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Trimethoprim and Sulfamethoxazole Residues in Untreated Wastewater used for Irrigation in Peri-urban Farms in Nairobi County, Kenya

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ABSTRACT

Untreated wastewater is often recycled in peri-urban subsistence farming. This water may contain residues of pharmaceutically active compounds discharged into the wastewater. Of concern are the residues of trimethoprim and sulfamethoxazole which are administered synergistically under the brand name septrin or cotrimoxazole. These two antibiotics are administered to both humans and livestock and belong to the group of medicines called 'anti-infectives'. This being the case, they are administered routinely to HIV-AIDS patients and to poultry and livestock as growth promoters, prophylaxis and to control microbial infections. Application of irrigation water with pharmaceutically active compounds may predispose microbial life to significant doses of the antibiotics for a non-limited span of time. This has been known to induce microbial resistance and thus minimize the drug sensitivity upon administration, an outcome that is harmful to human health and the environment. This study was carried out to determine if there are pharmaceutically active residues of these antibiotics by the use of high performance liquid chromatography coupled to spectroscopy. Untreated wastewater used for irrigation was collected during the dry season from various peri-urban farms in Ruai and Njiru from small scale farms along Ngong River. In the untreated wastewater, sulfamethoxazole levels ranged from 62.09 to 88.66 ng/L while trimethoprim levels ranged from 24.71 to 27.52 ng/L. In all cases the results in the farms upstream were higher than in those downstream. From these findings, it is recommended that wastewater should be treated prior to recycling in farms.

INTRODUCTION

Irrigation agriculture is currently practiced by farmers within the urban and peri-urban environs of Nairobi city, whereby most of them use untreated wastewater. Many urban families have low purchasing power due to inadequate income. This, along with the fluctuating prices of foods and inaccessibility to cheaper foods (Karanja et al. 2010) compels many urban families to look for alternative affordable foods by engaging in agricultural activities to provide food for consumption as well as selling to the local markets to raise income (Kaluli et al. 2011). Concerns are raised by consumers and exporters about the potential for negative health and environmental impacts with crops and recycled water and biosolids (Crute et al. 2003).

Approximately half of the wastewater discharged within the city and its environs ends up in wastewater treatment plants, while the rest is used untreated for irrigation (Githuku 2009). The untreated wastewater in the municipality comes from residential properties, commercial buildings, hospitals, industrial and agricultural processes (Gweyi & Osei 2011). The drugs administered therapeutically to both humans and animals, constitute the largest part of antibiotics detected in environmental waters and wastewaters.

The main route of antibiotic release on land is through the application of animal manure containing the excreted antibiotics to farms as manure (Kemper 2008). Antibiotics have often been detected in untreated and treated sewage wastewaters, and through irrigation with recycled wastewater, they can also get introduced into the farms (Gulkowska et al. 2008). Though the soil fertility is greatly improved by the presence of the essential plant nutrients in the untreated wastewater (Anjula 2011), this practice may have untoward outcomes affecting human health and the environment (Qadir et al. 2007).

Antibiotic ecotoxicological relevance (Rooklidge 2004) has not received as much attention as the effects of pesticides on agricultural land (Feng et al. 2009). However, microbial resistance has been accorded a lot of economic importance in terms of human and animal health because of the colossal sums that have to be ploughed into the management of drug resistant and multidrug resistant diseases.

The combination of sulfamethoxazole and trimethoprim obstruct two consecutive steps in the folic acid metabolism, thus interrupts the microbial synthesis of RNA and DNA (Pérez et al. 2005). The rate of loss of sulfamethoxazole and trimethoprim is enhanced in wastewater effluent due to indirect photolysis reactions, specifically reactions with hydroxyl radicals and triplet excited state effluent organic matter (Ryan et al. 2011). Photolysis in the presence of natural organic matter, however, does not lead to enhanced degradation of sulfamethoxazole. According to Kathryn (2004), trimethoprim was detected in untreated hospital wastewater in the concentration range of 2900-5000 ng/L. Similar studies done earlier by Kolpin (2002) reported a lower concentration range of 180-590 ng/L of trimethoprin in untreated wastewater.

Due to development of resistant bacteria against sulfamethoxazole, when it was used as a single substance, sulfamethoxazole is nowadays mainly used in combination with trimethoprim. In combination with trimethoprim, it is used to treat *Pneumocystis carinii* pneumonia, *Pneumocystis jirovecii* pneumonia and toxoplasmosis among the HIV-AIDS patients (De Ruiter et al. 2008). The free forms of sulfamethoxazole and trimethoprim are considered to be the therapeutically active forms (Kolpin 2002).

Sulfamethoxazole is water soluble, has a half life of 19 days under sunlight and is highly resistant to further biodegradation in the subsurface (Lam et al. 2004). It has a low K_{ow} (- 0.1 to 1.7) and is considerably hydrophilic and polar. Such properties enable sulfamethoxazole (SMX) to be transported over long distances without being adsorbed to sediments (Pérez et al. 2005). Under typical environmental pH conditions (pH 7-8) SMX is negatively charged, approximately 95-100%, a property that can increase its transport velocity in porous media due to anion exclusion.

MATERIALS AND METHODS

Study site: The study site was in Njiru/Ruai Districts 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 Southern by-pass road prior to crossing the Eastern by-pass road along the Kangundo road 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 kilometres behind a shopping centre referred to as Sewage Shopping centre, a site occupied by internally displaced people, which is a flood zone, and lower Ruai which is below 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 lush 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 the sampling sites are: S114.654 E36 57.255, S114.663 E36 57.260, S1 14.666 E36 57.269, and S114.669 E 36 57.273, S114.667 E36 57.198, S114.672 E36 57.206. The Figs. 1, 2 and 3 show the activities in the study site.

Cleaning of glassware and sample containers: All glassware and plastic containers used 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.

Fig. 1: The flowing river carrying the sewage and dumped materials.

Fig. 2: Wastewater along the river used for irrigating the vegetables.

Fig. 3: Cowpea vegetables irrigated with the untreated wastewater flowing right next in Njiru, Nairobi County.

Chemicals and solvents: All chemicals and solvents used for High Performance Liquid Chromatography (HPLC) were HPLC grade, i.e., acetronitrile HPLC grade, methanol HPLC grade, hexane HPLC grade, ethyl acetate HPLC grade, analytical grade potassium dihydrogen phosphate, ultra pure water, standards for sulfamethoxazole, trimethoprim. Distilled-deionized water was used.

Collection of water samples for antibiotic analysis: Untreated wastewater samples were collected from the farms irrigated with untreated wastewater using 500 mL plastic bottles that were washed with de-ionized distilled water. At the collection point, the bottles were rinsed with the untreated wastewater twice before filling them with the sample water. Untreated wastewater samples were collected for three days during two dry seasons in the year for three years. The untreated wastewater is pumped using a generator to flood the farmland 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 analysis by the protocol by Santos et al. (2005).

Method validation: The method 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 will be detected relative to that which was introduced in the sample.

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.

Table 1: The gradient of mobile phase.

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

Table 2: Percentage recoveries of standard material from biological reference preparations (BRP) under British Pharmacopoeia.

Parameter	USR (ppb)	SSR (ppb)	% Recovery
Trimethoprim	0.06	0.11	83.33
Sulfamethoxazole	0.06	0.12	100.00

USR-unspiked sample result, SSR-spiked sample result

Analysis of antibiotic residues: Prior to extraction, 500 mL of wastewater was filtered through Whatman filter paper No. 1 to remove suspended matter (Santos et al. 2005). 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 solid phase extraction (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 sulfamethoxazole and trimethoprim in a reverse phase C18 HPLC column (Table 1) and the wavelengths of detection used in the UV range are sulfame-thoxazole 260 nm and trimethoprim 230 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.

RESULTS

Percentage recoveries of the antibiotics: 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 2).

The method for analysis was reliable since the percentage recovery results fall between the acceptable recovery ranges of between 80-105% reported by Singh and Taneja (2010).

Limits of detection: Table 3 shows the limit of detection (practical) which was calculated as three times the standard deviation (3ó) of the blank signal (Jianrong & Khay 2001). The reference drugs were from Biological Reference Preparations (BRP) under British Pharmacopoeia.

Concentration of antibiotics in untreated waste water: The results for the concentration of the two PhACs, sulfamethoxazole and trimethoprim in water samples are given in Table 4. From the results, upper Njiru was found to contain significantly higher concentrations of sulfamethoxazole and trimethoprim as compared to the other sites ($P < 0.05$). The concentration decreased down the stream. As given in Table 4, the concentrations of the two pharmaceutically active compounds (PhACs) in different sites, in general followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai.

DISCUSSION

The antibiotics in the wastewater: The two target antibiotics, sulfamethoxazole and trimethoprim detected in the waste water are commonly used for a variety of therapeutic uses as antibiotics in the treatment of human infections. These antibiotics entered the irrigation water through untreated sewage effluent disposed to the river at upper Njiru. This is mainly because the wastewater came from household sewage. These antibiotics were detected in all matrices with the exception of control samples.

From the results, it was evident that the concentration of the antibiotics was significantly higher in upper Njiru concentrations as compared to the other sites ($P < 0.05$). In addition, the concentration of the two antibiotics decreased down the stream and followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai. The high levels of the antibiotics 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 these antibiotics is in tandem with the findings of Andreozzi et al. (2002) 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. In the wastewater samples, the concentration of the sulfamethoxazole drug was significantly higher than that of trimethoprim, which is consistent with the typical ratio in medications (5:1) containing the two synergistically acting antimicrobials (Pérez et al. 2005).

The present study clearly showed that the levels and detection frequencies of trimethoprim in wastewater samples were significantly lower ($P < 0.05$) in the all the four sites as compared to sulfamethoxazole. However, the concentration was slightly higher at upper Njiru than the other sites and this was consistent as with sulfamethoxazole.

A study carried out by Kathryn to determine the concentration of sulfamethoxazole in untreated hospital wastewater discharged into sewer systems found out that, the drug had high persistence and was detected at concentrations of 300

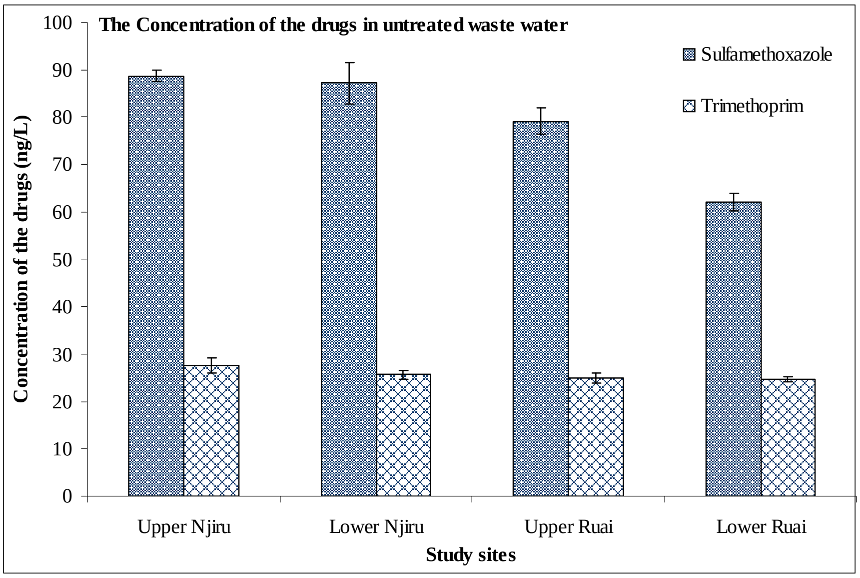

Fig. 4: Concentration of antibiotics in the waste water.

Table 3: Limits of detection.

Drug	Concentration (ng) (practical)
Sulfamethoxale[x]	0.02
Trimethoprim[xx]	0.02

[x] Drug code BP 314; [XX] Drug code BP 344

Table 4: The concentration of sulfamethoxazole and trimethoprim in water.

	Concentration of the drugs in untreated wastewater (ng/litre)	
	Sulfamethoxazole	Trimethoprim
Upper Njiru	88.66±1.23[a]	27.52±1.56[a]
Lower Njiru	87.21±4.32[a]	25.66±0.89[a]
Upper Ruai	79.15±2.81[b]	24.99±1.11[b]
Lower Ruai	62.09±1.77[c]	24.71±0.62[b]
Control	ND[d]	ND[c]

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

ng/L (Kathryn 2004). In addition to predictions regarding fate and persistence. Huang et al. (2001) 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 isolated in the untreated wastewater in the study region, but it is note worthy that the sampling in this case did not concentrate on hospital untreated wastewater, but rather a mixture of domestic, industrial, hospital and other urban sources of wastewater.

CONCLUSION

The wastewater used for irrigation is contaminated with the antibiotics sulfamethoxazole and trimethoprim. These antibiotics detected in the untreated wastewater were also present in the vegetables. Owing to the medical importance of these drugs in the management of opportunistic infections in HIV-AIDS patients, more stringent measures need to be applied in ridding the wastewater of these drugs, so that bacteria do not develop resistance due to persistence exposure.

RECOMMENDATIONS

The wastewater used for irrigation contains sulfamethoxazole and trimethoprim antibiotics. These two antibiotics are 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 drugs gradually leads to tolerance built in the body and this will render the drugs non-effective in disease management. Antibiotics should be discharged responsibly, especially from hospital wastes, since these have been found to be the key source of antibiotics in the environment.

RECOMMENDATIONS FOR FURTHER WORK

1. It is important for further research to be carried out on the levels of antibiotics in hospital wastewaters discharged into the environment
2. 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.
3. It is recommended 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.

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