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Sulfamethoxazole Residues in Vegetables Irrigated with Untreated Wastewater

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ABSTRACT

Untreated wastewater is used for growing of vegetables in small scale urban farming. Vegetables grown or irrigated with untreated wastewater may contain high levels of antibiotics residues that are detrimental to health. Sulfamethoxazole (SMX) is an antibiotic, administered in the management of *pneumocystis carinii* pneumonia, *pneumocystis jiroveci* pneumonia, toxaplasmosis and genitourinary tract infections in HIV-AIDS patients or in cases of oral thrush infections. It is cheap and readily available over the counter even through self-prescription for management of upper respiratory tract and genitourinary tract infections. The drug is also administered to poultry and livestock as a growth promoter, prophylactic and to control microbial infections. Its presence in vegetables could induce microbial resistance and minimize drug sensitivity. The concentration of sulfamethoxazole in untreated wastewater and vegetables collected during the dry season from various sites in Ruai and Njiru from small scale urban farms along Ngong River was determined. The samples for sulfamethoxazole residues underwent solvent extraction pre-analysis and the extracts were then analyzed using high performance liquid chromatography. The untreated waste water and vegetables were found to have sulfamethoxazole residues.
KEYWORDS: Wastewater Sulfamethoxazole Vegetables Human Health

1.0 INTRODUCTION

For most of the vulnerable populations living in the cities, farming is considered an important means of livelihood despite there being no access to agricultural land [1]. The farming carried out in urban and peri-urban sites in Nairobi is irrigated with untreated wastewater [2]. Untreated municipal wastewater comes from residential properties like houses and apartments as well as commercial buildings, hospitals, health centers, industrial and agricultural processes [3]. It can contain a wide variety of contaminants and presents a health hazard where humans or animals come into contact with it. It may overflow from a sanitary sewer after a period of very heavy rainfall passing through slums loaded with raw human wastes. Unlike pesticides used on agricultural land, antibiotics have not aroused attention as potential pollutants until fairly recently [4]. Bacterial resistance has been a big issue in terms of human and animal health; however, antibiotic ecotoxicological relevance is scarcely known because the potential effects of antibiotics in the environment are very limited [5]. In the Kenyan sewerage system, hospital, industrial and domestic wastes are discharged together.

The sulfonamide antibacterial group is considered to be one of the first antimicrobial drugs used and paved the way for the antibiotic revolution in medicine [6]. It was intensively manufactured since the mid 1940s. Sulfamethoxazole falls under the class of sulfonamide antibacterial group [7]. The medicinal use of sulfamethoxazole is for treatment of tuberculosis, malaria and urinary tract infections [8]. Its mode of action is prevention of folic acid synthesis in the bacteria [9].

The SMX is water soluble, has a half life of 19 days under sunlight and is highly resistant to further biodegradation in the subsurface [10]. It has a low K_{ow} (− 0.1 to 1.7) and is considerably hydrophilic and polar. Such properties enable SMX to be transported over long distances without being adsorbed to sediments [11]. Furthermore, under typical environmental pH conditions (pH ~ 7–8) SMX is negatively charged up to approximately 95–100%; a property that can increase its transport velocity in porous media due to anion exclusion.

Like other sulfonamides, SMX disrupts the folate biosynthetic pathway in bacteria, which was recently established as identical to that of plants, raising concerns over nontarget toxicity [9].

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In a study carried out to determine the concentration of sulfamethoxazole in sewage sludge, the concentration of SMX detected was in the range of 28 and 68 μg/kg [12]. The dominant pathway for antibiotic release in the terrestrial environment is through the application of animal manure and biosolids containing excreted antibiotics to agricultural land as fertilizer [13]. Antibiotics can also be introduced to agricultural land through irrigation with reclaimed wastewater, since they have been frequently detected in the raw and treated sewage wastewaters [14]. The effects of antibiotics on plants in soils are found to be different between compounds and between plant species [15].

2.0 MATERIALS AND METHODS

2.1 Study site

The study site was in Njiru/Ruai locations in Nairobi County located in the riparian region of the Ngong River that flows through Nairobi County. The sample collection points were upper Njiru, which is the area just past the Mwiki Police Station towards Njiru shopping centre in the Gituamba quarry site vicinity, Lower Njiru, which is the area that is within the vicinity of the Eastern by-pass road running at a point where two untreated water streams that make the Ngong-Nairobi River converge.

The two streams flow down, ridden with sewage into the Ruai sewage treatment site. Upper Ruai is approximately two kilometers behind a shopping center referred to as Sewage shopping centre, a site occupied by internally displaced people, which is a flood zone, and lower Ruai which is past the Sewage Shopping centre vicinity. The study site is arid, characterized by thorny shrubs and short thorny trees, in the middle of which there are the lash green vegetable farms. The geographical terrain of the study site is that Upper Njiru is upstream, with the river flowing through Lower Njiru, then Upper Ruai and finally Lower Ruai downstream at the lower end. The GPS bearing points for some of the sampling sites are: S114.654 E36 57.255, S114.663 E36 57.260, S1 14.666 E36 57.269, S114.669 E 36 57.273, S114.667 E36 57.198, S114.672 E36 57.206.

The vegetable farms directly get the untreated waste water from the exhausters in areas where exhausters discharge. This wastewater is then used for irrigation of agricultural crops and vegetables by the farmers. The study site also receives waste water from the Kariobangi small scale industries and part of the industries that are in the Njiru Township. Most of these industries deal with reconditioning of used cars, spray painting cars, smelting ores and there are several health centers and hospitals that discharge their waste directly into the River in the neighbourhood.

Seasonal variations were not considered because the farming takes place along the River banks of the Ngong River parallel to the Nairobi sewer lines and this is a flood zone during the rainy season. No farming is carried out during the rainy season until the rivers dry down and so the farmers use the banks exclusively in the dry season.

Plate 2.1: The sampling site where the vegetables are grown (a) The study site and background of the dry farms at Njiru where the vegetables are grown by irrigation. (b) Cow pea vegetables grown along the Ngong River polluted with wastewater used for irrigation.

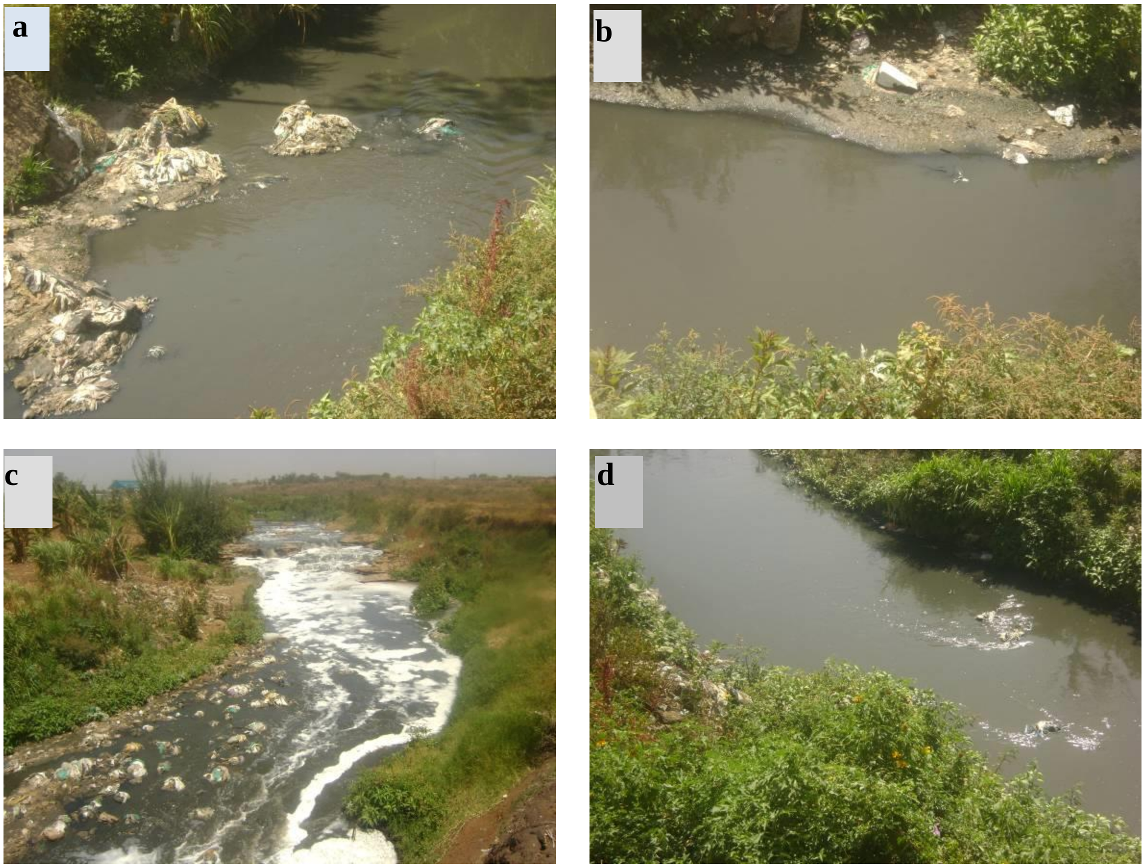

Plate 2.2: Foamy and dark coloured water of Ngong River used to irrigate the farms with vegetables. (a&b) Sewage and dumped materials disposed into a flowing river. (c) The flowing river carrying the sewage and dumped materials. (d) Still wastewater along the river used for irrigating the vegetables.

2.2 Research design

Quasi comparative study design among four areas was used, and there was a control plot at the Kenyatta University. The control plot comprised of potted plants that were grown on the soil collected from the study site but away from the farm irrigated with the untreated wastewater. The potable water used was from Ruiru dam which is the same water used by the population in the study area for domestic purposes.

2.3 Determination of sample size

The sample size was calculated using the formula reported by [16] and shown in equation 2.1

$$n = \frac{Z^2 P(1-P)}{d^2} \quad \ldots\ldots\ldots(2.1)$$

Where:

n = sample size

Z = statistic for a level of confidence (for the level of confidence of 95%, it is 1.96)

P = Expected prevalence or proportion (the proportion of farms where irrigation is carried out with sewage in Nairobi is 35% [2], this added to those who use untreated waste water 15% [2], P= 50% = 0.5)

d = precision (in proportion of 5 %, d = 0.25)

From the equation, a total of 30 vegetable samples and 30 untreated wastewater samples were to be from the farms in each study area; that is, Upper Njiru, Lower Njiru, Upper Ruai and Lower Ruai.

There were four study sites so 30 *4=120.

The number was then doubled to 240 because there are two dry seasons in the year when the irrigation is carried out and sampling was done. This increase of sample size minimized any confounding effects that arise due to use of very small sample size that may not be representative of the population. After sampling for the three consecutive years, the mean value concentration and standard deviation per study site was got from the samples collected per site.

The samples were collected on Monday, Wednesday and Friday during the dry season and for each of the three samples from the same site at the same season, they were homogenized. This was taken to be one sample and the procedure was repeated two more times during two other consecutive dry seasons in two following years.

Therefore the sampling was carried out in triplicate for three alternate days for three consecutive years during the two dry seasons till a total of the expected sample of 240 was collected per year. The respective vegetables grown in the sample farms were also grown in the control plot.

2.4 Chemicals and solvents

All chemicals and solvents used for HPLC were HPLC grade. Acetronitrile HPLC grade, methanol HPLC grade, hexane HPLC grade, ethyl acetate HPLC grade, analytical grade potassium dihydrogen phosphate, ultra pure water, standards for SMX.

2.5 Cleaning of glass ware and sample containers

All glassware and plastic containers were thoroughly washed with 10% HCl and rinsed thoroughly with de-ionized water and then oven dried. Prior to use, the glassware was rinsed out finally with 10% HCl acid solution. Storage self sealing bags used were clean and used as they were bought for packaging of vegetables.

2.6 Sampling and sample pretreatment

The samples of vegetables and water were taken from different plots irrigated with untreated wastewater during the dry season in areas namely; Upper Njiru, Lower Njiru, Upper Ruai and Lower Ruai. In each area, five farms that grew a specific vegetable of interest were identified and from each such farm, six samples of vegetables, water and soil were collected as described in the following subsections. The sampling was carried out in the morning before 8.00 am to limit the concentration effects of evaporation due to the hot sun during the dry season. The samples were collected on Monday, Wednesday and Friday during the dry season and for each of the three samples from the same site, they were homogenized. The the sampling was carried out in triplicate for three alternate days in the months of January and September for three consecutive years. The respective vegetables grown in the sample farms were also grown in the control plot. A total of 240 vegetable samples and 240 wastewater samples were collected. Samples from the control plot were also collected on the same days as the test samples in triplicate and homogenized prior to analysis just as the test samples.

2.6.1 Water samples

Water samples were collected using 500 ml plastic bottles that were washed with de-ionized distilled water and at the collection point, the bottles were rinsed with the untreated waste water twice before filling them with the sample water. The untreated waste water is pumped using a generator to flood the farm land and sampling was carried out in triplicate from each source point of irrigation of a particular vegetable. No additives to preserve the water were added to this water and upon arrival to the laboratory; it was subjected to solid phase extraction [17].

2.6.2 Vegetable samples

The vegetables were collected in triplicate from each of the four study sites. The vegetables collected were kales *(Brassica oleraceae),* spinach *(Spinacia oleraceae),* cow peas *(Vigna unguiculata),* pumpkin *(Cucubita pepo)* leaves. The fresh vegetables were collected in the morning between 8 and 9 am and packed in well labeled plastic paper bags. They were transported to the laboratory within 1 hour after collection. They were then put through solvent extraction.

2.7 Determination of the minimum detection limits

This was tested by gradually lowering the concentration of the standard material by diluting with double distilled de-ionized water and determining the concentration of sulfamethoxazole in the standard up to a point where the levels could not be further detected. The lowest concentration that the equipment was able to read was the minimum detection limit.

2.8 Analysis of antibiotic residues

Vegetables and untreated waste water were collected from the farms irrigated with untreated waste water for three days during the dry season in January and September and it was repeated two more times during the dry seasons in in two consecutive years. They were immediately delivered to the laboratory for processing as described in the following subsections.

2.8.1 Solvent extraction of vegetable material

For solvent extraction, initial methanol extraction was used [18]. Five grams of powdered plant samples was covered with 100 ml methanol in a flask and allowed to stand for 48-72 hours. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator at 60 °C until methanol free solid powder was obtained. The resulting extracts were then subsequently labeled as methanol extracts and preserved at 5 °C in airtight bottles until further use.

2.8.2 Untreated wastewater sample preparation

Prior to extraction, 500 ml of wastewater was filtered through Whatman filter paper No. 1 to remove suspended matter [17]. The solid phase extraction (SPE) cartridges were conditioned using 3 ml ethyl acetate, 3 ml methanol and 3 ml de-ionized water at a flow rate of about 3 ml/min. Water samples were transferred to the SPE cartridges through a Teflon tube at a flow rate of 15 ml/min using a vacuum pump. The loaded cartridges were rinsed with 3 ml of methanol/water (5:95) and 3 ml n-hexane at a flow rate of about 1 ml/min. The combined aliquots were evaporated to dryness in a gentle stream of nitrogen. The residues were then dissolved in 0.5 ml of methanol and injected into C18 HPLC system. The elution gradient for SMX in a reverse phase C18 HPLC column was as follows and the wavelengths of detection used in the UV range was 260 nm. Mobile phase of water and acetronile was adjusted to pH 3.3 by addition of formic acid at a flow rate of 0.75 ml/min.

Table 2.1 : The gradient of mobile phase

Time	% Water	% Acetonitrile
0	65	35
2	15	85
5	15	85
7	65	35
17	65	35

2.9 Method validation

The method of validation was also done using recovery analysis of the sample before spiking to know the concentration of the analyte of interest, then spiking the sample with a standard containing known concentration, and determining the concentration of the spiked material that was detected relative to that which was introduced in the sample.

2.10 Recoveries

The recovery was determined by spiking a sample whose concentration of antibiotic of interest was known with an analyte standard material putting the mixture through the HPLC analysis to determine the concentration. This evaluates the presence of background hindrances in detection relative to the solvent medium and other experimental conditions which may lower the sensitivity of the analytical procedure chosen.

2.11 Data management and analysis

Data was stored in both hard copy and electronically. MS excel was used as the data base. Table statistics were used to present data on sulfamethoxazole levels. Differences in mean concentrations of sulfamethoxazole was determined using ANOVA for analysis of more than 2 means. Within sample species and between sample species sulfamethoxazole differences were determined using ANOVA. Genstat was used as the analysis software. The means were deemed significantly different when $P \leq 0.05$ and insignificant when $P > 0.05$ at 95% confidence interval.

3.0 RESULTS

3.1 Percentage recoveries of the antibiotics

The detection of the percentage recoveries of the antibiotic reference samples are shown in Table 3.1 The percentage recovery was 100.00 %. The method of analysis chosen for determination of the concentration of antibiotics was acceptably effective because the percentage proportions of antibiotics recovered were within the statistically acceptable limits (Table 3.1).

Table 3.1: Percentage recoveries of standard material from biological reference preparations (BRP) under British Pharmacopoeia

Parameter	USR (ppb)	SSR (ppb)	% Recovery
Sulfamethoxazole	0.06	0.12	100.00
USR – unspiked sample result, SSR – spiked sample result			

The method for analysis was reliable since the percentage recovery results fall between the acceptable recovery ranges of between 80–105% reported by [19].

3.2 Limits of detection

Table 3.2 shows the limit of detection (practical) which was calculated as three times the standard deviation (3σ) of the blank signal [20]. The reference drugs were from Biological Reference Preparations (BRP) under British Pharmacopoeia.

Table 3.2: Limits of detection

Drug	Concentration (ng) (practical)
Sulfamethoxale[x]	0.02
[x] Drug code BP 314;	

3.3 Concentration of sulfamethoxazole in untreated wastewater

The results for the concentration of sulfamethoxazole in untreated wastewater samples are shown in Table 3.3

Table 3.3: The concentration of sulfamethoxazole in water

	Concentration of sulfamethoxazole in untreated wastewater (ng/litre) n=15
	Sulfamethoxazole
Upper Njiru	88.66±1.23[a]
Lower Njiru	87.21±4.32[a]
Upper Ruai	79.15±2.81[b]
Lower Ruai	62.09±1.77[c]
Control	ND[d]
Values are given as means of triplicates ± SD. Means with different small letters within a column are significantly different (*P* < 0.05). SD = Standard Deviation. ND = Not detected, n=15, (ẍ ±SD)	

From the results, upper Njiru was found to contain significantly higher concentrations of Sulfamethoxazole as compared to the other sites (*P* < 0.05). The concentration decreased down the stream. As shown in Table 3.3, the concentrations of Sulfamethoxazole in different sites in general followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai.

The concentration of of SMX decreased considerably as the untreated wastewater moved downstream.

3.4 The concentration of sulfamethoxazole in the vegetables

The mean concentration of SMX in the vegetables is reported in Table 3.4.

Table 3.4: The concentration of sulfamethoxazole in vegetables

	Concentration of Sulfamethoxazole in vegetables (ng/kg)			
	Cow peas	**Kales**	**Pumpkin**	**Spinach**
Upper Njiru	21.04±0.21[a]	7.26±0.88[c]	15.57±0.95[b]	22.64±2.01[a]
Lower Njiru	16.95±0.54[a]	6.64±0.25[c]	11.73±0.86[b]	18.86±2.25[a]
Upper Ruai	15.93±1.92[a]	5.16±0.41[b]	13.91±0.12[a]	14.35±1.33[a]
Lower Ruai	15.39±0.27[a]	4.93±0.98[c]	8.27±0.05[b]	12.56±1.00[a]
Control	ND	ND	ND	ND
Values are given as means of triplicates ± SD. Means with different small letters within a row are significantly different (*P* < 0.05). SD = Standard Deviation, n=15, (ẍ ±SD)				

For sulfamethoxazole in solvent vegetable extracts, the concentrations as reported in Table 3.4 ranged between 15.39 - 21.04 ng/kg, 4.93 - 7.26 ng/kg, 8.27 - 15.57 ng/kg and 12.56 - 22.64 ng/kg for cow pea, kales, pumpkin and spinach, respectively. The levels were significantly higher in the upper Njiru ($p<0.05$) as compared to the other sites. However, this is attributed to the direct discharge of untreated wastewater in upper Njiru. The level of sulfamethoxazole was significantly higher ($P < 0.05$) in spinach and cow pea and significantly lower in kales.

Figure 3.1 shows the concentration of SMX in the vegetables in the various study sites arranged from upstream to downstream for each of the four sites per cluster.

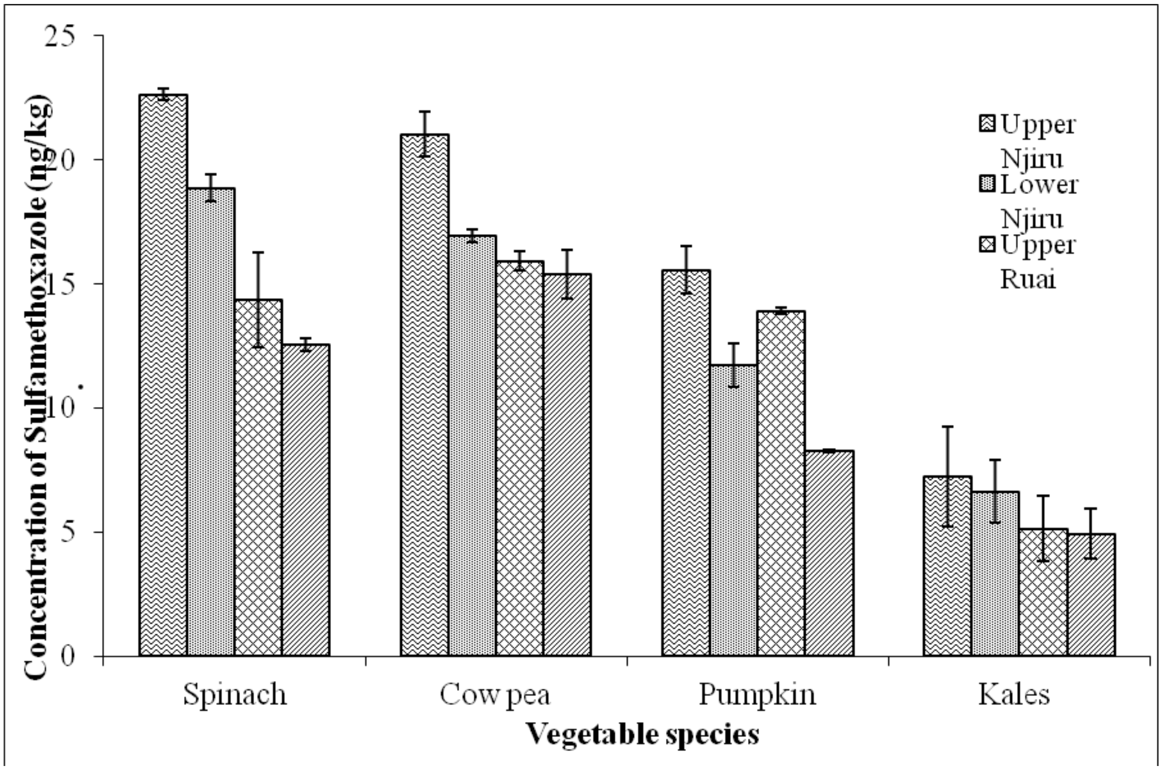

Figure 3.1: The concentration of sulfamethoxazole in vegetables

There was a general decrease in the concentration of SMX as one moved from upstream to downstream in the study region.

4.0 DISCUSSION

4.1 Sulfamethoxazole in untreated wastewater

The sulfamethoxazole antibiotic detected in the wastewater is commonly used for treatment of human infections. This antibiotic entered the irrigation water through untreated sewage effluent disposed to the river at upper Njiru. These antibiotics were detected in all samples with the exception of control samples that were grown with clean water from Ruiru dam. Sulfamethoxazole is resistant to breakdown and has been found in environmental ecosystems. [21]

From the results, it was evident that the concentration of the antibiotic was significantly higher in upper Njiru as compared to the other sites ($P<0.05$). In addition, the concentration of the antibiotic decreased down the stream and followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai. The high levels of the antibiotic in Upper Njiru resulted from direct discharge of untreated wastewater since the river flows from Upper Njiru and ends in Lower Ruai. The high concentration of the antibiotic is in tandem with the findings of [22] who reported that antibiotics are found more commonly in sewage effluent than in other recycled waters.

The concentration of sulfamethoxazole in the upper Njiru was also significantly higher than in the other sites. Its concentrations ranged from 62-89 ng/L in the four sites. This was much higher than the findings from a report by [23] of 10 ng/L , but in the case for the study, the concentration was after treatment of the wastewater. A study carried out by [24] to determine the concentration of sulfamethoxazole in untreated hospital waste water discharged into sewer systems found out that, the drug had high persistence and was detected at concentrations of 300 ng/l. In addition to predictions regarding fate and persistence, [25]. also reported the sulfamethoxazole concentrations in untreated hospital wastewater to range from 3.9 ng/l to approximately 27,000 ng/l. These findings are significantly higher than the levels obtained in this study but it is noteworthy that the sampling in this case did not concentrate on hospital untreated waste water but rather a mixture of domestic, industrial, hospital and other urban sources of waste water.

In comparison to other studies on the occurrence of sulfonamides in sewage treatment plants, [26] reported a sulfamethoxazole level in the primary effluent of a German Waste Water Treatment Plant (WWTP) of 2.4 μg/L, whereas analysis of the secondary treated sewage gave residual concentrations in a range from 0.3 to 1.5 μg/L. Another study monitoring a series of pharmaceuticals in sewage effluents found median concentrations of 0.40 μg/L for sulfamethoxazole [27]. In this study, the mean concentration range was from 62.09-88.66 ng/l for sulfamethoxazole in the untreated wastewater. These values are much lower than those detected in the study above. This could be due to the dilution effect of flowing water and improved disposal of drugs by the users and the health institutions.

4.2 The Sulfamethoxazole levels in the vegetables

The concentration of the antibiotic in waste water irrigated vegetables was significantly higher ($p < 0.05$) than in the control vegetables where none was detected. However, the concentration was higher in upper Njiru as it followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai. This is because Njiru farms were upstream of the Nairobi River, and sewage exhausters directly discharged the untreated sewage to the river at upper Njiru thus the effluents are carried from upper Njiru to lower Njiru, upper Ruai and all the way to lower Ruai. Therefore, this discharge was responsible for the highest levels of the antibiotics in the irrigation water. During irrigation, these antibiotics accumulated in the soil and thus got taken up by the vegetables.

Sulfamethoxazole levels were significantly higher in spinach and cow peas. However kales and pumpkin leaves reported significantly lower levels of both sulfamethoxazole ($P < 0.05$). This may be attributed to the fast growth of spinach and cow peas as compared to kales and spinach since the fast growing vegetables are able to pick up more organic matter. This is an indication of hyper accumulation in the spinach and cow peas and clearly explains that, the antibiotic concentration of vegetables not only depends on soil or media on which they grow but also depends on the type and nature of plant.

There was no significant differences ($p>0.05$) in the concentrations of the antibiotics in the upper and lower Njiru although the upper Njiru demonstrated a slightly higher amount than lower Njiru. This suggests that uptake of antibiotics in plants is higher when soils are irrigated with wastewater contaminated by sewage effluents and untreated wastewater.

Only the parent compound of sulfamethoxazole was investigated, and this was only done in wastewater and the common vegetables grown in the region. A control was set in Kenyatta University.

The presence of pharmaceutically active compounds food stuffs or drinking water may lead to antibiotic pathogen resistance [28] and affect the effect management of microbial related diseases in man.

It was not possible to state clearly the age of the vegetables but the sample taken was from the vegetables ready for market in the harvest season. Seasonal variations were not considered because irrigation is only carried out during the dry season since during the rainy season, the farms become flooded and no farming is carried out till the river levels go down.

4.3 Recommendations

The wastewater used for irrigation contains sulfamethoxazole residues. This antibiotic is used to manage human diseases and exposure of microbes to them may lead to development of resistance. The exposure of the human body to low quantities of the drug gradually leads to tolerance build in the body and this will render the drugs non-effective in disease management.

Antibiotics should be discharged responsibly, especially from hospital. This ensures that there is no contamination of the environment with harmful antibiotic residues.

The policy makers should have legislation banning use of untreated wastewater for irrigation in farms growing vegetables and fruits.

4.4 Recommendations for further work

i) It is important for further research to be carried out on the levels of antibiotics in hospital wastewaters discharged into the environment

ii) Research should be carried out on the impacts the antibiotics have on the bacterial and fungal pathogens that may be isolated from the soils in the farms and from the untreated wastewater, with intent to establish whether there is any variation to drug sensitivity that may be imparted to these pathogens by the small quantities of antibiotics they are exposed to.
iii) It is suggested that the antibiotic metabolites that may arise from the parent molecule and perhaps have some pharmaceutical activity be considered in a research to determine their quantities.
iv) Assessment of antibiotics in vegetables sold in markets in Nairobi county should be carried out.

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