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Uptake of selected antiretrovirals by pepper (*Capsicum annum*), radish (*Raphanus sativus*), and ryegrass (*Lolium perenne*) grown on two contrasting soils and fertilized with human urine-derived fertilizers

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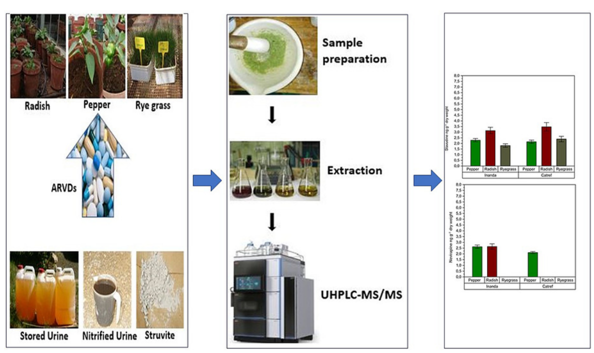
HIGHLIGHTS

- Plants fertilized with stored urine absorbed six of the nine target ARVDs.
- Nevirapine was the only ARVD detected in crops grown with nitrified urine and struvite.
- The ARVDs detected in the soils were significantly higher in the soil with high organic matter and clay content.
- Daily consumption of crops fertilized with stored urine does not pose a health risk to the consumer.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of urine-derived fertilizers has several economic and environmental advantages. However, there is concern that pharmaceutical residues present in urine could enter the food chain after plant uptake and pose potential risks to human and animal health. A pot experiment was conducted to evaluate the uptake of nine target antiretroviral drugs (ARVDs) by pepper (*Capsicum annum*), ryegrass (*Lolium perenne*) and radish (*Raphanus sativus*) grown in two soils of contrasting texture and organic matter content and fertilized with stored urine, nitrified urine concentrate (NUC), and struvite. Nevirapine was the only ARVD detected in crops grown with NUC and struvite on both soils, but the concentrations were below the limit of quantification. Plants fertilized with stored urine absorbed lamivudine, ritonavir, stavudine, emtricitabine, nevirapine, and didanosine, while abacavir, efavirenz and zidovudine were not detected. The ARVDs detected in the soils after harvest were significantly higher in the soil with high organic matter and clay content. To assess direct human exposure the estimated daily dietary intake (DDI) of ARVDs by consumption of the pepper and radish fertilized with stored urine was compared with the Threshold of Toxicological Concern (TTC) values based on the Cramer classification tree. The calculated DDI values for all ARVDs were about 300–3000 times lower than the TTC values for class III compounds. Therefore, daily consumption of these crops fertilized with stored urine does not pose a health risk to the consumer. Future research is required to assess the impact of ARVD metabolites, which may be more harmful to human health than the parent compounds.

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1. Introduction

The use of source-separated human urine as a fertilizer provides a viable option for managing waste, and minimising both environmental pollution and contamination of surface and groundwater. A variety of urine treatment systems have been developed with the goal of nutrient recovery, volume reduction, and pathogen and pharmaceutical elimination. In general, human urine is treated by either concentrating nutrients through eliminating water from urine or selectively extracting nutrients (Patel et al., 2020). Nutrient concentration techniques include membrane distillation, nitrification distillation, and forward osmosis, whereas nutrient extraction is accomplished through adsorption, ion exchange, stripping, and precipitation (Simha et al., 2020). Nitrified urine concentrate (NUC) is processed through biological nitrification and distillation. The nitrification process stabilizes the collected urine. The urine is oxidized into non-volatile nitrate (NO_3^-); and stabilized as non-volatile NH_4^+ (Udert et al., 2016). Struvite ($MgNH_4PO_4{\cdot}6H_2O$) is produced through urine precipitation, filtration, and drying (Li et al., 2019a). The latest scientific and technological achievements in the safe recovery of nutrients from human urine have been reviewed by Simha and Ganesapillai (2017) and Alemayehu et al. (2020). The use of stored urine as a fertilizer has been demonstrated in a variety of crops (Karak and Bhattacharyya, 2011; Pandorf et al., 2019; Alemayehu et al., 2020), while that of struvite was summarized in a meta-analysis review (Hertzberger et al., 2020). Nitrified urine concentrate was evaluated in hydroponics (Mauerer et al., 2018; Magwaza et al., 2020) and greenhouse studies (Bonvin et al., 2015; Mchunu et al., 2018). The efficiency of urine-derived fertilizers was reported to be comparable to that of mineral fertilizers for both nitrogen (N) and phosphorus (P) (Martin et al., 2020).

Although the use of urine-derived fertilizers has several economic and environmental advantages, treatment techniques are unable to completely remove pharmaceuticals, salts and heavy metals, so their widespread agricultural application remains problematic. However, due to a low heavy metal content in the human diet, their concentrations in urine-derived fertilizers have been reported as insignificant (Vinnerås and Jönsson, 2002). Additionally, (Antonini et al., 2012; Alemayehu et al., 2022) reported that heavy metals in human urine-derived fertilizers are much lower than in chemical fertilizers and far below the World Health Organisation's recommended limits for safe use of wastewater, excreta and greywater (World Health Organisation, 2006). A meta-analysis on the excretion pathways of 212 pharmaceuticals from the Swiss Pharmaceutical Compendium showed that 64 % of each pharmaceutical was excreted via urine while 35 % was via faeces (Lienert et al., 2007). This is of particular concern in fertilizers produced from source-separated urine as these pharmaceuticals could enter the food chain after plant uptake and pose potential risks to human and animal health (Abdel-Shafy and Mohamed-Mansour, 2013).

Plant studies have demonstrated the uptake of pharmaceuticals, however, in most cases plants were exposed to unrealistic concentrations (Carter et al., 2014). The anticonvulsant carbamazepine is the most studied pharmaceutical for uptake studies in various plant species (Goldstein et al., 2014; Malchi et al., 2014; Marsoni et al., 2014; Mordechay et al., 2018; Li et al., 2019b). Carbamazepine has been reported to be frequently detected in treated wastewater and biosolids (McClellan and Halden, 2010; Jelic et al., 2012), and to be relatively persistent in the environment (Mordechay et al., 2018). However, it is important to note that the use of pharmaceuticals is geographically specific and, consequently, location determines the type of pharmaceuticals detected at wastewater treatment plants (WWTPs) (Wood et al., 2015). Antiretroviral drugs (ARVDs) are a significant pharmaceutical class to consider for plant uptake studies in South Africa because the country has the highest use of ARV therapy in the world with approximately 5 million people receiving ARV treatment (UNAIDS, 2017). The presence of ARVDs has been reported at South African WWTPs (influent and effluent) (Abafe et al., 2018) and in surface waters (Wood et al., 2015). Nevirapine and efavirenz were detected in effluent sampled from WWTPs in Gauteng province (Schoeman et al., 2017), and Abafe et al. (2018) reported the persistence of atazanavir, efavirenz, lopinavir and nevirapine in effluent from selected WWTPs in KwaZulu-Natal province.

The aim of this study was to determine the uptake of some ARVDs, after application of urine-derived fertilizers, by pepper (*Capsicum annum* var. California Wonder), radish (*Raphanus sativus* var. cherry belle) and ryegrass (*Lolium perenne* var. Matilda) grown in two contrasting soils. Additionally, the exposure associated with the consumption of the pepper and radish was evaluated.

2. Materials and methods

2.1. Urine-derived fertilizers

Urine used for this study was collected from household urine diversion toilets in KwaMpumuza, Pietermaritzburg, KwaZulu-Natal, South Africa. The urine was stored for approximately two months before use at a temperature range of 20–25 °C. The struvite and NUC were produced at the Newlands Mashu research facility, Durban, South Africa (29° 46′ 3.94″ S; 30° 58′ 44.16″ E), following procedures described by Udert et al. (2016). The fertilizers were analysed for their chemical properties by following standard methods for water and wastewater analysis (Rice et al., 2012). The total N was determined with a Spectroquant® NOVA 60 photometer (Merck Millipore, Germany). Selected properties of the urine-derived fertilizers used are given in Supplementary Information Table S1.

2.2. Soils

Samples were collected at a 0–30 cm depth of a clay loam (Inanda (Ia) form; Rhodic Hapludox) and a sand (Cartref (Cf) form; Typic Haplaquept) (Soil Classification Working Group, 2018; IUSS Working Group WRB, 2015, respectively). The Cf was collected from Kwadinabakubo, South Africa (29° 44′ S; 30° 51′ E) under natural grassland and the Ia was from World's View, Pietermaritzburg, South Africa (29° 35′ S; 30° 19′ E) under a pine plantation. The soils were air-dried and sieved to <2 mm for analysis. The hydrometer method outlined by Huluka and Miller (2014) was used for the determination of soil particle size. The bulk density was determined from undisturbed soil cores following methods described by Blake (1965). Organic carbon was estimated by near-infrared reflectance applied to the air-dried, milled soil samples. Extractable P was determined by using Ambic-2 solution (Hunter 1974) followed by the molybdenum blue procedure (Murphy and Riley, 1962). Total inorganic N (NH_4^+ -N + NO_3^- -N) was extracted from freshly collected soils in a 1:5 soil:2 M KCl suspension followed by filtering through Whatman® No. 2 filter paper. The filtrates were subsequently analysed with a Spectroquant® Nova 60 photometer (Merck Millipore, Germany) according to standard methods (APHA 2005). Procedures outlined by Okalebo et al. (2002) were followed for the other soil analyses listed in Table 1.

Table 1
Selected chemical and physical properties of the Inanda and Cartref soils.

Property	Inanda	Cartref
pH (KCl)	4.11	4.21
Organic C (%)	6.0	0.5
Total N (%)	0.56	0.05
Extractable P (mg kg^{-1})	12.0	0.7
Acid saturation (%)	30	18
Exch. acidity ($cmol_c$ kg^{-1})	1.80	0.18
Extractable K ($cmol_c$ kg^{-1})	0.07	0.01
Extractable Ca ($cmol_c$ kg^{-1})	3.2	0.4
Exchangeable Mg ($cmol_c$ kg^{-1})	0.0	0.4
Total cations ($cmol_c$ kg^{-1})	5.9	1.2
Bulky density (g cm^{-3})	0.80	1.43
Clay (%)	23	12
Silt (%)	48	15
Sand (%)	29	73

2.3. Experimental set up and management

The soils were air-dried, sieved through an 8 mm sieve, and packed into experimental pots. Fertilizer application rates as recommended by the Soil Fertility and Analytical Services Division of the Department of Agriculture, Cedara, KwaZulu-Natal, South Africa, were employed (Supplementary Information Table S2). Stored urine and NUC were applied based on crop N requirements, while struvite was applied based on the crop P requirement. Muriate of potash was applied at a rate of 80 kg ha^{-1} and 50 kg ha^{-1} in Cf and Ia soils respectively to supplement for K in all treatments. The application rates for the various fertilizers mentioned were calculated based on crop nutrient requirements and residual soil fertility for each soil type and nutrient concentrations present within each fertilizer source. The liquid fertilizers (NUC and stored urine) were diluted with 1 L of water before application, while struvite was applied directly to the soil. Radish and sweet pepper were planted in 10 L pots, and ryegrass in 2 L pots, after fertilizer application. For the pepper trial, one seedling was transplanted into each pot in the evening to avoid heat stress and, thus, minimize the transplanting shock. Two seeds of radish were planted in each pot, while 5 g of ryegrass seeds were sown into each 2 L pot and lightly covered with soil. Plants were watered with tap water throughout the experiment by maintaining field capacity at 60–80 %. Soil moisture was measured with Irrometer moisture indicators (Irrometer Co., Riverside, California) based on the tensiometric method outlined by Kashyap and Kumar (2021). A DFM Technologies continuous soil moisture probe was used to inform irrigation decisions. Pepper fruits were harvested approximately 90 days after planting, while radish bulbs and ryegrass were harvested 60 days after planting. After harvest, pepper fruits, radish bulbs and ryegrass leaves were freeze-dried and ground, and soil samples were collected, air-dried and sieved (1 mm) for pharmaceutical analysis. The tunnel experiment was carried out under local environmental conditions, meaning that it was not possible to control climate factors such as light, temperature and humidity.

2.4. Experimental design

The experiment was laid out using a randomized complete block design (RCBD) and designed as a 3 × 3 × 2 treatment structure replicated three times to give 54 experimental units. The factors were three nutrient sources (stored urine, NUC, and struvite), three crops (pepper, radish, and ryegrass) and two soils (Cartref and Inanda).

2.5. Pharmaceutical analysis

2.5.1. Materials

Nine ARVD standards, namely, abacavir, didanosine, efavirenz, emtricitabine, lamivudine, nevirapine, ritonavir, stavudine, and zidovudine, and three isotope-labelled internal standards, namely, enrofloxacin-d_5 hydrochloric, flubendazole-d_3, and sulfamethoxazole-(phenyl-$^{13}C_6$), were purchased from Sigma Aldrich (South Africa). Nevirapine-d_4 isotope-labelled standard was purchased from Clearsynth Labs Ltd. (India). Ultra-pure methanol (> 99.9 %) and water (>18 MΩ, at 25 °C) were products of ROMIL SpS™. Formic acid (purity 99 %) was purchased from Merck (South Africa) and nylon syringe filters (0.22 μm) were used.

2.5.2. Sample extraction

Urine fertilizers (stored urine, NUC, and struvite), and plant and soil samples were analysed in an ISO17025 accredited laboratory at the Agricultural Research Council in Pretoria, South Africa. The extraction method of Garcia-Rodríguez et al. (2014) was modified for the extraction of ARVDs in solid matrices. Briefly, 0.2 g aliquots of each plant and soil sample was spiked with 50 μg L^{-1} internal standard mixture containing enrofloxacin-d_5 hydrochloric, flubendazole-d_3, sulfamethoxazole-(phenyl-$^{13}C_6$) and nevirapine-d_4. The samples were extracted with 12 mL of a 1:1 (*v*/v) methanol:water mixture in an ultrasonic bath set at 25 °C for 30 mins, followed by centrifugation at 6000 rpm for 10 mins. One millilitre of the supernatant was filtered through a 0.22 μm nylon syringe filter prior to injection into the UHPLC-MS/MS.

2.5.3. UHPLC-MS/MS method

The analysis of ARVD residues in the samples was achieved by using a PerkinElmer QSight™ 220 triple quadrupole mass spectrometer coupled with a PerkinElmer LX50 ultrahigh performance liquid chromatograph (UHPLC). The separation of ARVDs was achieved with a Shim-pack GIST C18 column (100 × 2.1 mm, 1.9 μm particle size) with a column oven temperature set at 25 °C and a mobile phase comprising of 0.1 % formic acid in water (A) and methanol (B) at a constant flow rate of 0.3 mL min^{-1}. The gradient elution program modified from (Abafe et al., 2018) is presented in Supplementary Information Table S3. The injection volume was 10 μL. The retention times of each of the ARVDs are presented in Supplementary Information Table S4.

The mass spectrometer was equipped with both positive and negative polarity electrospray ionization (ESI) sources, which were used for the analysis of multiresidue ARVDs by fast polarity switching. The electrospray voltage was set at 4000 V. Nitrogen was used as the drying and nebulizer gas, set at 150 and 400 arbitrary units, respectively. The optimized hot surface-induced desolvation temperature was set at 320 °C, while the ion source temperature was set at 350 °C. The acquisition of ARVDs was achieved by using the time-managed multiple reaction monitoring (MRM) mode. Data were acquired by using Simplicity™ 3Q software (version 1.4.1806.29651). Selection of the target ARVDs (Table 2) was based on those recommended for public sector ARV treatment in South Africa.

2.5.4. Quality control

Both the soil and plant samples were analysed in duplicate. For each batch of analyses, a method blank was included to monitor external contamination. None of the target ARVDs was measured above the method's limit of quantitation, hence, no blank correction was carried out. Quality control standards were run after every 15 injections, while solvent blanks were run after the injection of ten samples to monitor carryover effects. To test the accuracy and precision of the method, matrix spiked samples at three spiking levels (1, 10 and 100 μg L^{-1}) were analysed by using the analytical protocol described in Section 2.5.3. The accuracy measured in terms of average recoveries of the ARVDs in the spiked samples ranged from ~80 to 114 %, while precision measured in terms of relative standard deviations (RSDs) of duplicate measurements were generally <20 % for all the target ARVDs.

2.6. Bioconcentration

Bioconcentration factors (BCF) were calculated from Eq. (1):

$$BCF = \frac{C_{crop}}{C_{soil}} \tag{1}$$

where C_{crop} is the concentration of an ARVD in a specific plant organ (ng g^{-1} dry weight (dw)) and C_{soil} is its concentration in the soil (ng g^{-1} dw) at the end of the experiment.

2.7. Human exposure and risk assessment

The estimated daily dietary intake (DDI) of ARVDs by consumption of the pepper and radish grown from urine-fertilized soils was compared to the Threshold of Toxicological Concern (TTC) values based on the Cramer classification tree. This method has been used to assess the risks of drinking water that contains 10,11-epoxycarbamazepine (Houeto et al., 2012) and consumption of carrots and sweet potatoes irrigated with treated wastewater (Malchi et al., 2014).

The DDI of each chemical per kilogram bodyweight (ng kg^{-1} bw) was calculated (García et al., 2019) from Eq. (2):

$$\text{DDI} = \frac{C_{crop} \times consumption}{bw} \tag{2}$$

where C_{crop} is the detected ARVD concentration (ng g^{-1}), consumption is the South African average consumption of pepper and radish (35 and

Table 2
Selected physico-chemical properties of target antiretroviral drugs.

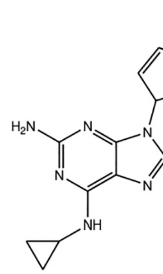
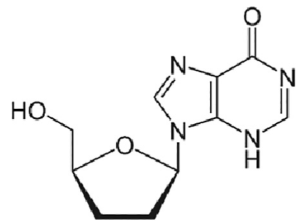
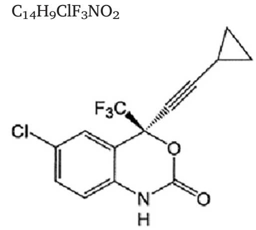
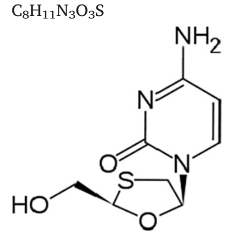
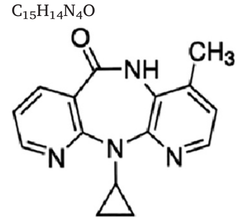
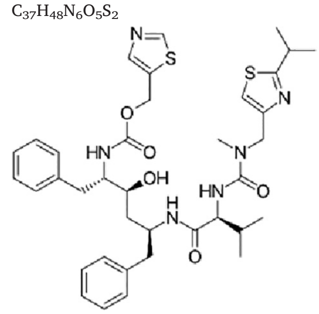
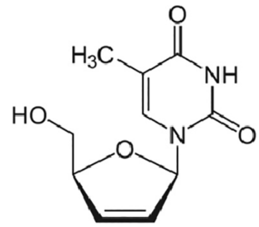
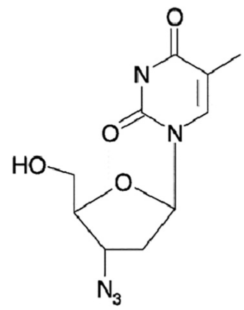
Compound	Pharmaceutical class	Structure and formula	Molar mass/g mol^{-1}	pK_a	log K_{ow}
Abacavir	Antiretroviral-nucleoside reverse transcriptase inhibitor	$C_{14}H_{18}N_6O$ 	286.33	5.01	1.22
Didanosine	Antiretroviral-nucleoside reverse transcriptase inhibitor	$C_{10}H_{12}N_4O_3$ 	236.2	9.13	−1.24
Efavirenz	Antiretroviral-non-nucleoside reverse transcriptase inhibitor	$C_{14}H_9ClF_3NO_2$ 	315.67	10.2	4.7
Emtricitabine	Antiretroviral-nucleoside reverse transcriptase inhibitor	$C_8H_{10}FN_3O_3S$ 	247.24	2.65	−0.43
Lamivudine	Antiretroviral-nucleoside reverse transcriptase inhibitor	$C_8H_{11}N_3O_3S$ 	229.26	4.3	−9.54
Nevirapine	Antiretroviral-non-nucleoside reverse transcriptase inhibitor	$C_{15}H_{14}N_4O$ 	266.30	2.8	3.89
Ritonavir	Antiretroviral-protease inhibitor	$C_{37}H_{48}N_6O_5S_2$ 	720.9	2.6	6.27

Table 2 (*continued*)

Compound	Pharmaceutical class	Structure and formula	Molar mass/g mol^{-1}	pK_a	log K_{ow}
Stavudine	Antiretroviral-nucleoside reverse transcriptase inhibitor	$C_{10}H_{12}N_2O_4$ 	224.21	9.95	−0.72
Zidovudine	Antiretroviral-nucleoside analogue and reverse transcriptase inhibitor	$C_{10}H_{13}N_5O_4$ 	267.24	9.6	0.05

Data from https://pubchem.ncbi.nlm.nih.gov/.

40 g d^{-1}, respectively) (W.H.O, 2002), and bw is the South African average bodyweight (70 kg) (National Department of Health 2019). The concentrations of ARVDs in the two crops were converted to a fresh weight basis by using the average water content of each crop (95.3 % for pepper, and 91.4 % for radish).

Compound classification and TTC values were determined using Toxtree software, which is an open-source software that was commissioned for development by the European Commission Joint Research Centre's European Chemicals Bureau (ECB) solely for the purpose of determining the Cramer classification of chemical compounds (Bhatia et al., 2015). The Cramer decision tree classifies materials into one of three classes (I – low, II – intermediate, and III – high). The TTC values are 30, 9, and 1.5 g kg^{-1} bw per day for Cramer Classes I, II, and III, respectively. The likelihood that chemicals might have negative impacts on health is low for exposures below the TTC levels (EFSA et al., 2019). A non-TTC technique is necessary to determine any potential negative health consequences if the expected exposure to a chemical is higher than the pertinent TTC value (Blackburn et al., 2020).

2.8. Data analysis

Data were subjected to analysis of variance (ANOVA) by using GenStat® Version 18 (VSN International, UK). Means of significantly different variables were separated by least significant differences (LSD) at $P = 0.05$.

3. Results and discussion

3.1. Antiretrovirals in plants

This research monitored the uptake of nine ARVDs by three plant species, namely, sweet pepper, radish, and ryegrass, grown on two different soil types (Cartref and Inanda) that were fertilized with three different urine-derived fertilizers.

3.1.1. Stored urine treatment

Pepper, radish, and ryegrass treated with stored urine absorbed six of the nine target ARVDs in detectable amounts. Lamivudine, ritonavir and stavudine were taken up by pepper, radish, and ryegrass grown in both soils (Table 3). Lamivudine and ritonavir were detected at significantly higher concentrations ($P < 0.05$) in pepper than radish and ryegrass grown in both soils (Fig. 1A, B). Stavudine was taken up in the order of radish > pepper > ryegrass on both soils (Fig. 1C). The only plant that absorbed emtricitabine was radish (Fig. 1D), with a significantly higher concentration ($P < 0.05$) when grown in Ia soil than Cf soil (2.43 and 1.77 ng g^{-1} dw, respectively). For the Cf soil, nevirapine was absorbed by both pepper and radish, whereas on the Ia soil it was only detected in pepper (Fig. 1E). Didanosine was taken up to the greatest extent of all the detected ARVDs with measured concentrations up to 7.8 ng g^{-1} dw (Fig. 1F). However, it was only found in pepper grown on both soils.

The uptake of a particular pharmaceutical by a plant depends on its physicochemical properties. Molar mass (MM) is an important factor associated with membrane permeability (Li et al., 2019b). Pharmaceuticals are readily absorbed by plants when their MM is <1000 g mol^{-1} (Zhang et al., 2017). The molar masses of the detected ARVDs range from 224.2 to 720.9 g mol^{-1}, with ritonavir having the largest MM (Table 2). The other five detected ARVDs with MM < 270 g mol^{-1} (lamivudine, stavudine, emtricitabine, nevirapine and didanosine) were absorbed at significantly higher concentrations than ritonavir. According to Goldstein et al. (2014), non-ionic pharmaceuticals can easily cross membranes while moving from the xylem to the phloem. As a result, they are mainly transported in

Table 3
Mean ARVD concentrations (ng g^{-1} dw) in plants treated with urine-derived fertilizers.

Antiretroviral	Cartref			Inanda		
	Pepper	Radish	Ryegrass	Pepper	Radish	Ryegrass
Stored urine						
Lamivudine	4.85	3.89	1.93	4.68	3.76	1.83
Ritonavir	1.65	1.63	2.02	1.67	1.65	1.68
Stavudine	2.28	3.14	1.81	2.25	3.43	2.38
Emtricitabine	ND	1.77	ND	ND	2.47	ND
Nevirapine	2.72	2.64	ND	2.13	ND	ND
Didanosine	7.81	ND	ND	3.17	ND	ND
NUC						
Nevirapine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Struvite						
Nevirapine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

< LOQ: below limit of quantification, ND: not detected.

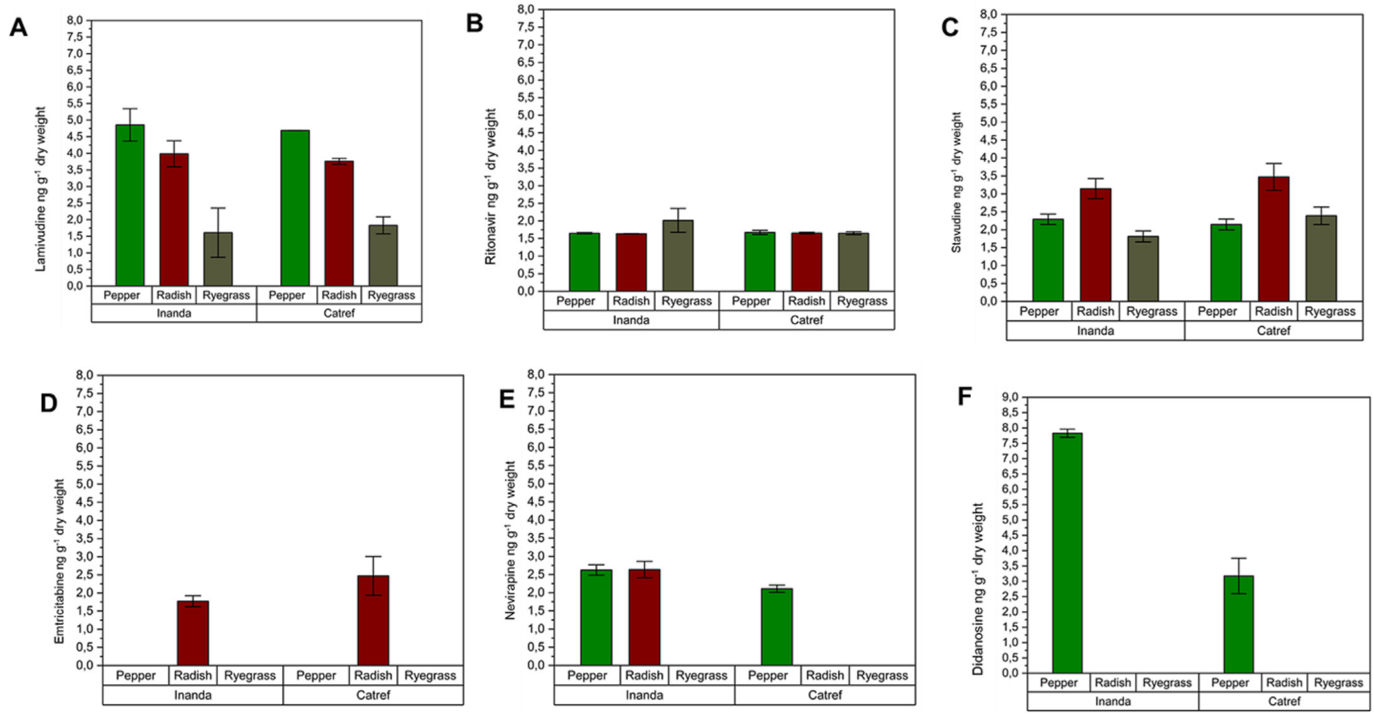


Fig. 1. Concentrations (ng g^{-1} dry weight) of (A) lamivudine, (B) ritonavir, (C) stavudine, (D) emtricitabine, (E) nevirapine, and (F) didanosine in pepper, radish, and ryegrass grown on Cartref and Inanda soils fertilized with stored urine. Results are presented as bar graphs (mean ± SE) $n = 3$.

the direction of the transpiration stream and primarily accumulate in the leaves. Ionic pharmaceuticals are repelled by the negatively charged cytosol and accumulate to a greater extent in the fruit. In this study ARVDs accumulated more in the pepper fruit and radish bulbs than the ryegrass leaves. The hydrophobicity of a pharmaceutical is typically used for interpretation of uptake of organic compounds into plant roots. A positive linear relationship between the root uptake and chemical hydrophobicity was reported for neutral compounds so that pharmaceuticals with high K_{OW} values are easily absorbed by plants (Wu et al., 2013). This current study demonstrates contrary results, hydrophobic ARVDs (K_{OW} 3.89–6.27) which include efavirenz, nevirapine and ritonavir were absorbed to a lesser extent than hydrophilic ARVDs (K_{OW} -0.72-1.22). This suggests that factors other than hydrophobicity might have an impact on how certain ARVDs are absorbed.

In addition to the properties of the pharmaceutical compounds, plant uptake is also influenced by the physical and chemical properties of the soil. With the exception of ritonavir and stavudine, ARVD concentrations detected in plants grown on Cf soil were significantly higher than found in those grown on Ia soil. This trend was probably due to the higher organic matter content of Ia soil (10.32 %) than Cf soil (0.86 %). High soil organic matter has been reported to reduce plant uptake of pharmaceuticals because compounds are strongly bound to soil organic matter, hindering availability for plant uptake (Fu et al., 2016; Paz et al., 2016; Mordechay et al., 2018). Concentrations of ARVDs available for uptake by plants may also be altered by abiotic and microbial transformation processes in the soil (Miller et al., 2016). Enzymes, such as laccases and peroxidases, are secreted by plants and rhizosphere-associated microbes, and they can transform pharmaceuticals (Martin et al., 2014). A higher microbial activity results from rhizosphere microorganisms utilising carbohydrates in root exudates as a carbon source.

The lipid and carbohydrate content of root cell walls, which impact the permeability of root cell membranes, are the main biological factors in plants that influence pharmaceutical uptake (Keerthanan et al., 2021). Pepper, radish and ryegrass have different root systems, leaf sizes and lipid content. Additionally, the ARVD analysis targeted the harvestable and edible components of the crops. Pepper fruits absorbed five of the six detected ARVDs. Radish bulbs followed the same trend except that they absorbed emtricitabine instead of didanosine, while ryegrass leaves only absorbed lamivudine, ritonavir and stavudine. The detected ARVDs were absorbed at significantly ($P < 0.05$) higher concentrations in pepper fruits than radish bulbs and ryegrass, except for stavudine and nevirapine. The differences in ARVD uptake between plant species may be attributed to differences in their metabolic systems, which may involve a network of enzymatic reactions, and growth and transpiration rates (Coleman et al., 1997; Wu et al., 2013). Ryegrass has been reported to have a high root lipid content (Huang et al., 2010), which may explain the limited uptake of ARVDs by ryegrass in comparison with pepper fruits and radish bulbs.

3.1.2. Nitrified urine concentrate and struvite treatments

Nevirapine was the only ARVD absorbed by the three crops fertilized with NUC and struvite in both soils, although all the concentrations were below the limit of quantification (< LOQ). Duygan et al. (2021) reported significant elimination of ARVDs, including atazanavir, darunavir and ritonavir, during the nitrification process of NUC production and this may account for the absence of the target ARVDs. Nevertheless, the current study's use of nitrification of urine alone does not guarantee complete elimination of pharmaceuticals (Etter et al., 2015). Of the nine antiretrovirals studied herein, only nevirapine was detected after nitrification and distillation treatment. However, the concentration was substantially degraded from 2000 ng L before treatment to 347 ng L after treatment (Table S5). Several post-nitrification processes for pharmaceutical removal have been developed such as microfiltration and electrodialysis in combination with nanofiltration (Pronk et al., 2006), and ozonation combined with ultraviolet light and hydrogen peroxide (Dodd et al., 2008). However, the reactivity of the oxidants with other compounds in the urine matrix limits the efficacy of both procedures (Zhang et al., 2015). Addition of powdered activated carbon as a post-nitrification process has been successfully tested to remove approximately 90 % of pharmaceuticals (Köpping et al., 2020). In contrast, struvite production has been reported to be inefficient in terms of pharmaceutical elimination. In the current study nevirapine, abacavir and didanosine were detected in struvite after treatment, with concentrations of 45.3 ng g-1 and below limit of quantification respectively (Table S5).

Table 4
Concentrations (ng g^{-1} dry weight) of ARVDs in Cartref and Inanda soils planted to pepper, radish, and ryegrass, and fertilized with stored urine, nitrified urine concentrate (NUC) and struvite.

Antiretroviral	Cartref			Inanda		
	Pepper	Radish	Ryegrass	Pepper	Radish	Ryegrass
Stored urine						
Lamivudine	< LOQ	2.305	<LOQ	0.925	< LOQ	ND
Ritonavir	2.211	2.118	1.784	ND	2.51	1.964
Stavudine	1.632	< LOQ	2.164	2.935	ND	2.823
Emtricitabine	0.972	< LOQ	0.674	2.516	< LOQ	1.964
Efavirenz	< LOQ	0.863	1.715	< LOQ	2.378	2.948
Nevirapine	3.102	1.634	2.981	3.951	3.473	3.378
Didanosine	1.219	4.838	4.540	2.348	3.353	5.632
NUC						
Nevirapine	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Struvite						
Nevirapine	0.671	0.659	< LOQ	0.710	0.795	< LOQ

< LOQ: below limit of quantification, ND: not detected.

According to a study by Ronteltap et al. (2007), 98 % of each of the seven drugs that were examined during the synthesis of struvite were still present in the liquid phase of struvite following precipitation. Also, the filtration and drying processes do not remove pharmaceuticals that remain after precipitation of struvite (Bischel et al., 2015). However, pharmaceutical plant uptake studies have demonstrated limited/no uptake from struvite. de Boer et al. (2018) determined bioaccumulation of five common pharmaceuticals (propranolol, diclofenac, sulfamethoxazole, ibuprofen and carbamazepine) by tomato fruits fertilized with spiked struvite and detected no pharmaceuticals. Results from the present study indicate that the uptake of ARVDs into pepper, radish and ryegrass fertilized with struvite and NUC under controlled conditions is limited.

3.2. Antiretrovirals in soils

At the end of the experiment, the concentrations of ARVDs in soils fertilized with stored urine showed a wide range from < LOQ to 5.638 ng g^{-1} dw (Table 4). Generally, ARVD concentrations detected in soil were significantly lower than those detected in plants. Lamivudine was only detected in quantifiable concentrations in the Cf soil planted to radish and Ia soil planted to pepper, while it was not detected or was < LOQ in the remaining treatments. Ritonavir was detected in all treatments except in Ia soil under pepper production. Ritonavir concentrations detected in the Ia soil were significantly higher ($P < 0.05$) than in the Cf soil. Stavudine was not detected in the Ia soil planted to radish and was < LOQ in the Cf soil planted to radish. In the remaining treatments the lowest and highest concentrations of stavudine were detected in the soils planted to pepper at 1.632 ng g^{-1} (Cf) and 2.935 ng g^{-1} (Ia) respectively. Nevirapine was detected in all treatments, with higher concentrations on the Ia soil than the Cf soil. Didanosine was also found in soils for all treatments at relatively high concentrations. In addition to the ARVDs identified in plant samples, efavirenz was detected in soil, although it was only quantifiable in soils planted to radish and ryegrass. Emtricitabine was < LOQ for both soils planted to radish, and the concentrations in Ia soils were higher than for Cf soils.

The main factors that influence pharmaceutical persistence in soil are a compound's photostability, binding and adsorption potential, rate of breakdown, and leaching (Wu et al., 2015). Pharmaceuticals undergo sorption/desorption and transformations after being introduced to soil. The production of non-exchangeable or bound residues with much lower bioavailability can be the outcome of a pharmaceutical's sorption and transformation (Wu et al., 2015). The most significant pharmaceutical sorption mechanisms include sorption to organic matter, surface adsorption to mineral components, ion exchange, complex formation with metal ions such as Ca^{2+}, Mg^{2+}, Fe^{3+} or Al^{3+}, and H-bonding (Tolls, 2001). Neutral compounds detected in soil have been reported to be more recalcitrant and persistent, while weakly acidic pharmaceuticals exhibit rapid degradation because they have carboxylic groups that are more susceptible to microbial transformations (Grossberger et al., 2014).

These results demonstrate the ability of the two soils to adsorb ARVDs from stored urine, potentially endangering environmental health especially of soil microbes. Antiretroviral drugs are created to be active at low concentrations, therefore, they may pose an ecotoxicological risk to the environment. Soil microorganisms would be of major concern especially those involved in key ecosystem services such as nutrient biogeochemical cycling (Caracciolo et al., 2015). A significant concern with regard to antimicrobial (antiviral, antibacterial, and antifungal) pharmaceuticals is the potential for resistance to be strengthened and propagated in a way that endangers crucial medications for human and animal treatment (Patel et al., 2019).

Nevirapine was detected and quantified in struvite-treated soils at the end of the experiment with concentrations ranging from 0.659 to 0.795 ng g^{-1} dw, while NUC-treated soils exhibited concentrations <LOQ (Table 4). There was no significant difference for nevirapine concentrations in either soil between those planted to pepper and radish. However, nevirapine concentrations were higher in the high organic matter, clay soil (Ia). The aromatic side rings of nevirapine can intercalate between layers of various clay minerals (Lambert, 2018) allowing the possibility for adsorption in the interlayer spaces of minerals in soils with a higher clay content, therefore, it would become less available for plant uptake.

3.3. Bioavailability of antiretroviral drugs in stored urine treated plants

Bioconcentration factor (BCF) values were computed based on the pharmaceutical concentration in bulk soil in order to better understand the accumulation potential of lamivudine, ritonavir and stavudine in pepper, radish and ryegrass (Fig. 2). The BCF was not calculated for NUC and

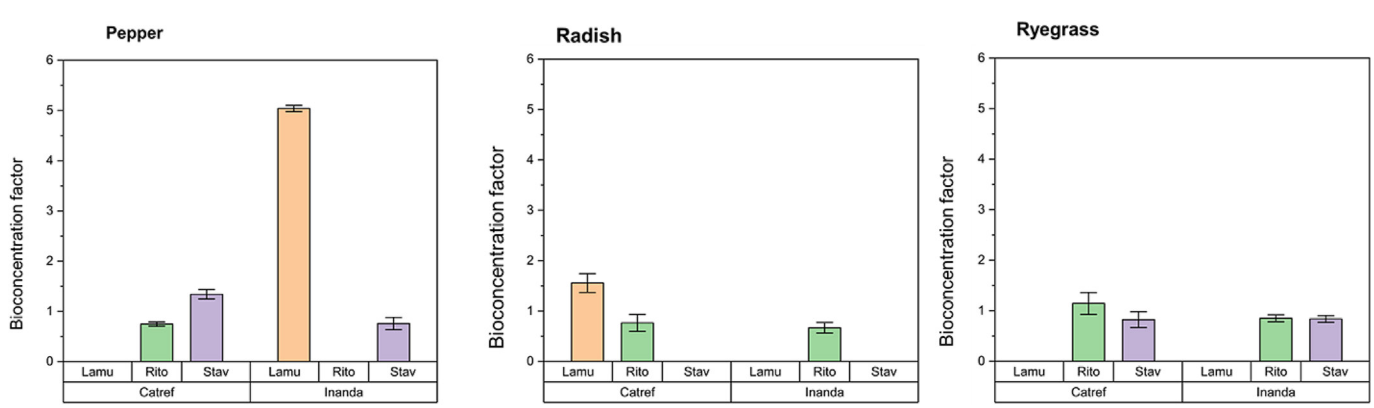


Fig. 2. Bioconcentration factors (mean ± SE) for lamivudine (Lamu), ritonavir (Rito) and stavudine (Stav) in pepper, radish and ryegrass grown in Cartref and Inanda soils and fertilized with stored urine, $n = 3$.

Table 5
Estimated daily dietary intake (DDI) of antiretroviral drugs by consumption of pepper and radish grown on two soils and fertilized with stored urine. In all cases the Threshold of Toxicological Concern (TTC) value was 1500 ng kg^{-1} bw day^{-1} and all antiretroviral drugs were in Class III (Cramer classification).

Soil	Antiretroviral drug	DDI (ng kg^{-1} bw day^{-1})		Adult intake to exceed TTC (kg day^{-1})	
		Pepper	Radish	Pepper	Radish
Cartref	Lamivudine	2.3	2.0	21.8	30.0
	Ritonavir	0.7	0.8	65.6	75.0
	Stavudine	1.1	1.6	47.7	37.5
	Emtricabine	nd	0.7	–	85.7
	Nevirapine	1.4	0.8	37.5	75.0
	Didanosine	3.7	nd	14.8	–
Inanda	Lamivudine	2.2	1.9	23.8	31.5
	Ritonavir	0.8	0.9	65.6	66.7
	Stavudine	1.2	1.7	43.8	35.3
	Emtricabine	nd	1.4	–	42.8
	Nevirapine	1.0	nd	52.5	–
	Didanosine	1.5	nd	35.0	–

nd: not detected.

struvite treatments as ARVDs were not detected or were <LOQ in both plants and soil. Bioconcentration factors for all three ARVDs were >1 in pepper. The stavudine BCF was significantly higher in peppers grown on Cf soil than those on Ia soil. Generally, the BCF for lamivudine was about two times higher than for ritonavir in radish on the Cf soil. Although both lamivudine and ritonavir are weak bases, ritonavir is a hydrophobic, thus, its bioavailability for plant uptake is limited (Fu et al., 2016). Ryegrass showed a higher BCF for stavudine than ritonavir on both soils. However, the BCF for ritonavir was <1, indicating its limited availability and accumulation in ryegrass. This is contrary to the proposed accumulation of non-ionic pharmaceuticals in transpiring organs such as leaves (Wu et al., 2013; Goldstein et al., 2014; Mordechay et al., 2018). The limited uptake of ritonavir compared with that of stavudine is probably a result of their contrasting log K_{ow} and MM values (6.02 and 720.2 g mol^{-1}; −0.72 and 224.1 g mol^{-1}, respectively).

3.4. Human exposure assessment

The assessment of human exposure through dietary intake of pepper and radish grown in soils fertilized with stored urine is presented in Table 5. Ryegrass was excluded from this assessment because it is primarily cultivated for livestock feed. The NUC and struvite treatments were also excluded from the evaluation due to the non-quantifiable ARVD concentrations in these treatments. All six ARVDs assessed were classified as Class III compounds based on the Cramer classification tree (Supplementary Information Figs. S1-S6). The DDI of ARVDs through consumption of pepper was highest for didanosine on Cf soil and lamivudine on Ia soil, and lowest for ritonavir grown on both soils (Table 5). In radish, lamivudine exhibited higher DDI values for both soils. The calculated DDI values for all ARVDs were about 300–3000 times lower than the TTC values for class III compounds. According to Prosser and Sibley (2015) for consumption to be considered safe, there should be a considerable gap (> factor 10 to factor 1000) between the DDI and the lowest therapeutic dose, in this study the TTC value. This is the case for all ARVDs assessed.

The amount of pepper required for daily consumption to reach the TTC value ranges from 14.8 to 65.6 kg in adults. The evaluated ARVDs would require an adult to consume approximately 30–86 kg of radish daily to reach the TTC value. Clearly this is an unrealistic consumption and, therefore, these results demonstrate that the daily consumption of pepper and radish fertilized with stored urine does not pose a health threat to the consumer.

4. Conclusion

This study investigated the plant uptake of a range of ARVDs from two contrasting soils fertilized with human urine-derived fertilizers. Plant uptake of these compounds from NUC and struvite was very limited. Soil application of stored urine resulted in uptake of some of the ARVDs into pepper fruits, radish bulbs, and ryegrass leaves. Antiretroviral drugs were more available for uptake in the plants grown on the soil with low organic matter and clay content. Nevirapine was the only ARVD quantified in struvite fertilized soils. The daily consumption of pepper and radish fertilized with stored urine does not present a health risk to the consumer. However, more field work and research are necessary to confirm these findings. Additionally, the eco-toxicological potential effects of human urine-derived fertilizers on soil microbiota remain a potential concern. Further studies are also necessary to evaluate the effects of ARVD metabolites that could have a greater impact on human health than the parent compounds.

CRediT authorship contribution statement

Sharon Migeri: Conceptualization, Investigation, Data curation, Writing – original draft. **Jeffrey Charles Hughes:** Conceptualization, Formal analysis, Writing – original draft. **Taruvinga Badza:** Formal analysis, Data curation, Writing – original draft. **Ovokeroye A. Abafe:** Methodology, Writing – review & editing. **Bice S. Martincigh:** Methodology, Writing – review & editing. **Alfred Oduor Odindo:** Conceptualization, Formal analysis, Writing – original draft, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164551.

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