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ASSESSMENT OF SELECTED ANTIBIOTICS IN UNTREATED WASTEWATER AND VEGETABLES IN URBAN AND PERI-URBAN FARMS IN EASTERN NAIROBI CITY, KENYA

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ABSTRACT

Background: Most urban and peri-urban farmers in Kenya use untreated wastewater to irrigate crops. The vegetables irrigated with this water may accumulate unsafe levels of pharmaceutically active compounds. This poses a health concern to the consumers because of the known health problems such as antimicrobial resistance that may arise from gradual un-intended exposure of the body to antibiotics over prolonged spans of time. The presence of antibiotic residues in the vegetables may lead to initiation of bacterial resistance to the drugs due to the unintended long exposure from the environment. Unlike pesticides used on agricultural land, antibiotics have not aroused attention as potential pollutants until fairly recently. There are no reports on levels of antibiotic residues in untreated wastewater and vegetables in Kenya. This study aimed to determine the concentration of sulfamethoxazole (SMX) and trimethoprim (TMP) in untreated waste water and vegetables irrigated with the untreated waste water in Ruai and Njiru suburb farms. **Methods**: Untreated wastewater and vegetables were collected during the dry season from various sites in Ruai and Njiru from small scale farms along Ngong River. The samples underwent solvent extraction pre-analysis. The antibiotic residues were determined by high performance liquid chromatography. **Results**: In the untreated wastewater sulfamethoxazole (SMX) concentration ranged from 62.09 to 88.66 ng/kg while the levels of trimethoprim ranged from 24/71 to 24.99 ng/kg. The vegetables TMP concentration ranged from 2.16 to 15.45 ng/kg while the sulfamethoxazole levels ranged from 4.93-22.64 ng/kg. **Conclusion**: The wastewater used for irrigation was contaminated with the antibiotics sulfamethoxazole and trimethoprim. These antibiotics detected in the untreated wastewater were also present in the vegetable and therefore it can be concluded that it is not safe to consume vegetables contaminated with the antibiotic even though it is in small quantities because it will lead to undue exposure and tolerenca build-up by microbes resulting in resistance to the drugs that have residues in the environment.

KEYWORDS: Antibiotics, Untreated waste water, Vegetables.

INTRODUCTION

Sulfamethoxazole and trimethoprim are the two choice prophylactic drugs prescribed to HIV/AIDS patients under the brand name cotrimoxazole. They are also administered prophylactically as a animal feeds component in the commercially produced animal feeds to ward off infections in poultry and livestock reared commercially. There has been an increase of other immunosuppressive conditions which are confounded by opportunistic infections managed by a wide array of pharmaceutical compounds that are eventually discharged through the body as metabolites or un-metabolized active forms. Hence there is cause for alarm on just what quantities of pharmaceutically active compounds may be in the wastewater effluent and subsequently transferred into the food consumed. Examples include antibiotics, analgesics and antidepressants.[1] Of concern related to PhACs is the initiation of microbial antibiotic resistance following discharge of antibiotics in the environment.[2]

The majority of antibiotics detected in environmental waters and wastewaters are drugs used for a variety of therapeutic purpose for both humans and animals.[3] Wrong disposal of pharmaceutical compounds is an important source of environmental contamination that needs to be noted with concern.[4] The dominant pathway for antibiotic release in the terrestrial environment is via the application of animal manure and bio solids containing excreted antibiotics to agricultural land as fertilizer.[5] 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.[1]

Most urban farmers in Kenya use untreated wastewater to irrigate crops. The vegetables irrigated with this water may accumulate unsafe levels of heavy metals and pharmaceutically active compounds. This poses a health concern to the consumers because of the known health problems that may arise from drug resistance as well the emergence and re-emergence of diseases. In addition, the presence of antibiotic residues in the vegetables may lead to initiation of bacterial resistance to the drugs due to prolonged exposure of enivironmental microbes to low doses of the drugs. Unlike pesticides used on agricultural land, antibiotics have not aroused attention as potential pollutants until fairly recently.[6] 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.[7] In the Kenyan sewerage system, hospital, industrial and domestic wastes are discharged together. No report in Kenya is available about the presence of antibiotic residues in the sewage and the vegetables. Therefore there is need to carry out assessment of antibiotic residues in different vegetables grown in the same locality and irrigated with the untreated waste water in order to determine whether there is variation.

In this study, the antibiotics quantified were sulfamethoxazole (SMX) and trimethoprim (TMP). Sulfamethoxazole falls under the class of sulfonamide antibacterial group.[8] The medicinal use of sulfamethoxazole is for treatment of tuberculosis, malaria and urinary tract infections.[9] Trimethoprim is a synthetic broad spectrum antibiotic that is used to treat urinary tract infections, respiratory infections and middle ear infections.[10] In synergy, they are administered to ward off opportunistic infections in both human and livestock.

METHODOLOGY

The current study was carried out 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.

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 farms 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. 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 the months of December, January, February, August and September. Therefore the sampling was carried out in triplicate for three alternate days in the months of January. 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 per session.

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.[11]

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 ad 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.

RESULTS

The concentration of sulfamethoxazole and trimethoprim in untreated water

Table 1: Shows the concentration of SMX and TMP in the untreated wastewater in the various study sites arranged from upstream to downstream.

	Sulfamethoxazole	**Trimethoprim**
Upper Njiru	88.66	27.52
Lower Njiru	87.21	25.66
Upper Ruai	79.15	24.99
Lower Ruai	62.09	24.71
Control	ND	ND

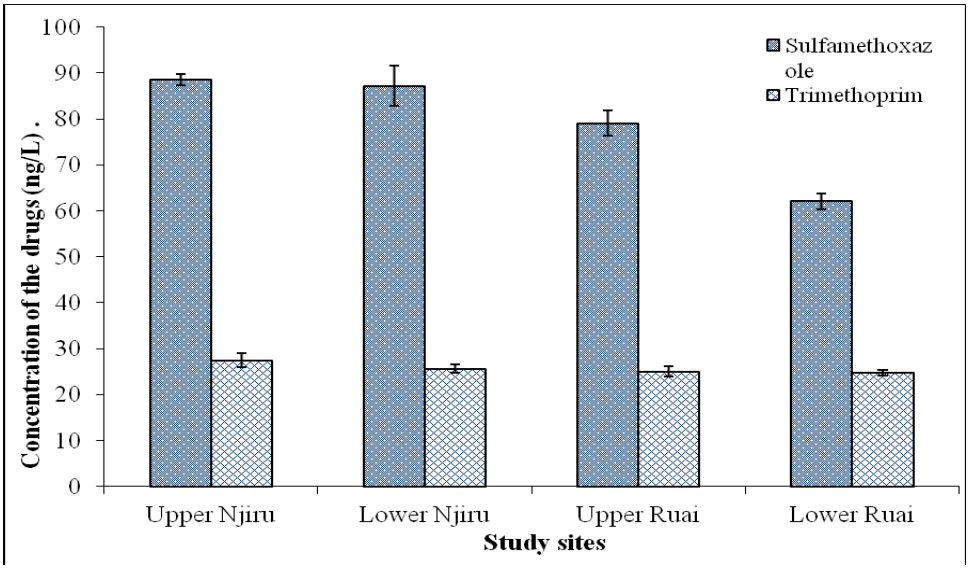

Figure 1: Shows the concentration of SMX and TMP in the untreated wastewater in the various study sites arranged from upstream to downstream.

Concentration of antibiotics in vegetables

Table 2: The concentration of trimethoprim in vegetables Concentration of trimethoprim in vegetables (ng/kg).

Cow pea	Kales		Pumpkin	Spinach
Upper Njiru	12.12	4.15	15.45	14.97
Lower Njiru	11.03	3.27	12.62	10.21
Upper Ruai	8.99	2.96	10.73	10.64
Lower Ruai	4.78	2.16	7.17	7.54
Control	ND	ND	ND	ND

The levels were found to be in the range of 4.78 - 12.12 ng/kg, 2.16 - 4.15 ng/kg, 7.17 - 15.45 ng/kg and 7.54 - 14.97 ng/kg for Cow pea, Kales, Pumpkin and Spinach, respectively. As it was for the water samples, the levels of trimethoprim were significantly higher in the upper Njiru as compared to the other sites. The direct discharge of untreated wastewater was responsible for the highest levels of the trimethoprim in upper Njiru. The level of trimethoprim was also significantly higher in pumpkin and spinach and significantly lower in kales ($P < 0.05$).

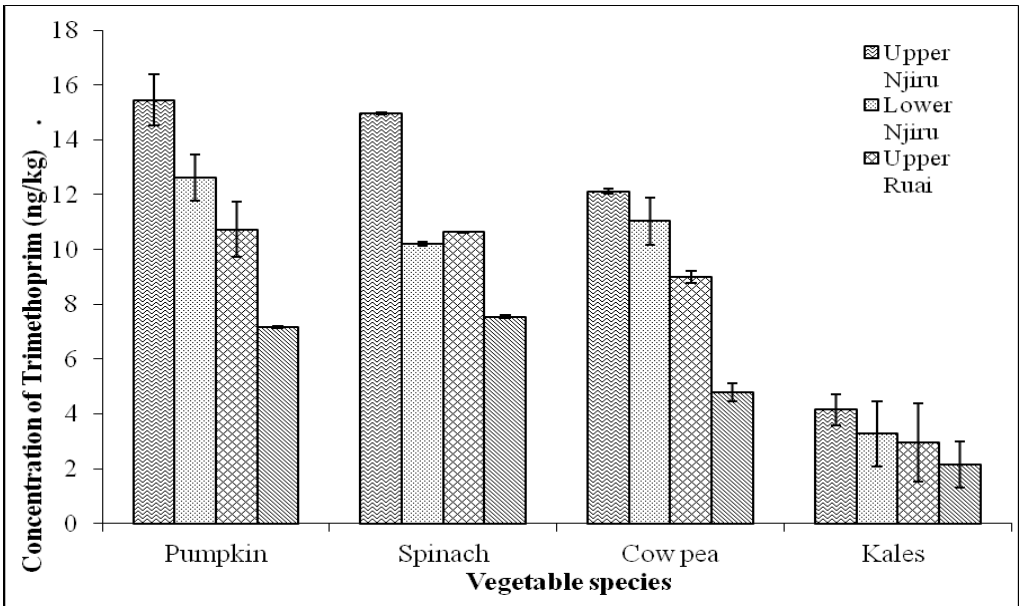

Figure 2: Shows the concentration of TMP in the vegetables in the various study sites arranged from upstream to downstream for each of the four sites per cluster.

The concentration of sulfamethoxazole in the vegetables

Table 3: The concentration of sulfamethoxazole in vegetables Concentration of Sulfamethoxazole in vegetables (ng/kg).				
Cow pea	**Kales**		**Pumpkin**	**Spinach**
Upper Njiru	21.04	7.26	15.57	22.64
Lower Njiru	16.95	6.64	11.73	18.86
Upper Ruai	15.93	5.16	13.91	14.35
Lower Ruai	15.39	4.93	8.27	12.56
Control	ND	ND	ND	ND

For sulfamethoxazole in solvent vegetable extracts, the concentrations as reported in Table 4.22 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 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. As compared to trimethoprim, the level of sulfamethoxazole was significantly higher in all the sites.

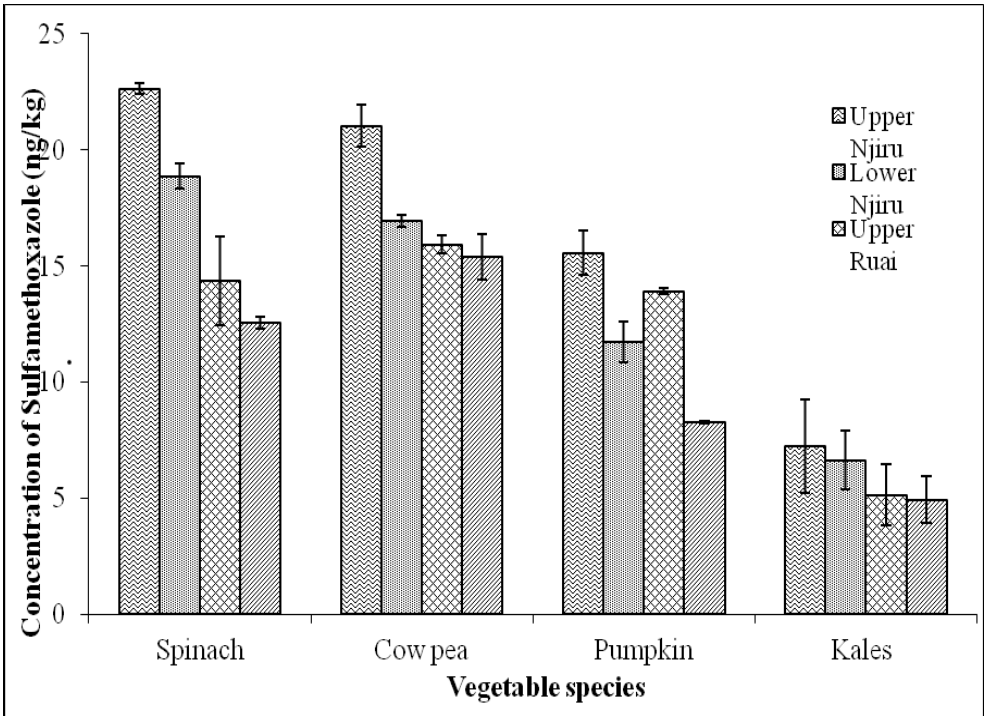

Figure 3: Shows the concentration of SMX in the vegetables in the various study sites arranged from upstream to downstream for each of the four sites per cluster.

Correlation analysis of antibiotics in untreated wastewater

A Pearson's correlation was done on the association between the concentration of antibiotics in untreated waste water and the vegetables irrigated with the untreated wastewater. It was found to be positive. Table 4 shows the correlation results.

Table 4: Pearson's correlation of antibiotics in untreated wastewater and vegetables. The correlation (r values) of antibiotics in wastewater and vegetables				
Cow peas	**Kales**		**Pumpkin**	**Spinach**
Trimethoprim	0.39	0.14	0.41	0.54
Sulfamethoxazole	0.32	0.25	0.43	0.59

The concentration of antibiotics in the waste water influenced the concentration of the antibiotics in the vegetables positively (Table 5). The vegetable that tended to accumulate the highest concentration of the two antibiotics was spinach, followed by pumpkin leaves and cowpeas. Kales had the lowest correlation value.

DISCUSSION

The antibiotics in the wastewater

The antibiotics, sulfamethoxazole and trimethoprim detected in the wastewater are commonly used for 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 samples with the exception of control samples. From the results, it was evident that the concentration of the antibiotics was significantly higher in upper Njiru 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[12] 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.[13]

The present study shows that levels and detection frequencies of trimethoprim in wastewater samples were significantly lower ($P < 0.05$) in all 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 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.[14] In addition to predictions regarding fate and persistence, another study also reported the sulfamethoxazole concentrations in untreated hospital wastewater to range from 3.9 ng/l to approximately 27,000 ng/l.[15] 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.

The antibiotics in the vegetables

The concentration of the antibiotics in waste water irrigated vegetables was significantly higher ($p < 0.05$) than in the control vegetables. 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 absorbed by the vegetables. The concentration of sulfamethoxazole drug was significantly higher than that of trimethoprim in all the vegetables. In addition, the level of trimethoprim was significantly higher in cow pea, pumpkin and spinach ($P < 0.05$) while for sulfamethoxazole the levels were significantly higher in spinach and cow pea. However kales reported significantly lower levels of both sulfamethoxazole and trimethoprim ($P < 0.05$). This may be attributed to the fast growth of spinach and cow peas as compared to kales since the fast growing vegetables are able to pick up more organic matter. Similarly, spinach appeared to bio accumulate significantly higher ($P<0.05$) concentrations of heavy metals as compared with the other vegetables. This is an indication of hyper accumulation in the spinach 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 as well as those irrigated with household-derived wastewater.

In the primary effluent, the concentration of the sulfamethoxazole was about four times higher than that of trimethoprim, which is consistent with the typical ratio in medications (5:1) containing the two synergistically acting antimicrobials.[16] A study done in Germany reported a sulfamethoxazole level in the primary effluent of a German 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.[17]

Another study monitoring a series of pharmaceuticals in sewage effluents found median concentrations of 0.40 µg/L for sulfamethoxazole and 0.32 µg/L for trimethoprim.[13] In the primary effluent, the concentration of the sulfa drug was about four times higher than that of trimethoprim, which is consistent with the typical ratio in medications (5:1) containing the two synergistically acting antimicrobials.[18] Traces of both compounds were still detectable in the treated sewage. In this study, the mean concentration range was from 62.09-88.66 ng/l for sulfamethoxazole and 24.71-25.66 ng/l for trimethoprim. 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. Unlike

sulfamethoxazole, trimethoprim has been reported to persist in sewage sludges mush longer.[13]

CONCLUSION

There is a positive correlation between the concentration of the two antibiotics in untreated waste water and the concentration in the vegetable. The wastewater used for irrigation was 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. All the vegetables contained residues of the drugs and therefore it can be concluded that it is not safe to consume vegetables contaminated with the antibiotic even though it is in small quantities because it will lead to undue tolerance to the drugs due to the unspecified exposure. Irrigation with untreated wastewater leads to production of vegetables that contain hazardous contaminants.

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