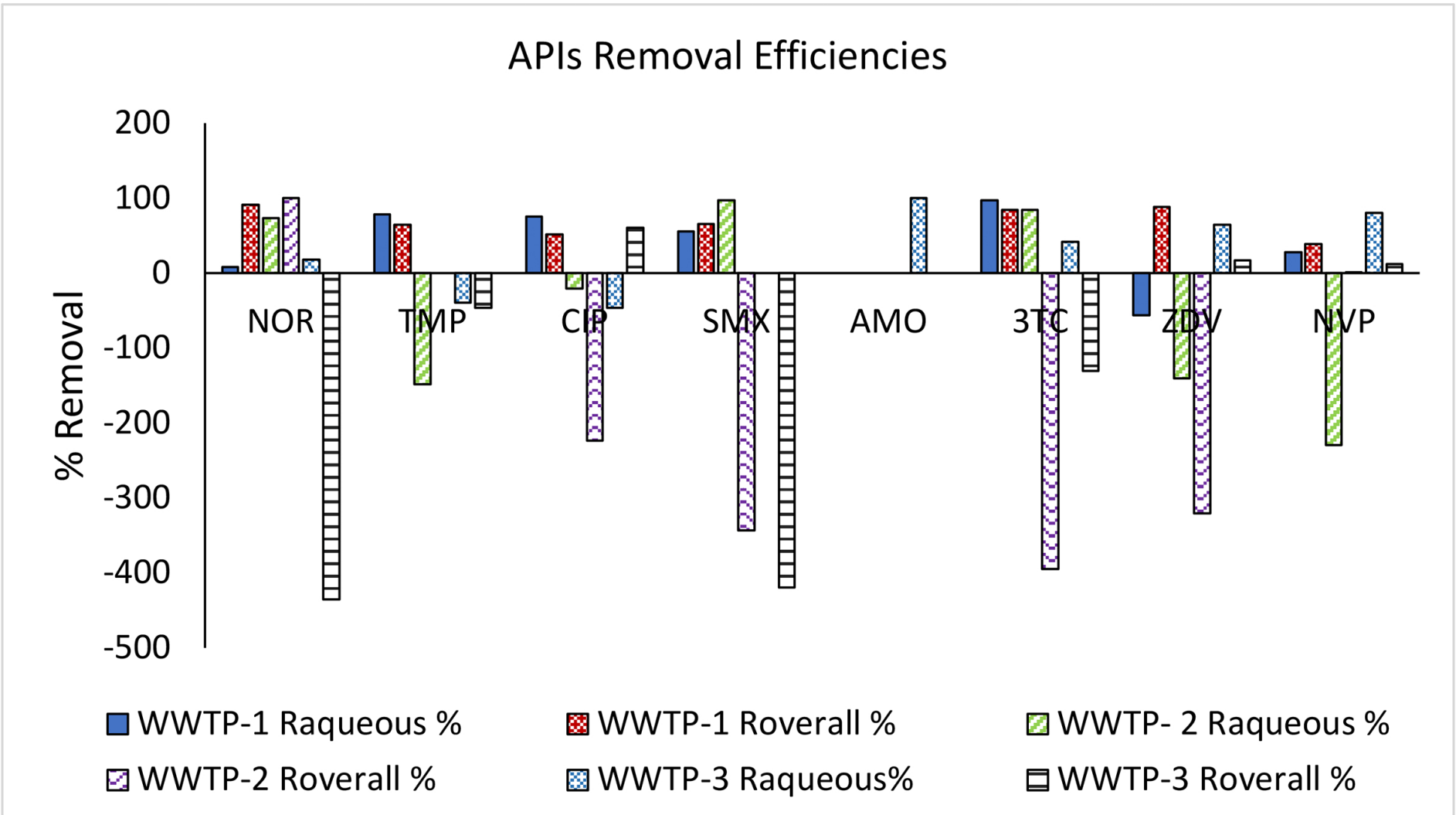
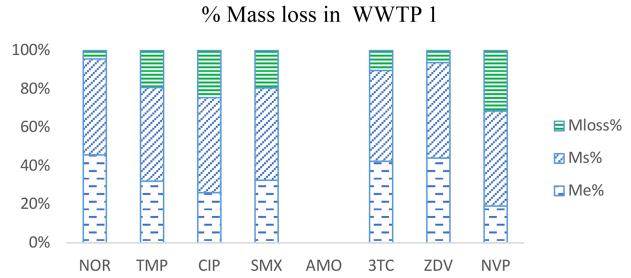
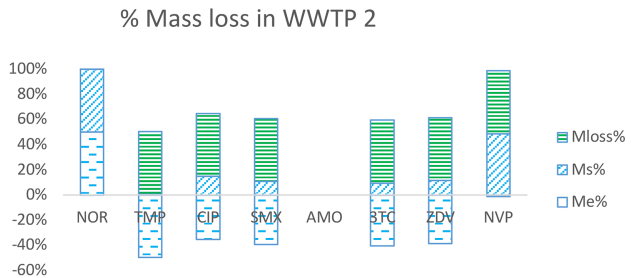

APIs Removal Efficiencies
200
100
0
-100
-200
-300
-400
-500
% Removal
NOR
TMP
CIP
SMX
AMO
3TC
ZDV
NVP
WWTP-1 Raqueous %
WWTP-1 Roverall %
WWTP- 2 Raqueous %
WWTP-2 Roverall %
WWTP-3 Raqueous%
WWTP-3 Roverall %

Figure 5


% Mass loss in WWTP 1
100%
80%
60%
40%
20%
0%
NOR
TMP
CIP
SMX
AMO
3TC
ZDV
NVP
Mloss%
Ms%
Me%


% Mass loss in WWTP 2
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
NOR
TMP
CIP
SMX
AMO
3TC
ZDV
NVP
Mloss%
Ms%
Me%

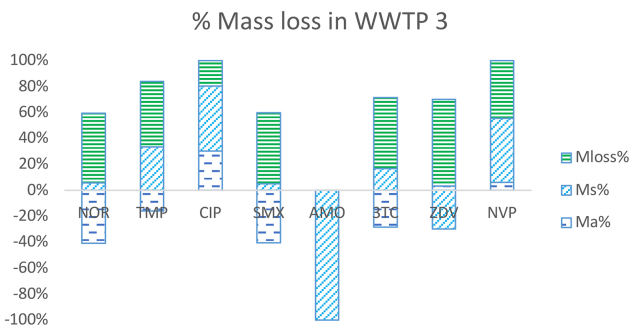

% Mass loss in WWTP 3
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
-80%
-100%
NOR
TMP
CIP
SMX
AMO
3TC
ZDV
NVP
Mloss%
Ms%
Ma%

Figure 6