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Modelling ecological risks of antiretroviral drugs in the environment

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ABSTRACT

The success of the antiretroviral therapy (ART) programme to manage HIV/AIDS in Sub-Saharan Africa (SSA) has inadvertently led to the release of antiretroviral (ARVs) into the environment. Consequently, ARVs have been detected in different countries across the globe, with the highest measured environmental concentrations in the SSA countries. Herein, we quantified ecological risks of ten regimen ARVs (six and four in first and second regimes, respectively) into environmental matrices in four spatial regions in Eswatini, namely: Manzini, Hhohho, Lubombo, and Shiselweni. Ecological risks (expressed as risk quotient (RQ)) were determined for different geographical regions by comparing the predicted environmental concentrations (PECs) to the predicted no effect concentrations (PNECs). PNECs were derived from ecotoxicological data generated using the Ecological Structure Activity Relationships (ECOSAR) model. PECs of ARVs in surface water in the Lubombo and Shiselweni regions were three-fold higher compared to those of the Manzini and Hhohho regions with RQs of three ARVs exceeding 10 (RQ > 10) to three taxa (fish, daphnia, and algae). ARVs of concern to the three taxa were ranked in descending order based on both acute and chronic toxicity based on RQ values as efavirenz (EFV) > lopinavir (LPV) > ritonavir (RTV) (all with RQs > 10). Two second regime ARV drugs (RTV and LPV) posed the highest risks to aquatic taxa though they had the least PECs, but were highly toxic with PNECs <1 μg/L. Due to dearth of toxicity data for ARVs on bacteria, their risks in wastewater (with the exception of TDF) could not be established. Results of this study are the first to quantify risks of ARVs in the environment using a modelling approach. The developed model can therefore serve as a first-tier screening tool. In addition, the results raise the need to examine the likelihood of antiviral resistance of ARVs linked to their high environmental concentrations.

1. Introduction

By 2021, approximately 38 million people were living with HIV (PLWHIV) worldwide, with 70% in the African continent [1], followed by 10% in South-East Asia, and 20% in other regions [1]. In addition, 66% of PLWHIV live in Sub-Saharan Africa (SSA) (thus, region with high HIV prevalence) where for example, Mozambique, Nigeria, Tanzania, Kenya and Zimbabwe each country with over a million PLWHIV, and South Africa with some 7.5 million infections [2]. HIV prevalence is the ratio of PLWHIV to the total population in a specified area at a specific time [3]. Therefore, globally Eswatini (formerly known as Swaziland) has the highest HIV prevalence at 27% (Fig. S1) [4]. Eswatini quantitatively has lower PLWHIV compared to South Africa but has a higher proportion of its population infected by HIV/AIDs.

The introduction of antiretroviral (ARV) drugs in 1987 to manage HIV using alternating drug combinations [5] has proven to be highly effective, for example, in rehabilitation of patients health under antiretroviral therapy (ART) after treatment initiation [6,7], and subsequent reduction in HIV/AIDs-related deaths [8]. On average, ARVs have been found to reduce the infectiousness of HIV/AIDS by some 92% – an estimate that incorporates real-world barriers to suppression including adherence and undetected drug resistance [9].

In the revised World Health Organization (WHO) guidelines on ART eligibility; the recommendation is that ART administration should be to every individual who tests positive for HIV regardless of their immunological status (Test and Start) [10]. In turn, this has exacerbated ARV treatment, especially in the SSA [11,12]. Subsequently, a large portion of SSA countries have adopted the 'Test and Start' policy in pursuit to attain the Joint United

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Nations Programme on HIV/AIDS (UNAIDS) 90–90-90 targets [13]. For example, between 2013 and 2018 estimates suggest that the number of patients under ART in Ivory Coast, Kenya, Lesotho, Malawi, Namibia, South Africa, Tanzania, and Uganda (all SSA countries) increased by about two times from 5,190,275 in 2013 (prior to the Test and Start program) to 9,240,111 in 2018 [14]. Globally, by 2020 Eswatini had the highest HIV prevalence (27%) but also the first country to achieve >95% of PLWHIV on ART, and therefore, being the first country to attain the 95–95-95 target [15].

ARVs are among frequently detected pharmaceuticals in regions with high HIV/AIDs prevalence [16,17]. This is because ARVs are not completely assimilated by the human body upon ingestion, and as a result, a portion of the parent drug and/or active metabolites are excreted via urine and faeces [18]. Dolutegravir (DTG), for example, has an excretion rate of 95%, and therefore, a large portion is eliminated from patients unaltered [19]. For this reason, ARVs are ubiquitously present in different environmental matrices due to incomplete elimination in wastewater treatment plants (WWTPs), from run-off linked to areas with no sanitation services, or improper disposal of unused ARVs [20]. With an exception of urban areas, large parts of the African continent primarily have limited or lack safely managed sanitation services [21], and where available, generally they are decentralized or onsite. This implies wastewater from peri-urban and rural areas are directly released untreated into surface and ground water systems with concomitant widespread pollution including that of ARVs [22–24].

Until now, ARVs have been quantified across the globe in different aquatic matrices mostly in WWTPs (Table S1), surface water (Table S2), groundwater (Table S3), and tap water (Table S4). Notably, the highest measured environmental concentration (MECs) are documented in the SSA – a region with the highest HIV/AIDS incidence globally [25–27]. Yet, at present in the SSA region, MECs of ARVs are limited and only documented in three countries (Kenya, South Africa, and Zambia) due to high analytical costs (Fig. S3). Therefore, in order to estimate the potential risks of ARVs in the SSA countries, it is practical to use modelling approaches to estimate predicted environmental concentrations (PECs) [28,29], and in turn, quantify their ecological risks.

Herein, the objective was to model the concentrations of ten ARVs in the environment, and their potential risks to organisms in surface water and wastewater compartments. To the authors' knowledge, the model provides the first assessment on the potential risks of ARVs to aquatic organisms using the modelling approach. This was done by comparing PECs to the predicted no effect concentration (PNECs).

2. Materials and methods

2.1. System background

The modelling of ARVs exposure in the environment was done following the European Chemical Bureau (ECB) approach [30]. The PECs were determined using the material flow analysis (MFA) approach with Eswatini as the case study country. ARVs flows were estimated for four regions, namely: Manzini, Hhohho, Lubombo, and Shiselweni of Eswatini with a temporal boundary of one year (2017). Reasons for the choice of country are outlined in the supplementary information (SI 3). The mass flows of ARVs for the first and second regime drugs, namely: atazanavir (ATV), efavirenz (EFV), lamivudine (3TC), lopinavir (LPV), didanosine (DDI), nevirapine (NVP), ritonavir (RTV), zidovudine (AZT), tenofovir (TDF) and DTG were estimated in surface water systems with WWTPs and runoff as the sources (Fig. S4). In Eswatini, both pit latrines and septic tanks serve about 72% of the population. In this study, possible flows of ARVs through groundwater with potential resultant contamination in surface waters [31] were not considered. This is due to lack of leachate values for ARVs into groundwater. Remarkably, septic tanks and pit latrines are known to leach out pharmaceuticals into groundwater resources [32–34]. Additionally, ARV flows into the aquatic environment through use of sludge for soil improvement was not considered. This is because sludge is not used for agricultural purposes in Eswatini.

2.2. Estimation of ARV flows

ARV flows were estimated in two compartments: wastewater and surface water. Each system was considered to be at steady state (homogenous). The key model input data used to quantify ARV flows were the number of PLWHIV, number of patients on ART, drug dosages per day, treatment adherence, drug excretion rate, specific drug use probability, sanitation access in the country (population connected to wastewater treatment systems), and the dilution factor (DF). Data on the number of PLWHIV was sourced from the University of Washington (UW) [35]. Next, data on the percentage of the population under the ART programme was sourced from UNAIDS [36], the daily drug dosage [37] under different treatment regimens employed in the country [38], and lastly, the excretion rate for each ARV was retrieved from technical and scientific literature, and are summarized in Table S5.

To improve the granularity of the model, each ARV's quantities were estimated and the constituencies (55 in total) were used as the basic areas. The constituencies in Manzini, Hhohho, Lubombo and Shiselweni were 16, 14, 11, and 14, respectively. Thereafter, masses obtained in constituents in a given region were aggregated to obtain total mass per region. The Manzini and Hhohho regions were classified as urban, with habitants having higher access to WWTP infrastructure compared to the latter two regions deemed asrural settings. The quantities of ARVs for each region were estimated using the expression:

$$M_{ARVSi,r} = \sum_{i=1}^{n} M_{ARVi,r} = PLWHIVr \times \text{ART coverage} \times \text{daily dosage}_i \times \text{DER} \times \text{DUP} \times \%\text{Adh} \times 365 \quad (1)$$

where $M_{ARVSi,r}$ is the sum of the masses of ARV i per region with n as the number of constituencies in a given region (in kg), PLWHIV is the number of people living with HIV/AIDS in a given constituency (whether urban or rural); daily dosage$_i$ is the quantity of an ARV$_i$ consumed per day by patients on ART in Eswatini; ART$_C$ is the percentage of the population under ART relative to the total number of PLWHIV; DER is the drug excretion rate for a specific ARV$_i$ (expressed as a percentage); DUP is the drug use probability by a portion of PLWHIV either under first or second regime drug treatments, and percentage adherence is the ratio of people on ART as per the prescription without default. Details of the model parameters are summarized in Table S5.

2.3. Ecological risk assessment

The ecological risk assessment of ARVs was predicted following the European Medicines Agency (EMA) guidelines [39] by calculating the risk quotient (RQ) of each individual compound. This was done in two environmental matrices (in wastewater and surface water) using the expression:

$$RQ = \frac{PEC_{i,k}}{PNEC_i} \quad (2)$$

where PEC$_{i,k}$ is the predicted environmental concentration of ARV$_i$ in compartment k and the PNEC$_i$ is the predicted no effect concentration of ARVs$_i$. PNECs were calculated using an assessment factor (AF) of 1000 [38] associated with high uncertainty of toxicity data for different taxa (fish, algae, and daphnia). Ecotoxicity data was estimated using the Ecological Structure Activity Relationships (ECOSAR) model. The PEC and PNEC values were determined following the procedures described in the supporting information (SI) in sections SI 4.4, and SI 4.5, respectively.

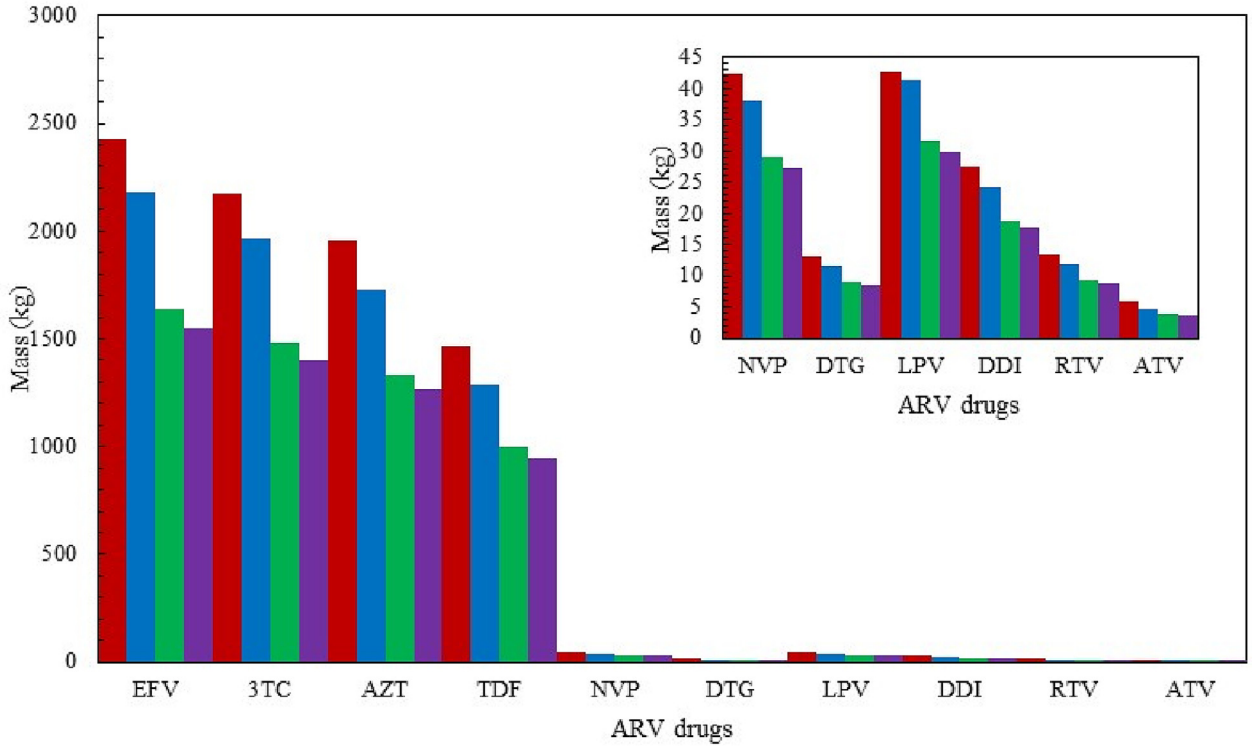

Fig. 1. Estimated quantities of the first and second treatment regimes of ARVs in different environmental matrices from four regions in Eswatini, namely: Manzini (red), Hhohho (blue), Lubombo (green), and Shiselweni (purple). The plotted values are the medial release scenario as were considered representative of likely ARV releases into the environment.

3. Results and discussion

3.1. Quantification of ARV flows

Quantities of ARVs released from the four regions of Eswatini are summarized in Fig. 1. Results indicate high variability between released quantities, and dependent on the number of HIV/AIDs patients under ART, access, or lack thereof to sanitation services, daily dosage, among other factors. In addition, the results demonstrate higher quantities of ARVs (irrespective of type) were released from urban regions (Manzini and Hhohho) into the environment. This is because their habitants accounts for about 62% of the Eswatini population [40]. For example, EFV (each region had >2000 kg) compared to those from the rural regions (each region had <1700 kg). This was attributed to differences in HIV/AIDs patients as well as population distribution disparity between urban and rural regions in Eswatini. This, in turn, points to a high portion of ART patients being resident in urban areas, and therefore consistent with data retrieved from the UW database [35].

In addition, quantities of ARVs released into the environment are also dependent on success rates for the ART coverage programme. For example, ART coverage increased from 48% in 2012 to 84.7% by 2017 [41]. Despite the HIV prevalence drop from 32% [34] to 27% [41], the number of patients under ART almost doubled in eight years reaching 90% by 2020 [42]. Based on the model inputs, ARVs released in high quantities were EFV, 3TC, AZT, and TDF (Fig. 1). These four drugs are used as first treatment regime drugs under different alternating combinations in Eswatini – a regime used on a preponderance portion of PLWHIV under the ART programme [38,43]. Our results indicate that three ARVs released into the environment in the four regions each exceeded a tonne (Fig. 1). EFV had the highest released quantities into the environment in all four regions o compared to other drugs – and especially in the Manzini region (2429 kg) (Fig. 1).

The release of high quantities of EFV were associated with a high administered dosage per day (600 mg/d) and an excretion rate of 62% [44]. Similar findings were observed for AZT linked to both its high daily dose prescription of 600 mg/d [45], and excretion rate of 75% [46]. In addition, AZT is used to prevent mother-to-child HIV transmission (PMTCT) in Eswatini [47–49]. Overall, the high release of AZT was due to the drug's high excretion rate (75%) [50] and high use as part of pre-exposure prophylaxis (PrEP)- to prevent HIV transmission [51,52]. 3TC is the most used ARV drug both in the first and second regime treatments in Eswatini, but was released at lower quantities when compared to EFV, yet it has a slightly higher excretion rate of 70% [53]. This was associated with a lower administered daily dosage (300 mg/d) [54] compared to the higher one of EFV (600 mg/d), and has relatively higher removal efficiency in WWTPs than the latter drug (Table S6).

Although both NVP and DTG are first regime drugs but were released at very low quantities into the environment irrespective of the region (< 14 kg). The reason being DTG is relatively a new drug in the management of HIV/AIDs [55,56], and is administered at a low daily dosage (100 mg/d) [57]. In turn, this may account for the lower releases relative to other first treatment regime drugs although almost all the drug is excreted from the body with only <2% adsorbed into the patient [58,59]. Conversely, NVP is predominantly assimilated in the patient's body, which ultimately leads to very low quantities of about 0.27% ending up into the environment [60], and therefore accounts for low estimated quantities of <42 kg (Fig. 1).

Estimates for the second treatment regime drugs were very low (< 45 kg) irrespective of the region, with LPV having the highest estimated quantities (Fig. 1). This is because of the drug's high daily dosage of 800 mg/d as well as higher excretion rate of 21% [61], for example, compared to 12.4 and 7% that of DDI [62] and ATV [63], respectively. Conversely, ATV had the least estimated releases into different environmental matrices in the four geographical regions of Eswatini (Fig. 1). Overall, these estimated quantities may partly account as to why second regime drugs are generally detected at very low concentrations in different environmental compartments globally [16,64]. This is plausibly for two-fold reasons. First, the low percentage of PLWHIV under the second treatment

regime (Table S7) e.g., for Eswatini was about 6.5% by 2017 [38]. Secondly, due to likelihood on the formation of transformation products (TPs) from these drugs although to date it is a poorly researched area.

3.2. Predicted no effect concentrations

PNECs were calculated using E(*L*)C_{50} values derived from acute and chronic toxicities to different taxa depending on the matrix of focus and by use of an AF [65]. At present, experimental toxicity data for ARVs are limited [16,64,66], with EFV being the most investigated drug to aquatic organisms at different trophic levels. For example; Almeida and colleagues exposed EFV to *Raphidocelis subcapitata* (algae) and *Ceriodaphnia dubia* (daphnia), and results demonstrated the drug to be highly toxic to both organisms with IC_{50}s of 34 μg/L and EC_{50} of 26 μg/L, respectively [67]. In other works, EFV effects were found to range from harmful to highly toxic to green algae with EC_{50}s in the range from 12 to 96,900 μg/L, and very toxic to blue algae (760 μg/L) [68]. Exposure of fish to a low concentration of EFV at 0.0103 μg/L was observed to severely damage the liver of *Oreochromis mossambicus* after 96 h [69].

In this work, E(*L*)C_{50} values were estimated using the toxicity predictive software ECOSAR (v2.0) for three taxa (fish, daphnia, and algae), and then were used to estimate PNEC values. PNECs derived using ECOSAR model are summarized in Fig. 2, and indicate five ARVs (LPV, RTV, EFV, NVP, and ATV) are highly toxic to the three taxa with PNECs <1 μg/L. The three drugs (LPV, RTV and ATV) are in the same therapeutic class – the protease inhibitors (PIs), and their hazard data indicate they are potentially highly toxic on the aquatic taxa. PIs have also been found to exhibit intolerable levels of toxicity to humans [70,71]. Conversely, EFV and NVP both non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs are less toxic to humans [72]. PNEC results also suggest that the toxicity of the two PIs (LPV and RTV) were one order of magnitude higher than that of NNRTIs (EFV and NVP) irrespective of the non-target taxa in question (Fig. 2). Conversely, four nucleoside reverse transcriptase inhibitors (NRTIs) (3TC, DDI, AZT, and TDF) had least toxicity (PNECs >100 μg/L) with daphnia as the most susceptible taxa.

ECOSAR model results indicate the potential toxicities of 3TC based on the PNECs were 47,662, 7927, and 332 μg/L to fish, algae, and daphnia (Fig. 2), respectively. Similar to our finding, 3TC was found to be moderately toxic to *C. dubia* and *R. subcapitata* with an EC_{50} of 1345 μg/L and IC_{50} 3103 μg/L, respectively [67]. AZT was observed to be moderately toxic on both *C. dubia* and *R. subcapitata* at EC_{50} of 5671 μg/L and IC_{50} of 5442 μg/L, respectively [67]. In another article, the toxicity of TDF was assessed on *Artemia salina* (marine organism), and the drug was found to be non-toxic with IC_{50} of 111,820 μg/L [71]. Since the species used in the experiment were not freshwater organisms, these experimental results were therefore not used in our model. Conversely, results for *Daphnia magna* toxicity studies demonstrated 3TC to be highly toxic to daphnia, where the tests yielded 100% mortality following 48 h exposure to 100 μg/L of 3TC [74].

Overall, toxicity data suggest that fish, algae, and daphnia taxa are all highly sensitive to LPV, RTV, EFV, and NVP (Fig. 2). Additionally, the hazard data estimated using the ECOSAR model indicated all four ARVs were highly toxic; however, available experimental-derived ecotoxicity data primarily is for EFV as earlier mentioned. Three taxa (fish, algae, and daphnia) investigated herein exhibited lower levels of sensitivity to the remaining six ARVs (except the four: LPV, RTV, EFV, and NVP) – with daphnia as the most sensitive. At the time of writing the paper, only a single toxicity study on bacteria had been reported, and for one ARV as documented by Silva and colleagues [73]. Results indicated that TDF is toxic to the bacteria *Aliivibrio fischeri* widely ubiquitous in aerobic wastewater treatment processes (IC_{50} of 14,830 μg/L) [73]. This, in turn, can plausibly induce adverse implications to the biological treatment processes in WWTPs including enhancing inefficient removal of different classes of pollutants.

3.3. Predicted environmental concentrations

PECs for ARVs used under ART in Eswatini were estimated using quantities released into the environment in each of the four regions (Fig. 1). Here, the PECs were estimated in wastewater and surface water based on quantities described in section SI 4.4. This was on the assumption that each ARV was equally distributed (at steady state) in the investigated compartments. Higher quantities of ARVs were likely to be present in the effluent post-treatment stage due to low removal efficiencies, with eventual entry into surface water. This assumption is plausible as is supported by evidence of low removal efficacies of ARVs in many WWTPs in Africa (as low as 3%) (Table S6).

3.3.1. PECs of ARVs in wastewater

The Manzini region had the highest estimated PECs in both the influent and effluent, with higher values in the former. Four ARVs (EFV, 3TC, AZT and TDF) had the highest PECs in the influent at concentrations >150 μg/L, and therefore, about two orders of magnitude higher relative

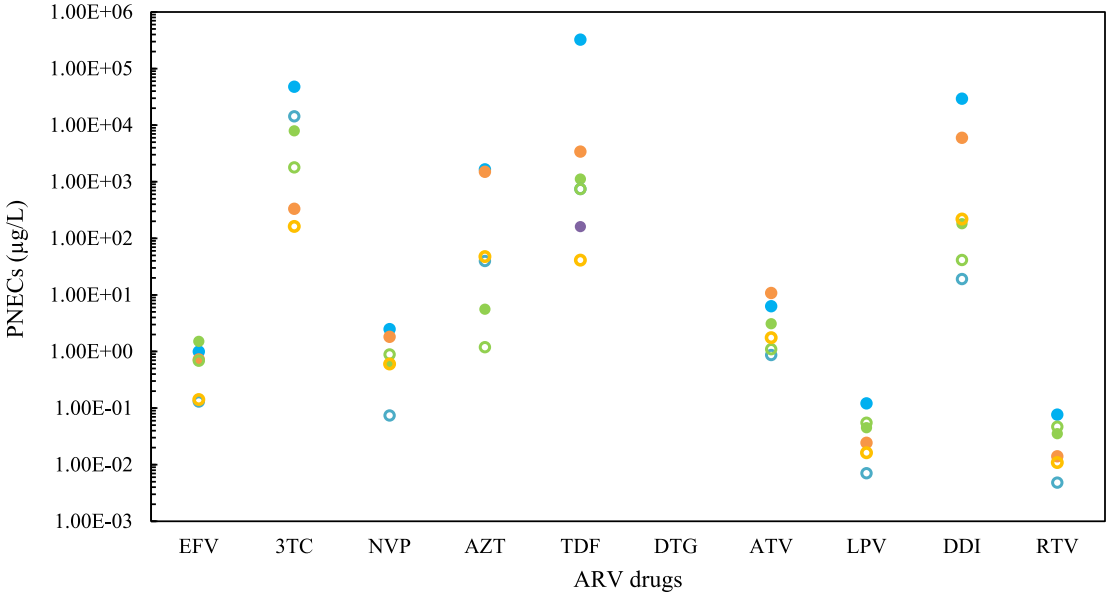

Fig. 2. Calculated PNECs for ARV drugs using acute (full circle) and chronic (open circles) toxicity data for fish (blue), algae (green), daphnia (orange), and bacteria (purple).

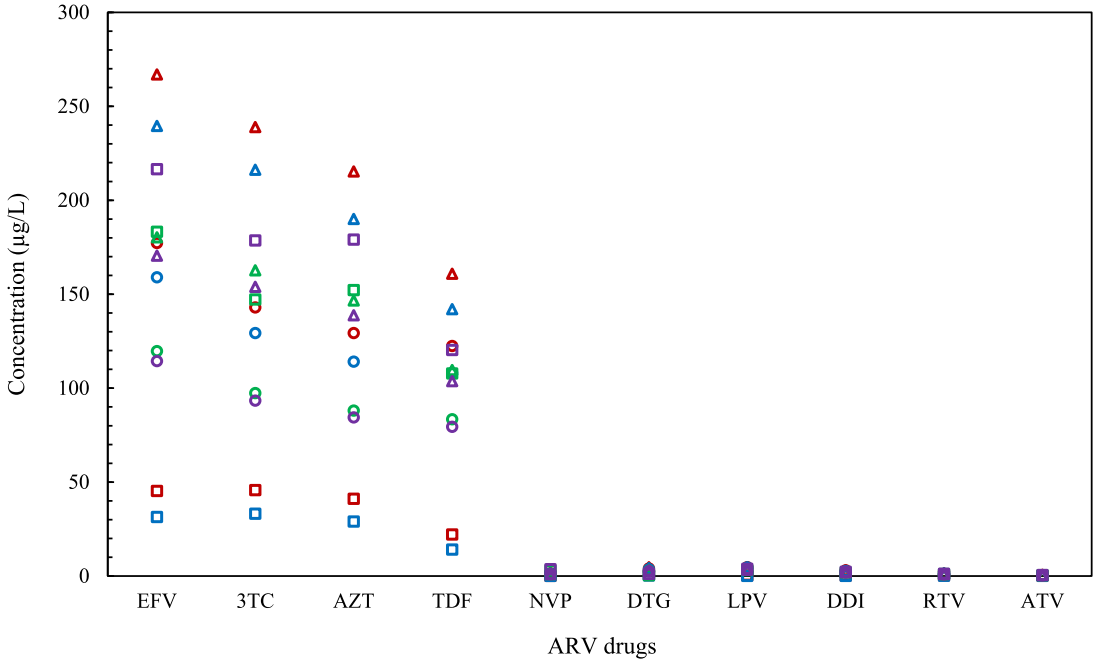

Fig. 3. Estimated PECs of different ARVs in the influent (triangles), effluent (circles) and surface water (squares) for the Hhohho (blue), Manzini (red), Lubombo (green) and Shiselweni (purple) regions of Eswatini.

to other ARVs (PECs <5 µg/L) (Fig. 3). These results are in good agreement with the use trends of ARVs, and previously measured environmental concentration (MECs). For example, very high MECs of these ARVs (Table S1) were documented in both the influent and effluent. In Kenya, for example, [24,66,75] MECs of AZT, 3TC and EFV were even above the predicted values herein for Eswatini. No significant differences in PECs of EFV and 3TC in both the influent and effluent were observed, and these findings depicted similar trends as for MECs documented in Kenya [24] and South Africa [76].

In several studies, the documented MECs were found to be higher in the effluent compared to the influent. For example, Mlunguza et al. [77] detected higher concentration of EFV in the effluent (3.63 µg/L) compared to the influent (1.37 µg/L). Schoeman et al. [78] obtained similar results and concluded the increase in EFV occurred after the chlorination process. This phenomenon has also been observed in many other pharmaceuticals, e.g.: acyclovir [79] as well as for diclofenac and carbamazepine [80]. This is unique in that EFV is hydrophobic with a log K_{ow} value of 4.7 [81], and low water solubility [82]; and therefore, should easily attach to flocculants, and be eliminated through sludge [78]. Previously, increase of parent compounds during the wastewater treatment process owing to the deconjugation of their TPs and metabolites has been attributed to this phenomenon, resulting to higher concentrations of the parent drug in the effluent compared to that in the influent [78,82]. For the ARVs, this aspect remains unclear and therefore, merits further investigation.

In the Manzini region EFV had the highest concentration in the effluent at 177 µg/L, and the least was for thef ATV (0.42 µg/L). Previously, EFV was detected in both influent and effluent in countries like Kenya, South Africa, and Zambia (Table S1 and S2). Mtolo et al. [83] reported higher concentrations of EFV (120.7 µg/L) in effluent than in surface water (0.380 µg/L) [77]. Notably, South Africa has the highest cases of PLWHIV globally (Fig. S2), and the largest ART program (with about 7 million patients on ART). However, the documented concentrations of ARVs in different aquatic systems (Fig. S3(b)) [17,78,84,85] were lower than the predicted concentrations in Eswatini. This is because a large portion of the population in the former country has access to sewage services, and therefore, may account for lower ARV concentrations in surface water. PEC results of AZT and 3TC showed they were reduced during the wastewater treatment process. For example, AZT and 3TC were reduced from 215 µg/L and 238 µg/L in the influent to 129 µg/L and 142 µg/L, respectively, in the effluent. Our results are similar to MECs for both ARVs in Zambia [34], but also differ markedly with values obtained in Kenya [24] as in the latter country they were almost completely removed (> 99%) (Table S6).

Notably, although the PECs for ARVs in the influent and effluent in Eswatini were <300 µg/L (Fig. 3), when compared to MECs reported in South Africa the former country's values were remarkably higher. This is astonishing since South Africa has 35-fold higher the number of people on ART compared to Eswatini. Remarkably, the estimated PECs in Eswatini were similar to the MEC values (>100 µg/L) reported in Kenya and Zambia. This is largely associated with a lack of sanitation services in Kenya and Zambia [24,34]; as similarly was observed to be the case in Eswatini. This raises the need for improved WWTP infrastructure in the SSA region especially given the high use of ARVs compared to other regions globally. The proposed approach can aid to achieve a fine balance between provision of health care to HIV/AIDS patients and protection of the aquatic environment. Furthermore, the high PECs of ARVs in influent in Eswatini may be associated with yearly cumulative regional PECs compared to MECs obtained from grab samples from specific locations in the SSA countries.

3.3.2. PECs of ARVs in surface water

Of the four probable scenarios based on DF used in this work (DF = 1, 3, 10, and 40), herein results for the PECs at DF = 3 are presented and discussed (Fig. 3). The year 2017 was drought-prone [86] with annual rainfall of 400–1200 mm, which in turn implies it had minimal to no precipitation [87]. Therefore, a DF < 10 [29] was selected and used in this study. Estimated PECs in surface water systems for different ARVs ranged from <1 to 220 µg/L (Fig. 3) and were generally higher in rural regions (Lubombo and Shiselweni) compared to urban regions (Fig. 3). This was associated with a lack of WWTPs in rural Eswatini; and therefore, accounts for the high concentrations of ARVs released into the aquatic environments.

This is plausibly due to three-fold reasons. First, 7 % of Eswatini's population in rural areas lack access to any form of sanitation [88], a similar aspect was observed in the slums of Kisumu [24], Nairobi [75], and Kibera [66] (all regions in Kenya) where high MECs of ARVs were detected. Secondly, rural regions (Lubombo and Shiselweni) have limited or no access

to WWTP infrastructure (< 10%) [89], therefore inevitably, raising the likelihood of high concentrations of ARVs in the rural aquatic environments. Finally, incomplete removal of ARVs in the effluent post-treatment processes before eventual release into surface water (Table S6); may also account for elevated estimated PECs of ARVs in surface water relative to other environmental matrices.

Similarly, four ARVs (EFV, 3TC, AZT, and TDF) estimated at elevated concentrations in the influent were also the highest in surface waters (Fig. 4). In addition, the same drugs were widely detected in surface waters across the SSA region (e.g., Kenya, South Africa, and Zambia). For example, very high concentrations of 3TC ≈ 150 μg/L were detected in River Ngong, Kenya [24], and are in the same range to those estimated in this study in the Lubombo (183 μg/L) and Shiselweni regions (216 μg/L). In sharp contrast, the highest MEC of 3TC in surface water in South Africa was 0.24 μg/L [17]. Therefore, higher concentrations in Eswatini and Kenya are justifiable and dependent on multifactorial factors including high HIV prevalence [89], high success of the ART programme [90], and poor sanitation services infrastructure [91,92].

3.3.3. Risk assessment

Here, ecological risks posed by ARVs in the four regions of Eswatini were estimated only in surface water. This was due to a lack of ecotoxicity data for organisms in the wastewater and soil compartments. In surface water, two scenarios were investigated based on acute and chronic effects of ARVs to aquatic organisms. To interpret and categorize ARVs ecological risks, here the Lemly framework [93] was adopted: with RQ < 0.1, 0.1 ≤ RQ < 1, 1 ≤ RQ < 10, and RQ ≥ 10, thus signifying none, low, moderate, and high risks, respectively. Results indicate that regions with limited access to WWTPs infrastructure (Lubombo and Shiselweni) had high ARV concentrations in surface water compared to regions with access to sanitation services (Hhohho and Manzini). These results further highlight the need for improved sanitation services. Our results indicate that ARVs pose wide levels of risks to different taxa in surface water spanning several ten orders of magnitude ($10^{-6} < RQ < 10^{4}$) based on RQ values (Fig. 3). For example, ARVs like 3TC, TDF and DDI posed no to low risk in all four regions. Conversely, EFV, LPV, and RTV were observed to pose moderate to very high risks to the three taxa in all four regions, irrespective of the exposure duration (acute or chronic). Additionally, the three ARVs (EFV, LPV, and RTV) have log K_{ow} values >4.5, and therefore, may be of environmental concern due to their high ecotoxicity and persistence since their log K_{ow} values >3 [94].

The chronic toxicity-based risks were generically one order of magnitude or greater compared to acute-associated risks. The chronic ARV risks ranked in descending order were as follows (for the top five ARVs): EFV > LPV > RTV > NVP > AZT. The estimated ecological risks ranged from moderate in the Hhohho and Manzini regions to high levels in the Lubombo and Shiselweni regions irrespective of the taxa type (Fig. 4). Similarly, ecological risks based on acute toxicity followed a pattern similar to that of chronic risk, with EFV having RQ values >10 to all taxa for Eswatini as a whole, irrespective of the region. Results herein are in good agreement with works of Almeida and colleagues where EFV yielded RQ > 1 [67]. Two ARVs (LPV and RTV) posed the highest risks based on chronic toxicity to fish in the Hhohho region; fish, and daphnia in the Manzini region, and to the three taxonomic groups in the Lubombo and Shiselweni regions. Similar trends were observed for acute toxicity-based risks in the Lubombo and Shiselweni regions; yet LPV and RTV had the least PECs irrespective of the region (> 5 μg/L) compared to other ARVs due to their high toxicity.

RQ results of LPV, RTV and ATV did not show marked difference between acute- and chronic-associated risk to algae and daphnia irrespective

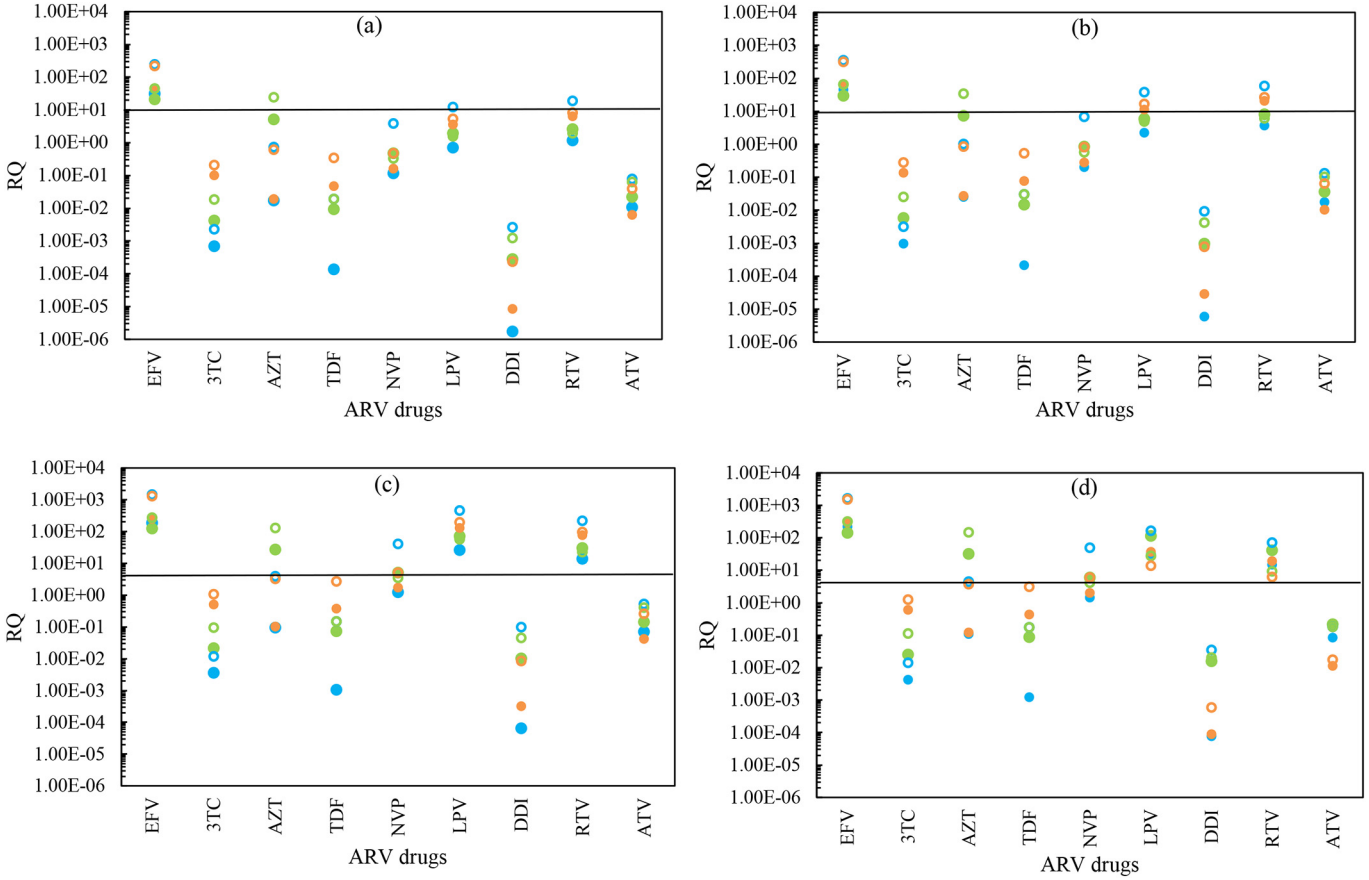

Fig. 4. RQs for surface water calculated using acute (full circles) and chronic (open circles) toxicity of ARV drugs to fish (blue), algae (green), and daphnia (orange) for the Hhohho (a), Manzini (b), Lubombo (c) and Shiselweni (d) regions in Eswatini.

of the region in question. A plausible explanation is that these drugs belong to the same class (PIs), and therefore, have the same mode of action to non-target organisms. Previously, high risks of RTV were observed to organisms at different trophic levels with algae as the most susceptible species with high RQ values of 52.6 [95] and 280 [96]. Elevated ecological risks of second regime ARVs (LPV, RTV and ATV) are of concern in cases where ART patients develop drug resistance as their prescriptions are altered from first regime to second regime ARVs [97]. Therefore, this raises the need to improve the environmental management of ARVs especially in the developing countries as they have limited sanitation services; but also carry the highest burden of HIV/AIDs and rapidly expanding ART programmes.

Irrespective of the region of focus, AZT posed the highest risk to algae (acute risk, RQ = 7.35 and chronic risk, RQ = 34.5) compared to fish (acute risk, RQ = 0.03 and chronic risk, RQ = 1.03) and daphnia (acute risk, RQ = 0.03 and chronic risk, RQ = 0.86). Similarly in earlier works, AZT was observed to pose very high-risk levels to algae with RQ calculated using MECs in surface water. For example, results of Muriuki et al. [98] for the Ndaragu river, and Ngumba et al. [66] for the Nairobi River Basin (both in Kenya) demonstrated that AZT posed very high risks with RQs of 1702 and 271, respectively, on algae. Therefore, our results are in good agreement with current literature and imply that AZT may pose deleterious effects on algae in freshwater systems. As the algae are primary producers, elevated risks due to certain ARVs can impair their functioning with possible disruption of the food chain.

Due to the lack of ARVs ecotoxicity data for bacteria, except for TDF [99], the potential risk posed by ARVs in wastewater could not be determined. For TDF, results showed it presented no risk to bacteria with very low (none) (RQ = 0.001). Nonetheless, absence of evidence is not evidence of absence [100]; therefore, lack of ARVs hazard data does not imply they do not pose risks to aquatic organisms. This, in turn raises the need to generate hazard data required to support the potential risks or lack thereof of ARVs to aquatic organisms in different environmental matrices. Such data are of existential significance especially in SSA countries characterized by increasing efforts to expand the ART programme, and habitant to some known highly biodiverse ecosystems around the globe. In addition, the findings herein raise concerns for a country like Eswatini where some 40% of the population lack access to safe drinking water [101]. For example, the population rely on surface water for drinking and other domestic uses. This can either induce adverse effects on the health of PLWHIV as ARVs in drinking water may trigger antiviral resistance [102] as previously documented for other pharmaceutical drugs e.g., antibiotics [103,104]. As a result, this can cause deleterious effects on ART patients including severely compromising the effectiveness of current ARVs in the market. In addition, this may not only compromise the current ART programme but also may require the development of new set of drugs – which in the interim can result to widely compromised public health concerning HIV/AIDs management.

Therefore, to improve ecological risk assessment of ARVs, occurrence data covering broader spatial and temporal scales across the continent and hazard data are needed. As risk assessment method outputs are dependent on a confluence of multifactorial parameters including differences linked to geographical regions, climates, demographics, and cultural background [105], in addition to marked distinctions in technological and financial capabilities between countries, for example, concerning the treatment of wastewater. Therefore, results derived from this study are considered to offer a practical approach as first-tier risk assessment for ARVs in ecological systems – and can be extended to other SSA countries in support to improve the health of PLWHIV under the ART programme, and concurrently protect ecological health.

4. Conclusions

In this work, modelling results on the potential risks of ARVs using the RQ approach are presented in surface water due to lack of effects data for organisms in wastewater and soil matrices. Results indicate that the PECs of ARVs in surface water differ markedly from region to region with Lubombo and Shiselweni regions being three-fold higher compared to those in Manzini and Hhohho regions due to lack of sanitation services in the former regions. The model shows that ARVs may pose risks to freshwater aquatic life; where based on RQ-values three ARVs exceeded 10 (RQ > 10) to three taxa (fish, daphnia, or algae). ARVs of concern to the three taxa were ranked in descending order (based on RQs) as: EFV > LPV > RTV (RQs > 10). ARVs exhibiting highest risk (RTV and LPV) are second regime ARVs, and this implies that as higher number of HIV/AIDs are prescribed second regime drugs, this in turn raises the possibility of increased risks to aquatic life. This study is the first to quantify the ecological risks of ARVs using a modelling approach, and the model developed can serve as a first-tier rapid screening tool. Also, the results raise the need to examine the likelihood of antiviral resistance of ARVs linked to their high environmental concentrations. The findings from this work can be beneficial to decision-makers in Eswatini and other SSA countries, for example, in pursuit to improve quality of lives for PLWHIV under the ART programme, without compromising the aquatic life.

CRediT authorship contribution statement

P. Ngwenya: Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – original draft. **N. Musee:** Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial or personal interests that plausibly appeared to influence the work on this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enceco.2023.06.001.

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