Health risk assessment of vegetables and garden soil contaminated with pharmaceutical residues from irrigation water

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ABSTRACT

The perseverance of pharmaceuticals in water has led to their occurrence in the agroecosystem potentially posing significant human health risks. This study examined the presence of efavirenz, ibuprofen, naproxen, sulfamethoxazole, and trimethoprim in river water, garden soils, cabbage, and spinach. Ultrasound-assisted extraction and solid phase extraction techniques were used to prepare solid and aqueous samples, respectively, before LC-qTOF-MS analysis. The sample preparation methods gave 79–119% recoveries upon validation. The method quantification limits for aqueous and solid samples were 0.07–5.4 ng/L and 0.28–25.3 ng/kg, respectively. The study detected efavirenz (2–181 ng/L), ibuprofen (34–460 ng/L), naproxen (<LOQ–980 ng/L), sulfamethoxazole (<LOQ–209 ng/L), and trimethoprim (10–183 ng/L) in river water. In the edible portion of vegetables, ibuprofen, sulfamethoxazole, and trimethoprim were detected in cabbage leaves, whereas only trimethoprim was detected in spinach leaves. Trimethoprim was the only drug detected in irrigation water (10–30 ng/L), while trimethoprim and ibuprofen were detected below quantification limits in garden soil samples. The concentrations of efavirenz and sulfamethoxazole in river water were found to be of significant risk to *Vibrio fischeri* and algae. However, all the detected pharmaceuticals in the edible portion of vegetables pose a negligible risk to human health. The study also tentatively identified 108 additional drugs, highlighting the need for more monitoring of pharmaceuticals in human food chains.

KEYWORDS

Pharmaceuticals; Agroecosystem; Aquatic environment; Ultrasound-assisted extraction; Solid-phase extraction; Health risk assessment

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INTRODUCTION

The global increase in medication accessibility has resulted in the continuous presence of pharmaceuticals in the aquatic environment. As drugs are ingested for therapeutic purposes, they are excreted in their unchanged state as a part of human waste, ultimately entering wastewater treatment plants (WWTPs), where they are partially treated and then released into the environment.[1] The constant detection of pharmaceuticals of different therapeutic groups in the environment is evidence of their release into the aquatic environment.[2] Furthermore, pharmaceuticals have been found in drinking water sources,[3,4] thus demonstrating the inability of conventional drinking water treatment processes to remove them. Many communities depend on drinking water suppliers to irrigate homestead crops, potentially leading to pharmaceutical residues in the human food chain and exposing individuals to these compounds. Notably, rivers heavily contaminated with pharmaceuticals are the primary source of these residues in drinking water suppliers.[5]

Several greenhouse and hydroponic studies have demonstrated the ability of vegetables to take up pharmaceuticals.[6–8] In such studies, vegetables are intentionally exposed to pharmaceuticals at certain concentrations that are sometimes not relevant to their environmental levels. Some studies have reported the presence of pharmaceuticals in vegetables cultivated under real conditions after being irrigated with reclaimed wastewater.[9,10] These investigations were conducted in light of the increasing popularity of utilizing reclaimed wastewater and sewage sludge in agricultural practices, driven by the belief in their high organic content, which is capable of increasing soil fertility and crop productivity.[11] However, reclaimed wastewater and sewage sludge are widely acknowledged for their high content of pharmaceuticals, thereby exposing vegetables to various organic pollutants.[12] Studies focusing on monitoring pharmaceuticals in vegetables cultivated under real conditions and irrigated with river water and/or tap water remain scarce. Additionally, pharmaceuticals can enter the agroecosystem through surface runoffs from industrial areas and direct disposal onto land surfaces. Therefore, comprehending the impact of contaminated river water on the presence of pharmaceuticals in garden soil and vegetables is essential for evaluating the associated risks to both food safety and environmental health.

The mechanism of pharmaceutical uptake by vegetables is a complex process as it is influenced by various factors, including soil type, plant physiology, and environmental conditions. However, chemical hydrophobicity stands out as the most critical factor affecting the uptake and accumulation of pharmaceuticals in vegetables.[13] Pharmaceuticals with an octanol-water partition coefficient (Log K_{ow}) ranging between 1 and 3.5 exhibit optimal uptake potential due to a balanced solubility in both lipid and aqueous environments.[14] In this regard, hydroponic experiments offer invaluable insights into how pharmaceuticals are translocated to the aerial segments of the plant as they exclude soil. The level and duration of exposure also play critical roles in influencing the uptake and accumulation of pharmaceuticals in vegetables. Since some vegetables are harvested earlier than others, the duration of exposure can significantly impact the extent of pharmaceutical accumulation. For instance, vegetables with shorter growth cycles may have had less time to accumulate pharmaceutical residues than those with longer growth periods. Overall, pharmaceuticals are taken up *via* the roots and subsequently translocated to the upper segments of the plants through the vascular tissues.[15] Furthermore, once within the plant, pharmaceuticals may undergo various metabolic processes or interactions with plant tissues, potentially affecting their distribution and persistence in edible parts.

The current study focused on evaluating the influence of river water on the occurrence of pharmaceuticals in cabbage and spinach as well as garden soil cultivated in real-field conditions. These vegetable species were selected because they can be planted during any season, they are readily available, and they can be consumed in their raw state, posing direct exposure if they are contaminated with pharmaceuticals. The

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selected pharmaceuticals were two antibiotics (sulfamethoxazole and trimethoprim), two non-steroidal anti-inflammatory drugs (NSAIDs) (ibuprofen and naproxen), and an antiretroviral drug (efavirenz) (**Table S1**). Most pharmaceutical monitoring studies in food samples are based on controlled experiments, wherein vegetables are exposed to high levels of pharmaceuticals. Therefore, the risk assessments derived from these studies may be inaccurate, as they are based on conditions that do not reflect typical environmental exposures. This study investigated the presence of pharmaceutical residues in vegetables and garden soil grown under real-field conditions and irrigated with tap water. It also evaluated the presence of pharmaceuticals in the Buffalo River, which feeds the Bridle Drift Dam, which serves as a water source for the Umzonyana water treatment works in East London. Moreover, the study further examined the effect of cooking on the levels of pharmaceutical residues in vegetables. This aspect of cooking's effect on contaminant levels is a novel contribution to the field, enhancing the understanding of human exposure and health risks associated with consuming contaminated food. This study aimed to reveal the potential health effects of pharmaceutical residues in food using rigorous scientific techniques and analysis, as well as contribute to advances in food safety and public health.

EXPERIMENTAL

Chemicals and materials

All the pharmaceutical standards used in this study (efavirenz (99.8 %), ibuprofen (99.6 %), naproxen (≥98 %), sulfamethoxazole (≥98 %), and trimethoprim (99.8 %)) were procured in powder form from Merck Chemicals (Pty) Ltd (Johannesburg, South Africa). The solvents: acetonitrile (99.9 %), ethyl acetate (99.8 %), formic acid (98 %), and methanol (99.9 %) were of LC-MS grade, procured from Merck Chemicals. Ultra-high purity water was generated at the University of South Africa laboratory at the Florida Science Campus. The Oasis® HLB 6cc/150 mg SPE cartridges from Microsep (Johannesburg, SA) were used in solid-phase extraction (SPE).

Sampling and sample pre-treatment

River water samples were collected from the Buffalo River in the Eastern Cape province of South Africa. Simultaneously, samples of vegetables and garden soils were collected from four households located near the same river. **Figure 1** illustrates the locations where both aqueous and solid samples were collected. The first sampling site (S1) for river samples is situated in King Williams Town, near the vicinity of a shopping center. This location served as the starting point for the collection of river samples along the Buffalo River. The second sampling site (S2) is within the Schornville community, preceding the point where the Buffalo River receives effluent discharges from the WWTP in Schornville. This location was strategically chosen to assess the river's condition before it received wastewater from a sewage treatment plant that was not operational during the time of sampling. The third sampling site (S3) is located downstream of the WWTP located in Schornville and considered the untreated sewage discharges into the river. This site is before the effluent discharge point of a WWTP located in Zwelitsha Township. The illegal dumping of solid wastes was observed adjacent to this site (S3), which has the potential to contribute to the occurrence of pharmaceuticals in the environment. The fourth sampling site (S4) is located downstream of the treatment plant in Zwelitsha Township, following the point where the Buffalo River merges with water from the Umkhanyiso River. Finally, the fifth sampling site (S5) is positioned downstream of the point where the Buffalo River converges with the Yellowwood River, near the Izeleni community.

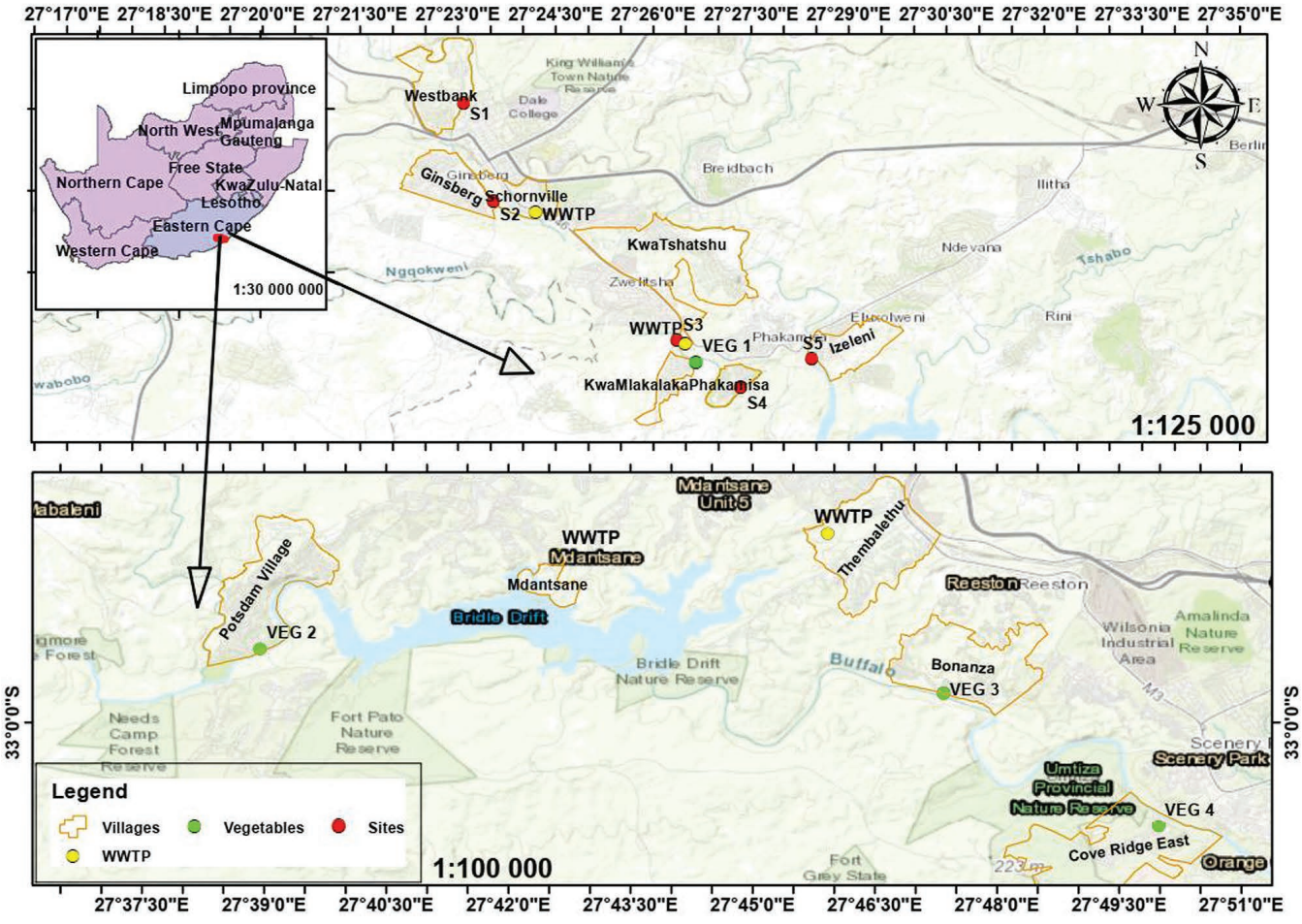

Figure 1: Illustration of the sampling sites for the investigated environmental samples.

Grab samples from the river were collected into 1-liter glass bottles. Meanwhile, vegetable samples were gathered intact with their roots and soil. At the time of sampling, residents asserted that these vegetables had been watered with tap water. Consequently, tap water samples were also collected to investigate the potential source of the identified pharmaceuticals. Resealable bags and aluminium foils were used to store sampled vegetables and soils, respectively. After that, samples were transported to the laboratory in an ice-filled cooler box. Upon arrival, aqueous samples (river water and tap water) were filtered through 11 µm Whatman filter paper to eliminate suspended solid materials before the extraction process. Meanwhile, vegetables were sub-divided into leaves, stems, and roots. After that, leaves and stems were segmented further into smaller pieces, rinsed with distilled water, and kept in a refrigerator at – 20 °C. All the sub-divided segments were then freeze-dried using Labcon FreeZone 2.5 freeze drier from Vacutec (Johannesburg, South Africa). The freeze-dried samples were then ground into powder using Ball Mill BM40 equipment from the POWTEQ Planetary (Beijing, China). The samples were then passed through a <150 mm sieve and kept in the refrigerator until the extraction process.

Sample Preparation

Extraction of water samples

Pharmaceutical residues in water samples were extracted using the automated Dionex™ Auto Trace™ 280 SPE device from Thermo Fisher Scientific (Waltham, United States) following the previously reported method.[16] In brief, the Oasis HLB 6cc/150 mg SPE cartridges were mounted to the SPE device and initially pre-conditioned with 5 mL of methanol and 5 mL of ultra-high purity water, flowing at a 1 mL/min rate. Thereafter, 250 mL of the aqueous sample was percolated through the cartridge at a flow rate of 5 mL/min. The interfering substances were then washed off the cartridge with 2 mL of ultra-high purity water flowing at 1 mL/min. This was followed by drying the cartridge with a stream of nitrogen gas for 5 minutes. Then 10 mL of methanol was used to elute the residual analytes at a flow rate of 0.8 mL/min. Afterward, the extracts were pre-concentrated by evaporating the methanol to near dryness. Subsequently, the pre-concentrated extracts were reconstituted with 1 mL of 0.1% formic acid in methanol and then subjected to LC-MS for the identification and quantification of analytes.

Extraction of solid samples

Pharmaceuticals in vegetables and garden soils were extracted using the ultrasound-assisted solvent extraction technique, following the method described by,[8] with modifications to enhance the efficacy of the method for the selected analytes. In brief, 100 mg of each solid sample was accurately weighed and transferred into a 15 mL centrifuge tube. Subsequently, 5 mL of extraction solvent was added to the sample, and the mixture was vortexed until a homogeneous solution was achieved. Methanol was used to extract the analytes in vegetables, while a mixture of ultra-high purity water and ethyl acetate (50:50, *v/v*) was employed to extract analytes in garden soils. The solution was subsequently sonicated for 5 minutes and centrifuged for 5 minutes at 3000 rpm. This procedure was repeated three times, and the resulting extracts were combined. Following this, the extracts were concentrated by reducing the extraction solvent to 1 mL through vaporization and then reconstituted with 250 mL of deionized water for sample clean-up, employing the same SPE procedure as described for water samples.

Liquid chromatographic analysis

The analysis of selected pharmaceuticals was conducted utilizing the Dionex Ultimate™ 3000 UHPLC system obtained from Thermo Fisher Scientific (Waltham, MA, USA). This system comprised a binary pump, online degasser, and autosampler. It was interfaced with an Impact II Quadrupole Time-of-Flight Mass Spectrometer (QTOF-MS) provided by Bruker (Bremen, Germany). The chromatographic separation of the chosen pharmaceuticals was accomplished using an XBridge™ C_8 column (3.5 µm, 3 x 100 mm) maintained at 30 °C. The mobile phase comprised 0.1% formic acid in ultrahigh-purity water and 0.1% formic acid in acetonitrile, operated under gradient elution. The elution process commenced with 2% organic solvent for 1.5 minutes, followed by a linear increase to 10% over 2.5 minutes, further increasing to 50% over 6 minutes, reaching 100% over 2 minutes, and subsequently returning to initial conditions. The sample injection volume and mobile phase flow rate were configured to be 10 µL and 0.300 mL/min, respectively. In the MS instrument, nitrogen gas produced by the Peak Scientific Genius nitrogen generator was used as both the nebulizer and auxiliary gas. The nebulizer gas was set to 1.8 bar, while the capillary voltage was set at 4500 V. Additionally, the drying gas (auxiliary gas) flow rate was maintained at 8 L/min, with a temperature of 220 °C. Data analysis was conducted using the Bruker QuantAnalysis software, where all analytes were processed under positive ionization conditions.

Validation of the analytical method

The analytical method employed underwent validation to ensure its effectiveness in extracting and analysing the target compounds from both aqueous and solid samples. This comprehensive validation process encompassed the assessment of method linearity, extraction efficiency, precision, and sensitivity, ensuring robustness and reliability across varied sample matrices. This was accomplished by fortifying both liquid and solid samples with a mixed standard solution at concentrations of 5 and 15 µg/L for liquid samples and 5 and 15 ng/g for solid samples. This fortification step was carried out prior to subjecting the samples to their respective sample preparation techniques. The extraction efficiency was then assessed by calculating the recoveries of each analyte in both aqueous and solid samples. This rigorous process was performed in triplicate to accurately ascertain the precision of the analytical method through the determination of relative standard deviation. The sensitivity of the analytical method was scrutinized by determining both the method detection limit (MDL) and method quantification limit (MQL). These limits were computed based on the concentrations at which the signal-to-noise ratios were 3 and 10, respectively. This assessment allowed for a comprehensive understanding of the method's sensitivity and its capability for detecting and quantifying analytes with acceptable precision.

Non-targeted screening of pharmaceuticals in the environmental samples

The environmental samples under investigation underwent screening to evaluate the presence of additional pharmaceuticals that did not form part of the target drugs under study. This comprehensive screening was aimed to provide a broader understanding of the pharmaceutical pollution within the samples. This was accomplished by first converting the mass spectrometry raw data files into the mzXML format using the MSConvert software. The mzXML files were processed using MZmine 2.53 software,[17] transforming the chromatographic information into essential parameters including the mass-to-charge ratio (m/z), retention time, and peak height for each potential compound. The processing parameters in the MZmine software were set per the methodology outlined in the previous report,[18] utilizing centroid techniques that involved mass detection, chromatogram builder, and peak deconvolution. Following MZmine processing, the aligned peak data was retrieved and uploaded to online databases (KEGG and MassBank) in CSV format for compound identification. In all cases, only matches with a confidence level exceeding 80% with compounds in the database were considered. Each identified compound was extracted from the chromatogram data to further confirm its identity, by assessing its fragmentation pattern.

Health risk assessment

Ecological health risk assessment

The risk posed by the detected concentration of each pharmaceutical present in river water toward the aquatic species was classified using the risk quotient approach.[19] This was done by determining the ratio between the pharmaceutical analyte's maximum detected concentration (MDC) and the predicted no-effect concentration (PNEC) for each analyte to that specific species based on equation 1. The PNEC values for each analyte were obtained from the literature.[16,20] These values are derived by dividing the Effective Concentration causing 50% effect (EC50) values obtained from ecotoxicological studies by an assessment factor of 1000.[21] The studied aquatic species were *Vibrio fischeri*, *Daphnia magna*, algae, and fish. The health risk posed by the presence of the selected pharmaceuticals was classified as low, medium, and high risk indicated by the risk quotient value of <0.1, 0.1 to 1, and ≥1, respectively.

$$\text{Risk quotient} = \frac{\text{MDC}}{\text{PNEC}} \quad (1)$$

Human health risk assessment

The concentrations of pharmaceuticals found in the edible parts of the vegetables were used to assess potential risks associated with consuming these vegetables. This involved the determination of the estimated daily intake (EDI) of the detected pharmaceuticals from vegetable consumption using Equation 2.

$$\text{EDI} = \frac{\text{C} \times \text{DR}}{\text{bw}} \quad (2)$$

where C, DR, and bw represent the analyte concentration, body weight of the consumer, and the average daily intake rate, respectively. It was assumed that an adult weighing 70 kg would consume 400 g/person/day of cabbage, while a child weighing 10 kg would consume 237 g/person/day of spinach. These assumptions were based on typical daily consumption rates of cabbage and spinach for both adults and children in South Africa.[22]

The resulting EDI values were applied in establishing the hazard quotient (HQ) values as shown in equation 3.

$$\text{Hazard quotient} = \frac{\text{EDI}}{\text{ADI}} \quad (3)$$

where ADI is an acceptable daily intake for each compound. The ADI represents the amount of a substance that can be safely ingested throughout a person's lifetime without causing harm.[23] ADI values used in this study were sourced from existing literature.[9,24] The risk was considered negligible if the calculated HQ value was below 0.01.

RESULTS AND DISCUSSION

Quality Assurance

The accuracy and precision of the analytical method were assessed by fortifying both aqueous and solid samples with a mixed standard solution containing all analytes at two distinct concentrations: 5 and 15 µg/L for aqueous samples and 5 and 15 ng/g for solid samples. This was done to assess the robustness and the extraction ability of all selected pharmaceuticals in real sample matrices. As observed in **Table 1**, for all the pharmaceuticals, acceptable recoveries ranging from 79 – 101% were observed for river water samples. The corresponding relative standard deviation (RSD) values were below 5%, illustrating the precision of the method. **Figure S1** further illustrates the chromatographic separation of the investigated pharmaceuticals by presenting the extracted ion chromatograms attained for the analysis of spiked environmental samples. This method also demonstrated high sensitivity towards the targeted pharmaceuticals, as evidenced by the MDL and MQL ranging from 0.02 to 1.76 ng/L and 0.07 to 5.35 ng/L, respectively.

Ultrasound-assisted extraction proved to be highly efficient in extracting the targeted pharmaceuticals from vegetables and garden soil. This efficacy is demonstrated by the recoveries ranging from 81 – 119% across all samples, with %RSD values consistently below 10% (**Table 2**). The reported MDL and MQL values across all samples showcased the capability of the analytical method to detect and quantify the selected analytes at parts per trillion levels.

Table 1: Method linearity (R^2), analytes recoveries in deionized and river water, relative standard deviation (±RSD) (n = 3), detection and quantification limits (MDL and MQL).

Pharmaceutical	R^2	Deionized water		River water			
		recovery ± % RSD		recovery ± % RSD		MDL ng/L)	MQL (ng/L)
		5 µg/L	15 µg/L	5 µg/L	15 µg/L		
Efavirenz	0.991	89 ± 0.68	85 ± 1.38	95 ± 0.14	101 ± 1.24	0.14	0.43
Ibuprofen	0.989	85 ± 0.66	87 ± 1.08	79 ± 3.25	84 ± 2.34	1.35	4.08
Naproxen	0.949	103 ± 3.17	94 ± 4.62	92 ± 0.67	98 ± 0.15	1.76	5.35
Sulfamethoxazole	0.998	102 ± 0.47	93 ± 1.65	84 ± 0.75	88 ± 0.31	0.25	0.76
Trimethoprim	0.991	98 ± 1.93	90 ± 0.43	96 ± 0.94	99 ± 1.11	0.02	0.07

The standard calibration range to construct the calibration curves was 0.01 – 1 mg/L.

Table 2: Analytes recoveries, relative standard deviation (±RSD), method detection limit, and method quantification limit.

Analytes	Cabbage				Spinach				Garden soil			
	Recovery ± RSD		MDL ng/kg	MQL ng/kg	Recovery ± RSD		MDL ng/kg	MQL ng/kg	Recovery ± RSD		MDL ng/kg	MQL ng/kg
	5 ng/g	15 ng/g			5 ng/g	15 ng/g			5 ng/g	15 ng/g		
Efavirenz	94 ± 4.81	96 ± 1.60	0.24	0.72	96 ± 4.79	95 ± 7.29	0.36	1.16	80 ± 3.37	83 ± 6.03	0.48	1.48
Ibuprofen	85 ± 3.80	81 ± 8.10	4.92	14.92	82 ± 7.32	82 ± 7.42	8.36	25.32	95 ± 7.88	101 ± 9.33	4.92	14.9
Naproxen	102 ± 5.42	90 ± 8.68	2.00	6.00	111 ± 7.37	119 ± 3.03	2.44	7.44	90 ± 5.00	101 ± 5.80	1.80	5.40
Sulfamethoxazole	104 ± 7.51	90 ± 8.82	0.32	0.96	91 ± 4.50	101 ± 4.01	0.48	1.4	88 ± 3.34	94 ± 3.84	0.16	0.52
Trimethoprim	87 ± 6.80	96 ± 8.44	0.12	0.40	90 ± 5.44	98 ± 7.70	0.08	0.28	91 ± 2.54	87 ± 2.18	0.20	0.64

It is well known that SPE plays a crucial role in extracting and pre-concentrating analytes from complex matrices, thereby enhancing sensitivity and selectivity in analytical techniques.[25]

Identification of selected pharmaceuticals in the environmental samples

The identification of pharmaceuticals within the investigated environmental samples was conducted through a rigorous analytical approach. Initially, the analyte's retention times and ionized masses in samples were compared with those of established reference standards, serving as the primary means of identification. Subsequently, to further validate the identity of these compounds, their MS/MS fragmentation patterns were analysed and compared with those obtained from the standard references. **Figure S2** presents the MS/MS spectra of the selected pharmaceuticals and their respective fragmentation patterns. Notably, the precursor ions of efavirenz (316.0315 m/z), ibuprofen (207.1376 m/z), naproxen (231.1016 m/z), sulfamethoxazole (254.0597 m/z), and trimethoprim (291.1450 m/z) exhibited close agreement with values reported in the literature.[4,26,27] This concordance reinforced the confidence in the accurate identification of these pharmaceuticals within the environmental samples under investigation.

Occurrence and prevalence of selected pharmaceuticals in Buffalo River

The presence of pharmaceuticals in the environmental waters has been extensively studied in South Africa,[28,29] thereby providing valuable insights into the extent and dynamics of pharmaceutical contamination in South African aquatic environments. The concentrations of the selected pharmaceuticals observed in this study are presented in **Table 3**. Traces of efavirenz and ibuprofen were detected across all surveyed sites, indicating widespread contamination of these pharmaceuticals in the Buffalo River. These compounds were found within the concentration ranges of 2 – 181 ng/L and 34 – 460 ng/L, respectively. The 100% detection of these compounds could be attributed to consumption patterns. For instance, South Africa has an extensive number of people benefiting from HIV treatment,[28] and ibuprofen, an over-the-counter drug, accessible without a medical prescription is mostly used to treat cold symptoms.[30] Since the human body is unable to metabolize these drugs after consumption, substantial quantities are excreted as human waste, contributing to their consistent detection across the sampled sites. Naproxen was found in 80% of the investigated sites and it recorded the highest concentration of 980 ng/L at site 5. The high detection of naproxen could be linked to its accessibility as it is normally obtained as an over-the-counter medication, which facilitates its widespread use, and results in its environmental presence. Moreover, a study conducted by Sibeko *et al*, reported comparable concentrations of naproxen in the Mbokodweni River, ranging from 1 to 3.8 µg/L.[31]

Sulfamethoxazole and trimethoprim were also found in 80% of the sampled sites, with the concentrations ranging from <LOQ – 209 ng/L and 10 – 183 ng/L, respectively. The significant detection of these antibiotics in this study comes as no surprise, as they are consistently found in the South African aquatic environment. These drugs are primarily employed in treating animal infections, particularly for combating coccidiosis in poultry.[32] The relatively high concentrations of these antibiotics in river water could be attributed to both the inadequate maintenance of nearby WWTPs and the utilization of outdated technologies during the treatment process which allows for the disposal of these drugs into the surface water. The detected concentrations of sulfamethoxazole and trimethoprim are relatable to those found in previous studies.[34,35,36] For instance, a study conducted by Mhuka *et al*. reported a high detection of these antibiotics in Apies River (South Africa), with a concentration of 300 ng/L and 100 ng/L, respectively.[34] Another study conducted in a neighbouring country, Zambia, reported considerably higher concentrations of these antibiotics in surface water in Chunga Lusaka, with levels reaching 11.8 µg/L.[36] This could be attributed to the prescribed dosages across the region, as well as anthropogenic activities occurring in the vicinity of the river, such as improper disposal of expired medication and agricultural runoff from farms where these antibiotics are commonly used. Overall, the presence of pharmaceuticals in investigated samples significantly contributes to the degradation of freshwater ecosystems, posing a serious threat to aquatic species. Given that many South African communities rely on river water for domestic purposes, including drinking and crop irrigation, this issue becomes even more pressing. Consequently, it adds to the global challenge of antimicrobial resistance, intensifying the spread of drug-resistant pathogens and compromising public health on a broader scale.

Occurrence and prevalence of pharmaceuticals in irrigation water, garden soil, and vegetables

Irrigation water

All the investigated households used tap water to irrigate the vegetables. Hence, tap water also underwent analysis to ascertain the origin of pharmaceuticals found in the vegetables and the results are presented in **Table 4**. Trimethoprim was the sole analyte detected in the tap water used for irrigating cabbage and spinach samples with the concentration reaching 30 ng/L. The detection of trimethoprim in tap water indicates that conventional drinking water treatment processes are not effective in terms of removing pharmaceuticals. Similar observations were previously reported in a previous study.[4] This issue often arises when water treatment plants abstract water from rivers and dams that are heavily polluted with pharmaceuticals, leading to the presence of compounds like trimethoprim in the tap water supply. The occurrence of pharmaceuticals in soil and vegetables was examined to determine if contamination originated from sources other than irrigation water. Additionally, most rural communities in South Africa experience inconsistent water supply. Consequently, river water might sometimes be used to irrigate homestead crops, which could contribute to the presence of pharmaceuticals in the vegetables. This comprehensive analysis was essential to ensure that all potential contamination pathways were considered.

Table 3: Detected concentrations of selected pharmaceuticals in Buffalo River.

Sampling sites	Detected concentrations (ng/L) ± SD				
	Antiretroviral	Antibiotic		NSAIDs	
	Efavirenz	Sulfamethoxazole	Trimethoprim	Ibuprofen	Naproxen
Site 1	77 ± 0.003	nd	10 ± 0.697	111 ± 0.002	nd
Site 2	15 ± 0.481	<LOQ	nd	50 ± 0.031	<LOQ
Site 3	2 ± 0.000	23 ± 0.024	58 ± 0.332	34 ± 0.221	169 ± 0.332
Site 4	141 ± 0.008	184 ± 0.018	100 ± 0.006	320 ± 0.218	498 ± 0.459
Site 5	181 ± 0.070	209 ± 0.038	183 ± 0.026	460 ± 0.068	980 ± 0.585

nd; not detected, <LOQ; below limit of quantification.

Table 4: Detected concentrations of selected pharmaceuticals (ng/kg, dw ± SD) in irrigation water, garden soil, cabbage, and spinach samples (n = 3)

Matrices		Household 1					Household 2				
		Efavirenz	Ibuprofen	Naproxen	Sulfamethoxazole	Trimethoprim	Efavirenz	Ibuprofen	Naproxen	Sulfamethoxazole	Trimethoprim
Irrigation water*		nd	nd	nd	nd	10 ± 0.001	nd	nd	nd	nd	16 ± 0.000
Garden soil		nd	nd	nd	nd	<LOQ	nd	nd	nd	nd	<LOQ
Cabbage	Leaves	nd	nd	nd	nd	51 ± 1.402	nd	nd	nd	nd	131 ± 6.303
	Cooked leaves	nd	nd	nd	nd	nd	nd	nd	nd	nd	<LOQ
	Stem	nd	nd	nd	nd	nd	nd	nd	nd	nd	<LOQ
	Roots	nd	nd	nd	nd	<LOQ	nd	nd	nd	nd	664 ± 15.786
Spinach	Leaves	nd	nd	nd	nd	172 ± 2.140	nd	nd	nd	nd	431 ± 2.433
	Cooked leaves	nd	nd	nd	nd	<LOQ	nd	nd	<LOQ	nd	<LOQ
	Stem	nd	nd	nd	nd	283 ± 0.781	nd	35 ± 0.020	nd	nd	305 ± 1.399
	Roots	<LOQ	nd	nd	nd	<LOQ	328 ± 0.113	272 ± 0.141	nd	nd	180 ± 6.975
		Household 3					Household 4				
Irrigation water*		nd	nd	nd	nd	30 ± 0.000	nd	nd	nd	nd	nd
Garden soil		nd	<LOQ	nd	nd	nd	nd	nd	nd	nd	<LOQ
Cabbage	Leaves	nd	<LOQ	nd	993 ± 0.059	874 ± 0.065	nd	629 ± 0.790	nd	265 ± 0.001	1501 ± 0.104
	Cooked leaves	nd	nd	nd	<LOQ	<LOQ	nd	<LOQ	nd	nd	<LOQ
	Stem	nd	21 ± 0.311	nd	14 ± 1.210	55 ± 0.554	nd	52 ± 1.021	nd	16 ± 2.154	<LOQ
	Roots	nd	121 ± 0.010	nd	431 ± 0.245	661 ± 0.332	nd	147 ± 0.002	nd	66 ± 0.001	521 ± 0.255
Spinach	Leaves	nd	nd	nd	nd	62 ± 0.037	nd	nd	nd	nd	nd
	Cooked leaves	nd	nd	nd	nd	<LOQ	nd	nd	nd	nd	nd
	Stem	nd	nd	nd	nd	24 ± 0.001	nd	nd	nd	nd	nd
	Roots	nd	nd	nd	nd	134 ± 0.005	nd	nd	nd	nd	nd

*the presented concentrations of irrigation water are in ng/L,nd; not detected, <LOQ; below limit of quantification.

Garden soil

Commonly, vegetables uptake pharmaceuticals when irrigated with contaminated water or when grown on contaminated soil.[8] Garden soil can become contaminated with pharmaceuticals through various pathways, including the direct disposal of medications onto the land surface, the use of fertilizers that are contaminated with pharmaceutical residues, and the excretion of human and animal waste into the ground. All these activities are common in East London communities where there is limited infrastructure related to farming and proper ablution facilities. When pharmaceuticals are disposed of improperly, either by being flushed down the toilet or discarded onto the ground, they can leach into the soil over time. Additionally, if fertilizers are derived from sources exposed to pharmaceutical contamination, such as sewage sludge or animal manure from medicated livestock, they can introduce pharmaceutical residues into the soil when applied to gardens.[37] However, only ibuprofen and trimethoprim were detected in the analyzed garden soil samples, though at unquantifiable concentrations (**Table 4**). Thus, indicating the persistency of these drugs in the environment. Analytes that were not detected in garden soil samples may have been influenced by factors such as dilution, degradation, transformation into metabolites, and uptake by plants.

Vegetables

In all analysed vegetable segments, trimethoprim was the most dominant analyte with the highest detection found at 1501 ng/kg, dw in cabbage leaves from household 4 (see **Table 4**). The second highest detection observed was sulfamethoxazole in cabbage leaves from household 3. Sulfamethoxazole and trimethoprim are commonly used in combination, typically in a ratio of 5:1 to effectively treat a wide range of bacterial infections in animals.[38] This might be attributed to the usage of animal manure to increase soil fertility, leading to the accumulation of these antibiotics in vegetables. In the analysed spinach samples, only trimethoprim was detected in the edible segments at relatively lower concentrations compared to those found in cabbage. This variance could potentially be attributed to the duration of exposure during cultivation. Cabbage typically requires a longer growth period, ranging from 12 to 20 weeks before reaching maturity and being ready for harvest. Conversely, spinach has a shorter growth cycle, typically taking only about 6 to 8 weeks to reach harvest readiness.[39] This shorter period may result in less time for pharmaceutical residues to accumulate in the spinach plants, thus leading to lower contamination levels than cabbage.

The uptake of pharmaceuticals by vegetables is a complex mechanism that is impossible to cover in detail within this study. Initially, the process begins with the absorption of pharmaceuticals by the roots of the plant. Subsequently, these absorbed pharmaceuticals are transported to the aerial parts of the plant, contributing to their presence in the aerial tissues.[40,41] Hence, it is crucial to monitor the distribution of pharmaceuticals in various plant segments. In cabbage stem, pharmaceutical levels were observed to be mostly below quantification levels, indicating limited retention of pharmaceuticals within the stem tissue. However, in spinach stem, trimethoprim was mostly found at quantifiable concentrations recording a maximum of 305 ng/kg, dw, suggesting some degree of uptake in the stem tissue. Efavirenz emerged as the least detected analyte across all samples, exclusively appearing in spinach roots. This observation can be attributed to its distinctive characteristics, particularly its low water solubility (10 mg/L) and relatively large molecular size (315.68 g/mol), which limit its permeability through cell membranes. As a result, efavirenz faces difficulty in translocating to the aerial parts of the plant, leading to its exclusive detection in spinach roots.[42,43] The highest detection of efavirenz in spinach roots was 328 ng/kg, dw. However, a study by Akenga *et al*. reported a significantly higher accumulation of 3463 ng/g in spinach roots following exposure to a concentration of 100 µg/L in a hydroponic experiment.[8] This contrast highlights the influence of factors such as biodegradation and exposure levels on the accumulation of pharmaceuticals in vegetables cultivated under environmental conditions. Ibuprofen and trimethoprim were also detected in spinach roots at concentrations of 272 and 180 ng/kg, dw. Meanwhile, analytes detected in cabbage roots were observed to follow the order of ibuprofen < sulfamethoxazole < trimethoprim.

Impact of cooking vegetables

The study also investigated the effects of cooking on the presence of pharmaceuticals in vegetables. In this experiment, both cabbage and spinach leaves were boiled for 5 minutes and then analysed. This takes into consideration the limited cooking time that is mostly explored by many consumers. As shown in **Table 4**, all the analytes were found to be below the limit of quantification in the cooked samples except for trimethoprim which was not detected in the cooked cabbage sample from household 1. This suggests that the process of cooking may lead to the thermal degradation of pharmaceutical residues or their dissolution from vegetable materials into the boiling water, possibly due to the high-water solubility of the pharmaceuticals. However, naproxen, which was not initially detected in spinach leaves from household 2, was found to be present below the quantification limits in the cooked sample. This unexpected finding could suggest that naproxen transformed during the cooking process, potentially becoming more biologically active. This warrants more research to better understand these changes and their implication for food safety and human health. This aspect could be explored by analysing the water used for cooking the vegetables, a factor that was not considered in this study. Furthermore, this study showcased a need to monitor the metabolites of these pharmaceuticals and the parent compounds in vegetables before and after cooking. This is necessary to understand the transformation of these drugs during exposure to high temperatures.

Overall, the results presented in this study highlight the differential accumulation and distribution of pharmaceutical residues within different parts of cabbage and spinach plants. Leaves exhibited the highest concentrations of pharmaceuticals, followed by roots, with stems showing the lowest levels across all the samples. These findings underscore the importance of understanding pharmaceuticals' uptake and translocation mechanisms in plants, particularly leafy greens like spinach and cabbage, and their implications for food safety and human health. Further research is necessary to elucidate the factors influencing pharmaceutical uptake in plants and to develop strategies for mitigating their potential impact on consumer health and the environment.

Translocation of pharmaceuticals to aerial parts of the plant

Pharmaceuticals that were found present in the aerial parts of the plant (stem and leaves) were ibuprofen, sulfamethoxazole, and trimethoprim. The extent to which these analytes were transported to aerial segments was assessed by computing the translocation factor for each analyte. This was accomplished by dividing the concentration into the aerial segments by that found in the roots using equations 4, 5, and 6.

$$T_f = \frac{C_{stem}}{C_{root}} \tag{4}$$

$$T_f = \frac{C_{leaves}}{C_{roots}} \tag{5}$$

$$T_f = \frac{C_{stem\ +\ leaves}}{C_{roots}} \tag{6}$$

where T_f, C_{stem}, C_{leaves}, and C_{roots}, represent translocation factors, pharmaceutical concentration found in the stem, leaves, and roots, respectively.

The resultant translocation factors are presented in **Table 5**. Efavirenz and naproxen were not detected in the stems and leaves of both cabbage and spinach, prohibiting the computation of translocation factors for these analytes. It has been documented previously that lipophilic compounds like efavirenz and naproxen often exhibit limited mobility within plants.[44] This limitation may stem from their high affinity for lipid-rich environments and lower water solubility, factors that could inhibit their translocation from roots to leaves.

As indicated in **Table 5**, the translocation factors of analytes from roots to stems in cabbage plants were predominantly less than 1, except for trimethoprim, which recorded a translocation factor of 1.30 in spinach. On the contrary, the translocation factors of all analytes from roots to leaves were found to be greater than 1, suggesting a high propensity for analytes to migrate from roots to leaves.[8] Ibuprofen was not detected in spinach leaves, hence its translocation factor from roots to leaves was not computed. Overall, all the analytes demonstrated a translocation factor greater than 1 from the roots to the aerial parts of both cabbage and spinach. This observation raises concerns, as both cabbage and spinach are often consumed fresh from the garden, potentially exposing individuals directly to pharmaceutical residues.

Statistical analysis was conducted to evaluate the potential factors influencing the accumulation of pharmaceutical residues in the aerial parts of the plant. This involved converting the octanol-water partition coefficient (Log K_{ow}) to the pH-adjusted octanol-water partitioning coefficient (log D_{ow}) using a pH value of 6.5. This pH was chosen based on the preference of both cabbage and spinach for slightly acidic to neutral soil pH, which promotes their optimal growth.[45] The resultant correlation observed between Log TF and Log D_{ow} in both cabbage and spinach presented a magnificent statistical relation ($r^2 > 0.87$) (**Figure 2**). Thus, the accumulation of these pharmaceuticals in roots and their translocation to aerial parts might be influenced by the hydrophobicity of the analytes. It has been documented elsewhere that compounds with intermediate polarity (Log D_{ow}) in the range of 0.5 – 3, are readily distributed within the plant to the aerial parts of the plants.[46] However, factors such as exposure time, compound concentration, vegetable type, and soil type also play crucial roles in the migration of analytes to the upper parts of the plants.

Table 5: Translocation factors of the selected pharmaceuticals from the roots to stem and leaves of cabbage and spinach.

Vegetable	Pharmaceutical	Translocation factor (TF)		
		Roots to stem	Roots to leaves	Roots to stem and leaves
Cabbage	Ibuprofen	0.27	4.69	2.40
	Sulfamethoxazole	0.06	2.53	1.30
	Trimethoprim	0.09	2.56	1.18
Spinach	Ibuprofen	0.13	-	0.13
	Trimethoprim	1.30	1.41	1.36

Health risk assessment

Assessment towards aquatic species

The potential ecological impact of the selected pharmaceuticals on aquatic species was assessed through the computation of the risk quotient. **Table 6** presents the resulting risk quotient values for the selected pharmaceuticals concerning aquatic species. Despite naproxen being detected at the highest concentration (980 ng/L) compared to other pharmaceuticals, the observed level poses no health risk to *Vibrio fischeri*, *Daphnia magna*, algae, and fish. However, efavirenz which was detected at the lowest concentration of 181 ng/L was observed to be of high risk to *Vibrio fischeri*. Also, the maximum concentration of sulfamethoxazole was observed to be of high risk to algae. These findings highlight the necessity to constantly monitor the presence of efavirenz and sulfamethoxazole in aquatic environments. The continuous discharge of pharmaceuticals from WWTPs and improper disposal, along with other possible sources, could increase the concentrations of pharmaceuticals that are deemed harmless to aquatic species in this study. Furthermore, other pharmaceuticals may be present in much higher quantities potentially presenting certain risks to aquatic species. Hence, it is imperative to expand the analytical

Table 6: Ecological health risk assessment towards aquatic species.

Pharmaceutical	Maximum concentration (µg/L)	PNEC (µg/L)				Risk quotient			
		Vibrio fischeri	*Daphnia magna*	Algae	Fish	*Vibrio fischeri*	*Daphnia magna*	Algae	Fish
Efavirenz	0.18	0.13	4.4	-	-	1.38	0.04	-	-
Ibuprofen	0.46	35.7	9.06	5.7	170	0.01	0.05	0.08	0.00
Naproxen	0.98	21.2	25	626	34	0.05	0.04	0.00	0.03
Sulfamethoxazole	0.21	-	25	0.0027	563	-	0.01	77.8	0.00
Trimethoprim	0.18	-	121[b]	16	100	-	0.00	0.01	0.00

PNEC values attained from[16] except one value [b] which was sourced from[20]

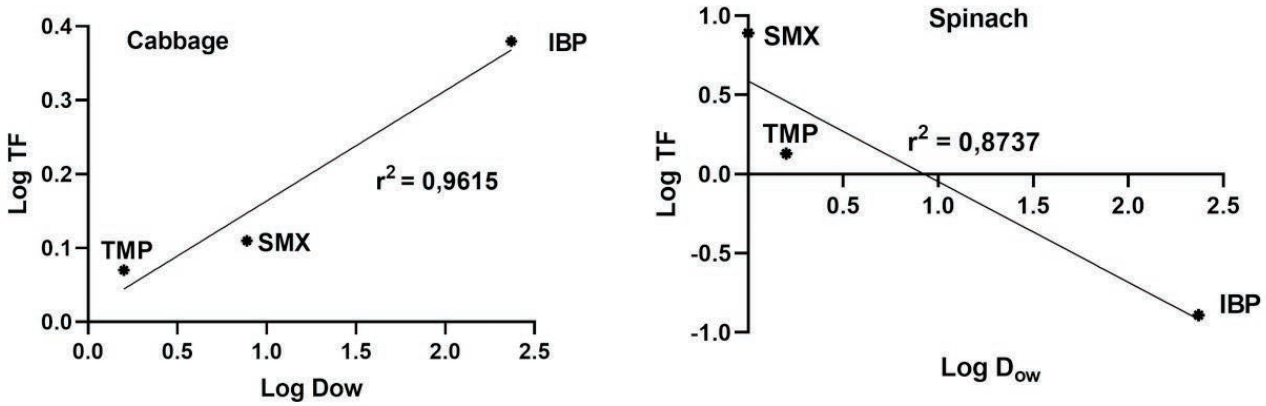

Figure 2: Relation between Log TF and Log D_{ow} of ibuprofen (IBP), sulfamethoxazole (SMX), and trimethoprim (TMP) in cabbage and spinach.

Table 7: Estimated daily intake of detected pharmaceuticals in cabbage and spinach leaves, their acceptable daily intake, and hazard quotient for both adults and children.

Vegetable	Pharmaceutical	Detected concentration (ng/kg, dw)	Estimated daily intake (ng/kg/day)		Acceptable daily intake (ng/kg/day)	Hazard quotient	
			Adults	Children		Adults	Children
Cabbage	Ibuprofen	629	3.59	14.84	110 000[a]	$3.26x10^{-4}$	$1.35x10^{-3}$
	Sulfamethoxazole	874	4.99	20.71	130 000[b]	$3.84x10^{-5}$	$1.59x10^{-4}$
	Trimethoprim	1501	8.58	35.57	4200[b]	$2.04x10^{-3}$	$8.47x10^{-3}$
Spinach	Trimethoprim	431	2.46	10.21	4200[b]	$5.86x10^{-4}$	$2.43x10^{-3}$

ADI values were obtained from [a 24] and [b 46]

scope to encompass a wide range of pharmaceuticals in environmental waters as well as consider the effect of low-dose chronic exposures. This expansion will enable more enhanced risk assessments of aquatic species exposed to pharmaceuticals. Since some aquatic species form part of human food chains, their high exposure to pharmaceutical residues might also expose humans to these pollutants.

Assessment towards humans

Human health risks associated with the consumption of cabbage and spinach contaminated with pharmaceuticals were evaluated. This was accomplished through the computation of estimated daily intake (EDI) which was derived from the maximum concentration detected in both cabbage and spinach. The resultant EDI values for each analyte were then compared with the acceptable daily intake for each pharmaceutical and the results are shown in **Table 7**. As observed in **Table 4**, trimethoprim was the only drug that was found present in spinach leaves, hence no EDI for the other four analytes was obtained. In all cases, the EDI of pharmaceuticals was observed to be higher for children compared to adults across all the studied vegetables. For instance, the EDI of trimethoprim for children through consumption of cabbage was found to be 35.57 ng/kg/day, whereas adults are estimated to be consuming 8.58 ng/kg/day of trimethoprim. This difference is mainly because the EDI was computed as per individual body weight. Therefore, children, with their body weight, exhibit higher EDI even with the same level of exposure. This observation aligns with the findings of the study conducted by Tadić *et al.* despite their focus on vegetables intentionally irrigated with reclaimed wastewater.[46] Although the concentrations of pharmaceuticals detected in the vegetables do not appear to pose immediate harm to human health, remaining uncertainties persist regarding potential health risks associated with prolonged low-dose exposures over time. The shift in bioavailability after cooking highlights that not enough is known in this regard. Thus, continued research is warranted to comprehensively assess the long-term implications of pharmaceutical residues in food crops and their potential effects on human health.

Suspect screening of pharmaceuticals in environmental samples

A total of 108 pharmaceuticals were identified in the investigated samples. As shown in **Table S2**, 94 compounds were detected in the studied vegetables, while 65 drugs were found in the aqueous samples. Among these, antibiotics were the most prevalent therapeutic group, followed by cardiovascular agents. The high prevalence of antibiotics in South African environmental matrices is a well-documented issue.[18,47,48] However, there is currently a lack of information on the presence of cardiovascular medications in South African matrices. Pharmaceuticals detected in aqueous samples are listed in **Table S3**. The compounds predominantly detected in these samples include acetaminophen, bicalutamide, dronedarone, norgestrel, sulfadiazine, and sulfasalazine. These compounds were found in more than five aqueous samples, showing a high prevalence in river water samples. However, the tap water collected from household 4 was found to contain 17 pharmaceuticals.

In the case of vegetables (**Table S4**), the dominant compounds identified were allopurinol, bicalutamide, cocaine, cyproterone, and thioridazine. These compounds appeared in more than six cooked and uncooked vegetable samples. However, most were not detected in the cooked samples, indicating thermal degradation or dissolution in the cooking water. This suggests that cooking may reduce the presence of certain pharmaceutical residues in vegetables. For instance, only 10 compounds were detected in the cooked cabbage sample from household 3, whereas 19 compounds were detected in the uncooked samples. A similar trend was observed with spinach, where 7 compounds were detected in the cooked spinach from household 4, compared to 19 in the uncooked samples. However, certain compounds that were undetected in raw samples were found in cooked samples, indicating that these compounds may have been activated through biological processes during cooking. These findings emphasize the need for further pharmaceutical monitoring studies on vegetables to ensure food safety and understanding of potential health risks associated with consuming uncooked and cooked vegetables.

CONCLUSIONS

The current study assessed the occurrence of the selected pharmaceuticals in river water, cabbage, and spinach, as well as in the garden soil cultivated under real environmental conditions. This investigation was predicated on the understanding that river water serves as a principal reservoir of pharmaceutical contaminants, commonly utilized for abstraction in drinking water treatment facilities, and subsequently utilized for vegetable irrigation. The observed concentrations in the edible parts of the studied vegetables were found to be of no harm to both adults and children. Despite conducting this survey considering the worst-case scenario of consuming vegetables daily, the presence of pharmaceutical residues in vegetables is of concern as it directly exposes individuals to these pollutants, potentially contributing to the emergence of antimicrobial resistance. In addition to the targeted pharmaceuticals, 108 other drugs were detected across the investigated samples. The findings of this study call for the immediate establishment of pharmaceutical monitoring programs and mitigative measures to prevent the occurrence of pharmaceuticals in the environment as they are finding their way into human food chains.

SUPPLEMENTARY INFORMATION

The datasets resulting from the current study are available from the author in the case of a reasonable scientific request. Additionally, some datasets are provided in the supplementary material.

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AUTHOR CONTRIBUTIONS

Credit: Ronewa Netshithothole data curation, formal analysis, investigation, methodology, software, writing original draft; Lawrence Mzukisi Madikizela funding acquisition, project administration, resources, supervision, writing-review & editing.

DECLARATION

The authors declare no competing financial interest.

DECLARATION OF GENERATIVE AI

Authors declare the use of Grammarly check AI assistance.

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