International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Occurrence and Removal of Three Antibiotics in Two Wastewater Treatment Plants in Morogoro Municipality, Tanzania

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Abstract: ***Urban wastewater treatment plants (UWTPs) are among the main sources of antibiotics release into various compartments of environment worldwide. These antibiotics have aggravated increasing anxiety, particularly as no legitimate requirements have been set for discharge into surface water bodies of these ubiquitous, persistent and biologically active substances. Massive quantities of antibiotics are used in human and veterinary medicine in all parts of the globe to treat diseases with bacterial, fungal and parasitic origins. These are also largely used in animal operations for growth promotion and for disease prophylaxis .These are often partially metabolized depending on type of antibiotics. After administration a significant fraction of the antibiotic can be excreted as a parent compound, metabolites or in conjugate forms that can be converted back to parent antibiotic. The residual antibiotics from human and animal use can enter the environment via various pathways, including wastewater, runoff from land in which manure or human waste has been applied and leaching. In this study occurrence and removal of three selected antibiotics, tetracyclines, sulfonamides and quinolones were studied in two large wastewater treatment plants in Morogoro Municipality using ELISA technique. Results indicated that the highest mean concentrations were 27.2753±2.83878µg/l, quinolones 48.7615±7.9343.91µg/l tetracyclines and 18.7492±5.4906µg/l sulfonamides. The elimination of antibiotics through these wastewater treatment plants was incomplete. The removal efficiency was 28.50% tetracyclines, 11.33 % quinolones and 82.32% sulfonamides at mafisa wastewater treatment plant. At Mzumbe wastewater treatment plant removal efficiency was 2.5% tetracyclines, 7.259% quinolones and 2.28% sulfonamides.***

Keywords: Antibiotics,, Removal, wastewater, Morogoro, Tanzania

1. Introduction

As a class of emerging environmental micropollutant contaminants, antibiotics have been of increasing concern over the past decade. Antibiotics play an important role in treating and preventing diseases in humans and animals. The overuse of antibiotics sometimes is inevitable in preventing and treating infectious diseases in humans and animals. Antibiotics are also used as growth promoters in livestock and aquaculture. Approximately 210 million kilograms of antibiotics are produced annually in China (Su *et al.*, 2012) and 16 million kilograms of antibiotics are used annually in the USA (United States of America) for human and agriculture use (Sarmah *et al.*,2006).

Antibiotics are only partially absorbed after administration, and nearly 75% of antibiotics are excreted as original compounds or metabolites by humans and animals (Luo *et al.*,2011).After consumption, such antibiotics are eventually discharged in the environment through wastewater discharge, sewage effluent, most in their original form (Kummerer,2009; Larson *et al.*, 2007, Threedeach *et al.*, 2012).In the environmental waters, the long-term residues of antibiotics may cause potential risks to the aquatic organisms (Liu *et al.*, 2009) and may lead to occurrence and prevalence of biological resistance genes (Tao *et al.*, 2010). Also antibiotic residues generate selective pressure to bacteria in the environment, thus contributing to proliferation of antibiotic resistance genes. The occurrence of antibiotic resistance changes the composition and structure of microbial community and increase the potential risks to humans health and environment (Kim *et al.*,2004; Akinbowale *et al.*,2007). Once bacteria acquired antibiotic resistance can compromise the effectiveness of antibiotic therapy.

Li and Zhang, 2011; Leung *et al*.,2012; Aydin and Talnli, 2013 have gathered extensive literatures on the concentration levels of pharmaceuticals in aqueous phases such as wastewater and surface water. In wastewater treatment plants removal percentage, which is based on concentrations of pharmaceuticals in the influent and the effluent is the only parameter available for calculating the pharmaceutical removal efficiency in wastewater treatment plants currently (Leung *et al.*, 2012).

Aquatic environments made by (or just influenced by) humans may serve as a reservoirs for antibiotic resistant genes (Negreanu, 2012). Even antibiotic concentrations below minimal inhibitory concentration (MIC) can promote development of resistance (Gullberg,2012).This suggest that occurrence of trace amounts of antibiotics into environment may generate an increase in development of antibiotic resistant bacteria (Negreanu, 2012). But ,the permissible levels of antibiotics in environmental waters are still not yet established. As plenty of antibiotics flowing into rivers, wastewater treatment plants, soils and manure, probably can affect the environment, hence persistent exposure to antibiotics to humans, animals and aquatic organisms at low

doses. Permissible levels for antibiotics in aqueous environment need to be established.

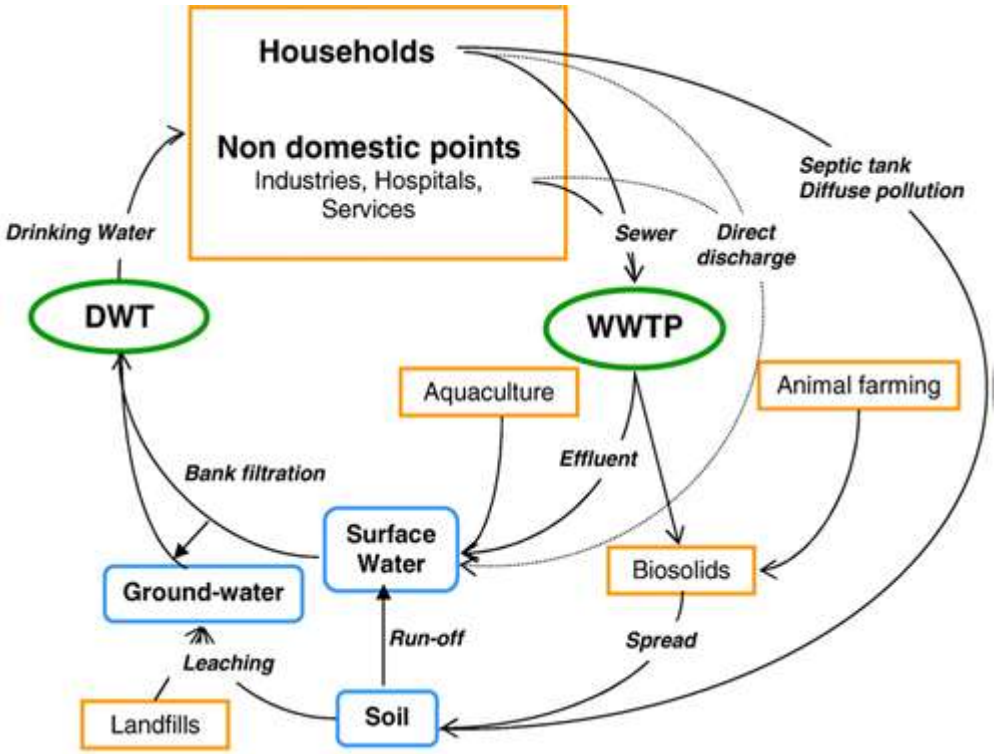

Figure 1: Possible routes of antibiotics into the environment (DWP-Drinking water treatment plants, WWTP-Wastewater treatment plants)

From the figure above, antibiotics from households and non domestic points like industries, hospitals and services move to wastewater treatment plants, finally to surface water and soil. From soil move to ground water through leaching .Ground water is used as a source of drinking water, hence are taken back by humans and animals through drinking water .Also biosolids from animals and aquaculture antibiotics are taken to surface water , move to drinking water treatment plants and taken by humans and animals through drinking water.

2. Materials and Methods

2.1. Location

Mafisa and Mzumbe wastewater treatment plants are located in Morogoro municipality, Tanzania. Mafisa wastewater treatment plant has eight ponds which are connected from entry (first) to the exit (eight), Mzumbe wastewater pond has only three ponds, but also connected together from the entry (first) to the exit (third). Morogoro is town with approximately 2,218,492 inhabitants according to 2012 census located 200km km inland from Dar es- Salaam. Mafisa is located next to the Morogoro river in northern part of the city, in an area with housing and farming, receives wastewater from the city of Morogoro. The mafisa wastewater treatment system consists of two receiving ponds (gravity receiving pond and track receiving pond) and six sedimentation ponds. While pond one is anaerobic pond, second pond is aerobic stabilization pond, ponds three, four, five and six are stabilization maturation ponds. The ponds have different functions as well as different dimensions. The dimension, flow rate and pH are summarized in Table 1.Sewage water is guided through Mafisa wastewater treatment plant, finally it joins the Morogoro river. During dry season, the water in the river is low, hence water from Mafisa is used for irrigation of fields, mainly rice surrounding Mafisa and river. In rainy season the water joins the river immediately after outlet. Mzumbe wastewater treatment plant is located on the western part of the city and receives wastewater from Mzumbe University community. Mzumbe wastewater treatment plants has only three ponds, the first one is a receiving pond, second is sedimentation pond, while the third one is maturation as well as exit pond. Water from last pond is used for irrigation of vegetables especially during dry season.

Table 1: The dimensions, dynamics, flow rate (Q) and pH of Mafisa wastewater treatment plant

	1	2	3	4	5	6
Width(m)	48	59	59	59	59	59
Length(m)	72.2	133	133	133	133	133
Depth(m)	1.62	1.534	1.064	1.132	1.156	1.188
Q(m^3/sec	0.034	0.031	0.031	0.038	0.039	0.027
Volume(m^3)	5614	12037	8349	8883	9071	9322
Q(m^3/24h)	2938	2678	2678	3283	3370	2333
Q/V	0.5232	0.2225	0.3208	0.3696	0.3715	0.2502
pH	7.4	7.2	7.6	7.5	7.8	7.3

2.2 Sampling

Ten sampling points were identified and implemented at mafisa wastewater treatment plant, and ten sampling points at Mzumbe wastewater treatment plant. Sampling was done in duplicates in both wastewater treatment plants. Water was collected at each sampling point in 2.5l glass amber bottles. pH was adjusted on site to 3 using Sulphuric acid (Carl-

Erba). pH was measured using universal pH indicator strips. The samples were transported to laboratory within one hour where they were filtered twice. The first filtration was through a grade 5 filter paper from Munktell with particle size retention of 20µm.The second filtration was through a grade 120H filter paper also from Munktell with particle size retention of 1-2µm. After filtration the samples was divided into 2x 800ml amber bottle.

2.3 Solid phase extraction

800 ml water sample, pH adjusted to 3 was loaded into Oasis®HLB 6m³ 200mg (30ml) cartridges from Waters (Milford, MA,USA) using a vacuum manifold and pump. The vacuum manifold was a VacMaster from IST(Sweden) and the pump was from ScanVac (Denmark). The drop rate was adjusted to 1.5ml/min. Prior to loading cartridges were preconditioned with 2ml MeOH followed by 2ml distilled water. After loading the samples and cartridges were air-dried using vacuum and stored at -18c° before analysis.

Prior to analysis, antibiotics were eluted from cartridges with 8ml (formic acid in MeOH) after washing with 2ml 5% MeOH in water. The eluent was evaporated to dryness under a gentle stream of nitrogen at 33c°. Afterwards, the samples were reconstituted to 100µl (0.01% formic acid in MeOH) and 900µl water. Elution and evaporation was done in 12ml amber bottles. Samples were then transferred to eppendorf tubes centrifuged and supernatant were used for analysis.

2.4 Chemicals/Reagents

Pure antibiotics salts of sulphonamides, tetracyclines and quinolones were purchased from Sigma -Aldrich (Augsburg, Germany). Ridascreen kits (Ridascreen sulfonamides (R3004), Ridascreen tetracyclin (R3505) and Ridascreen quinolones/chinolones (R3113) were purchased from R-Biopharm AG, (Darmstadt, Germany).Analytical grade methanol was purchased from Sigma-Aldrich (Augsburg, Germany).

2.5. Enzyme- linked immumosorbent Assay Basis

2.5.1 Sulfonamides

The basis of the test was antigen-antibody reaction. The microtitter wells were coated with capture antibodies directed against anti-sulfonamide antibodies. Standard or sample sulfonamides conjugate and anti-sulfonamide antibodies solutions were added. Free sulfonamides and sulfonamide conjugate compete for the sulfonamides antibody binding sites (competitive enzyme immunoassay). At the same time , the anti-sulfonamide antibodies were also bound by the immobilized capture antibodies. Any unbound conjugate was removed in washing step. Substrate/chromogen was added to the wells and incubated .Bound conjugate converted the chromogen into a blue product. The addition of the stop solution changed the colour from blue to yellow .The measurement was made photometrically at 450 nm using ELISA reader. The absorption was inversely proportional to the sulfonamide concentrations in the sample.

2.5.2 Tetracyclines

The basis of the test was antigen-antibody reaction. The microtitter wells were coated with tetracycline-protein-conjugate. Tetracyclines standards or sample solutions and anti-tetracycline antibodies were added. Free tetracyclines and immobilized tetracyclines compete for tetracyclines antibody binding sites (Competitive Enzyme immunoassay).Any unbound antibody was removed in washing step and enzyme labelled secondary antibody, which was directed against the anti-tetracycline antibody was added. After removing unbound enzyme labelled antibodies by a washing step, substrate/chromogen was added to the wells and incubated. Bound conjugate converted the chromogen into a blue product. The addition of the stop solution changed colour from blue to a yellow. The measurement was made photometrically at 450nm using ELISA reader. The absorption was inversely proportional to the tetracyclines concentration in sample.

2.5.3 Quinolones

The basis of the test was antigen-antibody reaction. The wells were coated with a capture antibodies directed against anti-quinolones antibodies. Standards or sample solutions of ciprofloxacin enzyme conjugate and anti-quinolones antibodies were added. Free quionolones and ciprofloxacin conjugate compete for the quinolones antibody binding sites (Competitive enzyme immunoassay). At the same time the anti-quinolone antibodies were also bound by the immobilized capture antibodies. Any unbound conjugate was removed in washing step. Substrate/chromogen was added to the wells and incubated .Bound conjugate converted the chromogen into a blue product. The addition of stop solution changed colour from blue to yellow. The measurement was made photometrically at 450nm using ELISA reader. Absorption was inversely proportional to the quinolones concentrations in the sample.

3. Analytical Procedure

3.1 Sulfonamides

Fifty microliters of each standard or prepared sample were added to microplate wells of ELISA plate in duplicate. Fifty microliters of conjugate was added to each well. Then fifty microliters of antibody was added to each well mixed gently. The mixture was incubated for one hour at room temperature. The solution in the wells was discarded and the microplate was tapped three times in blotting paper to ensure complete removal of solution from wells. The wells were filled with 250µl of washing buffer. The liquid was poured out and the wash step was repeated three times. One hundred microliters of substrate/chromogen was added to each well, incubated for 15 minutes at room temperature in the dark. One hundred microliters of stop solution was added. Absorbance was read at 450nm using ELISA reader.

The results of were expressed in percentages of the maximum absorbance (B/B0%) using the following equation.

$$\frac{B}{B0}\% = \frac{\text{Absorbance standard/sample}}{\text{Absorbance at Zero standard concentration}} \text{X100}$$

B/B0% values were interpolated on the calibration curve built with six sulfonamide standard solutions (0, 1, 3,10,10 and 100µg/l).Multiplied by dilution factor to obtain the final concentrations of sulfonamides.

3.2 Tetracyclines

In order to obtain the tetracycline concentrations in the sample, the B/B0% values were interpolated on the calibration curve built with six tetracycline standard solutions (0, 0.05, 0.15, 0.3, 0.6 and 1.8µg/l). Multiplied by dilution factor to obtain the final concentrations of tetracyclines.

3.3 Quinolones

In order to obtain the quinolone concentrations in the sample, the B/B0% values were interpolated on the calibration curve built with six tetracycline standard solutions (0, 0.5,1.5, 3, 6 and 18µg/l). Multiplied by dilution factor to get the final concentrations of the quinolones.

4. Results and Discussion

4.1. Recovery and Detection limit

Recovery rates were 70-87%, 75-114% and 80-110% for sulfonamides, tetracyclines and quinolones respectively, these rates were within the range stated in ELISA kits used. The detection limit of the method ranged from 1.5µg/l, 1.2µg/l and 0.5µg/l sulfonamides, tetracyclines and quinolones respectively

Table 2: Mean concentrations of antibiotics and removal efficiency at Mafisa wastewater treatment plant
Cluster1- Influent, anaerobic and facultative ponds, Cluster 2-maturation ponds and Cluster 3-Effluent and exit point

Antibiotic	Cluster	Mean	Removal efficiency
Quinolones	Cluster 1	27.2753±2.83878	11.33%
	Cluster2	24.0660±.5.1280	
	Cluster3	24.1841±2.14841	
Tetracyclines	Cluster1	48.7615±7.9343	28.50%
	Cluster2	41.5620±8.43186	
	Cluster3	34.8635±5.17469	
Sulfonamides	Cluster1	18.7492±5.4906	82.32%
	Cluster2	10.3945±1.77246	
	Cluster3	3.3136±1.35145	

Table 1, shows that the concentrations of antibiotics generally was high in the first cluster and low in the third cluster. Also mean concentrations of tetracyclines was high followed by quinolones, while the concentration of sulfonamides was lowest among the three antibiotics. There was a reduction of antibiotics concentrations from influent, maturation and exit ponds ,more was for sulfonamides (figure 2, 3 and 4).

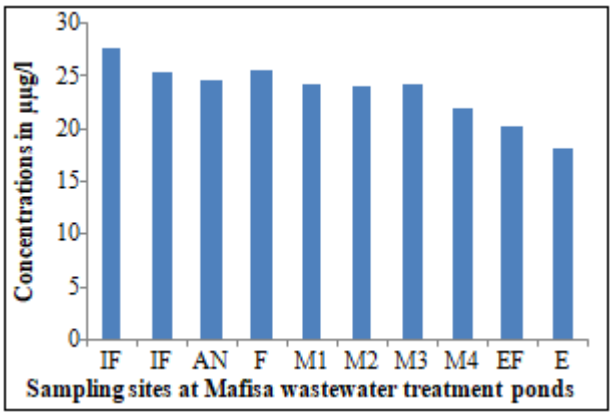

Figure 2: Mean concentrations of quinolones in mafisa wastewater treatment plant
IF-Influent, AN-Anaerobic, M1-M4-Maturation ponds, EF-Effluent and E-Exit point

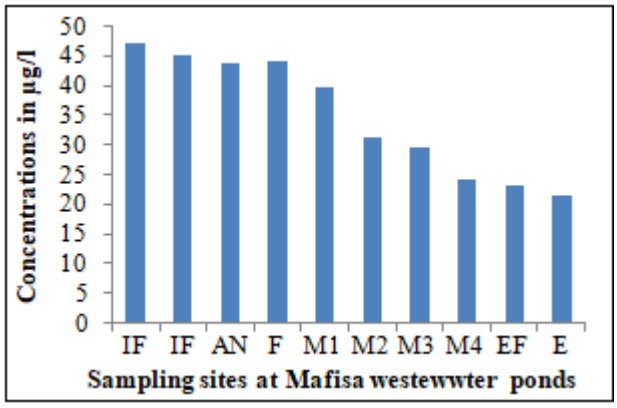

Figure 3: Mean concentrations of tetracycline in mafisa wastewater treatment plant
IF-Influent, AN-anaerobic, M1-M4-Maturation ponds, E-Exit point

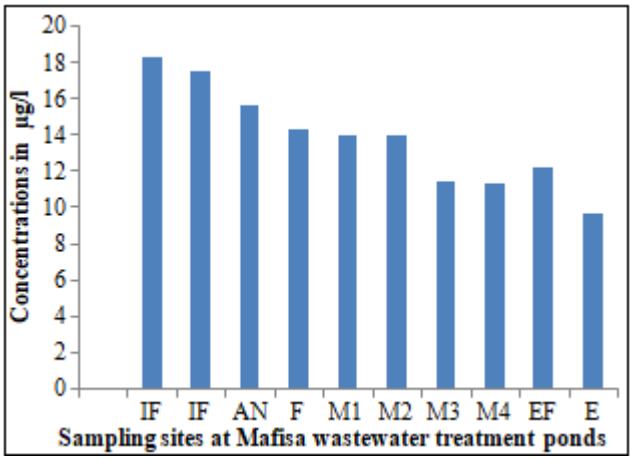

Figure 4: Mean concentrations of sulfonamide in mafisa wastewater treatment plant
IF-Influent, AN-Anaerobic, M1-M4 Maturation ponds, EF-Effluent, and E-Exit point

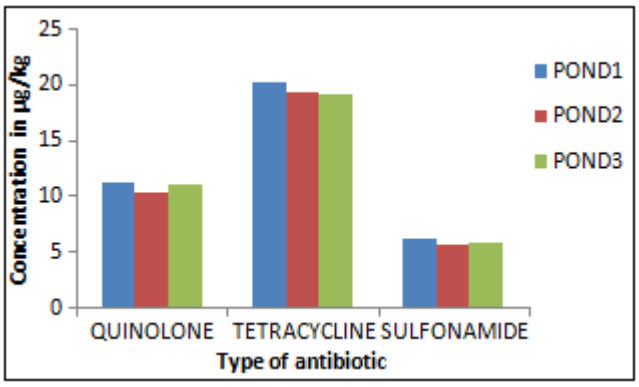

Figure 5: Mean concentration of antibiotics at Mzumbe wastewater treatment plant

5. Discussion

Wastewater treatment plants play an important part in the life cycle of antibiotics in society, and their removal may influence the sorrounding environment. Except for antibiotics application in livestock ,followed by fertilization with manure (Zarfl *et al*, 2009) and direct discharge of wastewater from livestock and human (Tang *et al*, 2015), the removal efficiency of wastewater treatment plants could be another important reason for the presence of antibiotics in the environment (Watkison *et al*, 2009; Zhang *et al*, 2015).

In this study the removal efficiency of all selected antibiotics was high at Mafisa wastewater treatment plant than at Mzumbe wastewater treatment plants.The removal efficiency at mafisa wastewater treatment plant was higher for the sulfonamides,followed by tetracyclines and lowest for quinolones.At mzumbe wastewater treatment plant the removal efficiency was high for quinolones, but for both sulfonamides and tetracycline was low and generally the removal efficiency at Mzumbe wastewater treatment plant is poor. From this study it is clear that the Mafisa wastewater treatment plant sysytem is efficient in removing antibiotics, probably due to a large number of sedimentation and maturation ponds compared to mzumbe wastewater treatment plant, hence mafisa system can be adopted for wastewater treatment in developing countries.
For tetracyclines, the removal efficiency ranged from 25.62% to 50.74% in Jiulongjiang River Basin, in South China. In USA tetracycline have been reported after secondary treatment and chlorination with removal efficiencies of 78% and 67%. Li and Zhang reported removals of 24-36% at two plants, while higher removals of 67.9-100% were reported by Karthikeyan and Meyer in four Taiwanese wastewater treatment plants.Tetracyclines are reported to be interact strongly with clay, natural organic matter and metal oxides by cation exchange, surface complexation, bridging hydrophobic partitioning, and electron donor-acceptor interactions. Normally sulfonamides are easy to be degraded during aerobic decomposition compare to tetracyclines and quinolones,hence high removal efficiency.

Removal efficiencies for antibiotics appear to vary with wastewater treatment plants, affected by their set up, operational and environmental factors. The main operational factors that can influences the biological removal of antibiotic residues in wastewater treatment are biochemical oxygen demands, existence and size of anoxic and anaerobic compartmentss, suspended solids, hydraulic retention time,sludge retntion time, food/microorganism ratio, pH,temperature of the raw sewage. In this study study removal efficiency of antibiotics was low compare to convetional advanced wastewater treatment plants in developed countries.

Concentrations of antibiotics in the influents and effluents are summerized in table 2. All the target antibiotics were detected in samples. The selected antibiotics were tetracyclines, sulfonamides and quinolones. Among the three selected antibiotics, concentrations of tetracyclines were high, followed by quinolones, probably because these drugs are commonly used in human and veterinary medicine for treating different infectious diseases.Also these two drugs are difficult to degrade compare to sulfonamides.

The concentrations of detected antibiotics were higher than those detected in different studies in the world, like USA (Meyer, 2006), Autralia (Wtkison *et al*, 2007), Beijing, China (Chang *et al*, 2008), UK (Kasprzyk-Horden *et al*, 2008), Switzerland (Gobel *et al*, 2005) and Fjian, China (Zhang *et al*, 2015). This mght be due to poor removal efficiency of the studied wastewater treatment plants.

6. Conclusion

All the selected antibiotics were found in the inffluents and effluents at two studied wastewater treatment plants.The highest level of total antibiotics was found in Mafisa wastewater treatment plant, which receives wastewater from large population inhabiting the morogoro municipality, compare to to Mzumbe wastewater treatment plant which receives wastewater mainly from a small population of Mzumbe University community.

The removal of antibiotics by the two wastewater treatment plants was incomplete, especially at Mzumbe wastwawater treatment plant.Sulfonamides were removed relatively more efficiently compared to other studied antibiotics, especially at Mafisa wastewater treatment plant. As for the occurrence and removal of antibiotics from the two studied wastewater treatment plants, these were found to be within the range of other wastewater treatment plants.Probably antibiotics from studied wastewater treatment plants are sources of water bodies like rivers contamination in Morogoro municipality.

7. Acknowledgements

This work is financially supported by DANIDA through Urban and Peri-urban Livestock farming in Tanzania. We declare that there is no conflict of interest between funding organization and this research work.

References

[1] Akinbowale O.L, Peng H, Barton M.D. 2007. Diversity of tetracycline resistance genes in bacteria from aquaculture sources in Australia. *Journal of Applied Microbiology. 103:2016-2025.*

[2] Facazio MS, Kolpin DW, Barnes KK, Furlong ET, Meyer MT. (2008). A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States-II) untreated drinking water sources. *Sci.Total. Eviron.402:201-216*
[3] Gobel A, Thomsen A, Mcardell CS, Joss A, Giger W. (2005). Occurrence and sorption behaviour of sulfonamides, macrolides and trimethoprim in activated sludge treatment. *Envoron.Sci. Technol 39:3981-3989.*
[4] Homem, V, Santos, L, 2011. Degradation and removal methods of antibiotics from aqueous matrices- a review. *Journal of Environmental Management 92, 2304-2347.*
[5] Kasprzyk-Hordem B, Dinsdale RM, Guwy AJ (2008). Multiresidue methods for analysis of pharmaceuticals, personal products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography-electrospray tandem mass spectometry. *Anal.Bioanaly Chem.391:1293-1308*
[6] Kim, S.R, Nonaka L, Suzuki S. 2004. Occurrence of tetracycline resistance genes tet(M) and tet (S) in bacteria from marine aquaculture sites. *FEMS Microbiol Lett. 237(1):147-156.*
[7] Kim, Y, Eichhorn, P, Jensen J.N, Weber A.S, Aga, D, 2005. Removal of antibiotics in wastewater; Effects of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental Science and Technology, 39, 5816-5823.*
[8] Kummeeer, K 2009. Antibiotics in aquatic environment-a review-part II. *Chemosphere,75,435*
[9] Larson D.J.G, de-Pedro C, Paxeus N. 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *J. Hazard. Mater. 148:751-755.*
[10] Leung H.W, Minh T.B, Murphy M.B, Lam J.C.W, So M.K, Martin M, Lam PKS, Richardson B.J. 2012. Distribution, fate and risk assessment of antibiotics in sewage treatment plants in Hong Kong, South China. *Environ.Int.42:1-9*
[11] Li, B, Zhang, T, 2011. Mass flow and removal of antibiotics in two Municipal Wastewater treatment plants. *Chemosphere 83, 1284-1289*
[12] Liu ,M, Zhang Y, Yang M, Tian Z, Ren L, Zhang S. 2012. Abundance and distribution of tetracycline resistance genes and mobile elements in an oxytetracycline production wastewater treatment system. *Environ. Sci.Technol. 46 (14):7551-7557.*
[13] Luo Y, Xu L, Rysz M, Wang Y, Zhang H, Alvarez P.J. 2011. Occurrence and transport of tetracycline, sulfonamide, quinolones and macrolide antibiotics in the Haihe River Basin, China. *Environ.Sci.Technol. 45:1827-1833.*
[14] Sarmah A.K, Meyer M.T, Boxall A.B. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics in environment. *Chemosphere.65:725-755.*
[15] Su H.C, Ying G.G, Tao R, Zhang R.Q, Zhao J.L, Liu Y.S. 2012. Class 1 and 2 integrons, Sul resistance in Escherichia coli isolated from Dongjiang River, South China, *Environ. Pollut.169;42-49.*
[16] Tao R , Ying G.G, Su H.C, Zhuo H.W, Sidhu J.P.S. 2010. Detection of antibiotics resistance and tetracycline resistance genes in Enterobacteriacea isolated from Pearl rivers in South China.*Environ.Pollut.158:2101-2109.*
[17] Threedeach S, Chiemchaisri W, Watanabe T, Chiemchaisiric, Honda R, Yamamoto, K . 2012. Antibiotics resisitance of Escherichia coli in leachates from municipal solid waste landfills: Comparison between semi-aerobic and aerobic operations. *Bioresour Technol 113:253-8.*
[18] Watkison AJ,Murby EJ, Costanzo, SD (2007). Removal of antibiotics in conventional and advanced wastewater treatment: Implications for environmental discharge and wastewater recycling. *Water Res. 41:4164-4176*
[19] Zarfl C, Klasmeier J, Matthies M (2009). A conceptual model describing the fate of sulfadiazine and its metabolites observed in manure- amended soils. *Chemosphere 77;720-726.*
[20] Zhang H, Du M, Jiang H, Zhang D, Lin L, Ye H, Zhang X (2015) .Occurrence, seasonal variation and removal efficiency of antibiotics and their metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China. *Environmental Science Processes and impacts 17:225-34.*