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β-lactams and Fluoroquinolone Antibiotics in influents and effluents of Wastewater treatment plants, Dar es Salaam, Tanzania

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Abstract

The outcomes of the wastewater contaminated with antibiotics entering the WWTPs of Dar es Salaam city were examined. This study aimed at evaluation of the quality of the disposed treated wastewater in order to find out the fate of the antibiotics in the city WWTPs. Antibiotics amoxicillin, ampicillin and ciprofloxacin concentrations ranged from below detection limits (bdl) to 0.367 mg/l and bdl to 0.037 mg/l were measured in wastewater influents and effluents samples, respectively. In all the WWTPs, effluents concentrations were lower than their corresponding influents. Variations in levels of antibiotics in WWTPs were considered to be attributed to removal efficiency of individual WWTP and antibiotics prescription patterns in different locations of the city, which determine or affect influent concentrations. All the parameters were measured by standard methods.

Keywords: Antibiotics, wastewater treatment plant, influents, effluents.

Introduction

In previous study[1], it was reported levels of heavy metals passing municipal wastewater treatment plants (MWWTPs), and getting into streams and rivers environments. In continuation with investigation for quality of the disposed treated wastewater of Dar es Salaam City this paper reports analyses of concentrations of antibiotics entering and leaving the Dar es Salaam MWWTPs. Antibiotics are suspected to be persisting in the environment due to their inability to naturally biodegrade or continued prevalence because of continuous release. In developing countries the continuous release of antibiotic in the environment is due to their overuse in public and private healthcare services. Antibiotic resistance, increase burden of infectious diseases and low spending of healthcare are possible cause for the overuse[2].

Antibiotics and other domestic chemicals are normally released to the environment after going through treatment in wastewater treatment plants, or domestic septic systems, which often are not designed to remove antibiotics[3]. An increasing number of antibiotics have been found in the aquatic environment and the issue has become one of most urgent emerging human and environmental health concern. Their behaviour as well as that of their degradation products are not clearly known, potential chronic effects in the aquatic life forms, in particular effect on endocrine system due to long term and low level exposure threaten the sustainability of the ecosphere[4]. Long term exposure to antibiotics can result not only in subtle effects on aquatic species but also could pose a risk to human health through consuming contaminated drinking water[5]. Potential effects include development of resistant bacteria, increased toxicity of chemical mixtures in the environment, increased incidences of cancer, abnormal physiological processes and reproductive impairment[6].

Wastewater treatment plants (WWTPs) are considered to be among the most important source of pharmaceuticals contaminants in the water system. Many researches on the fate of these chemicals in WWTPs have been done in developed countries[7]. In Tanzania, limited or no researches have been conducted to understand the fate of antibiotics and other pharmaceuticals in WWTPs and aquatic environment. Therefore, this study was conceived in order to understand the outcome of the antibiotics entering the WWTPs in Dar es Salaam city.

Material and Methods

Study area and sampling: The study was conducted in seven WWTPs of Dar es Salaam City, Tanzania (figure-1). The WWTPs are used to treat wastewaters originating from domestic, institutions, hospitals and industrial areas. The treated wastewaters are eventually flow into Indian Ocean. Twenty eight samples of wastewater were collected between March and June 2013. Two samples were collected from the influent and effluent points of individual WWTP. The names of the WWTP, geographical position, wastewater origin and the receiving river/stream are presented in table 1. Samples were collected using one litre sampling glass bottles with Teflon stop corks. The bottles were previous washed with detergents, rinsed with deionised water and acetone, followed by drying at 170°C for 2 hours. During sampling, the glass bottles were first rinsed with the water samples then filled just to overflowing through fibre glass filters to remove sand and debris, without passing or trapping air bubbles in sealed bottles. The samples were

acidified to pH 2.5 with HCl to inhibit biological activity, and were kept cold in ice while transporting to the laboratory for analysis.

Sample preparation: Samples of water were extracted by Liquid-Liquid Extraction (LLE) method. Each water sample (1000 ml) was quantitatively transferred to a 1 L separating funnel and the sampling bottle was rinsed with dichloromethane (30 mL) which was then combined with the water sample in the separating funnel. The combined contents were then successively extracted with dichloromethane (3 x 50 mL). The organic layer was filtered through plug wool containing anhydrous sodium sulphate (30 g) for drying. Sodium sulphate was later rinsed with dichloromethane (2 x 3 mL) and the combined extract concentrated in *vacuo* at 30°C ready for analysis. The water extract appeared clean and were not subjected to further clean up.

Analytical methods: Reverse phase HPLC coupled with UV/Vis detector was used for the analysis of the three antibiotics. All the separations were performed by C_{18} column (0.25 m long and 4 mm diameter) packed with silica gel. The mobile phase was obtained by dissolving 2.5 g of NAH_2PO_4 in 1000 ml of distilled water and then mixed with 1.94 ml phosphoric acid to make a buffer solution. The elution was then done by isocratic mixture of 85:15 buffer:acetonitrile. Determination of antibiotics compounds was enabled by comparing chromatograms of samples and external reference standards.

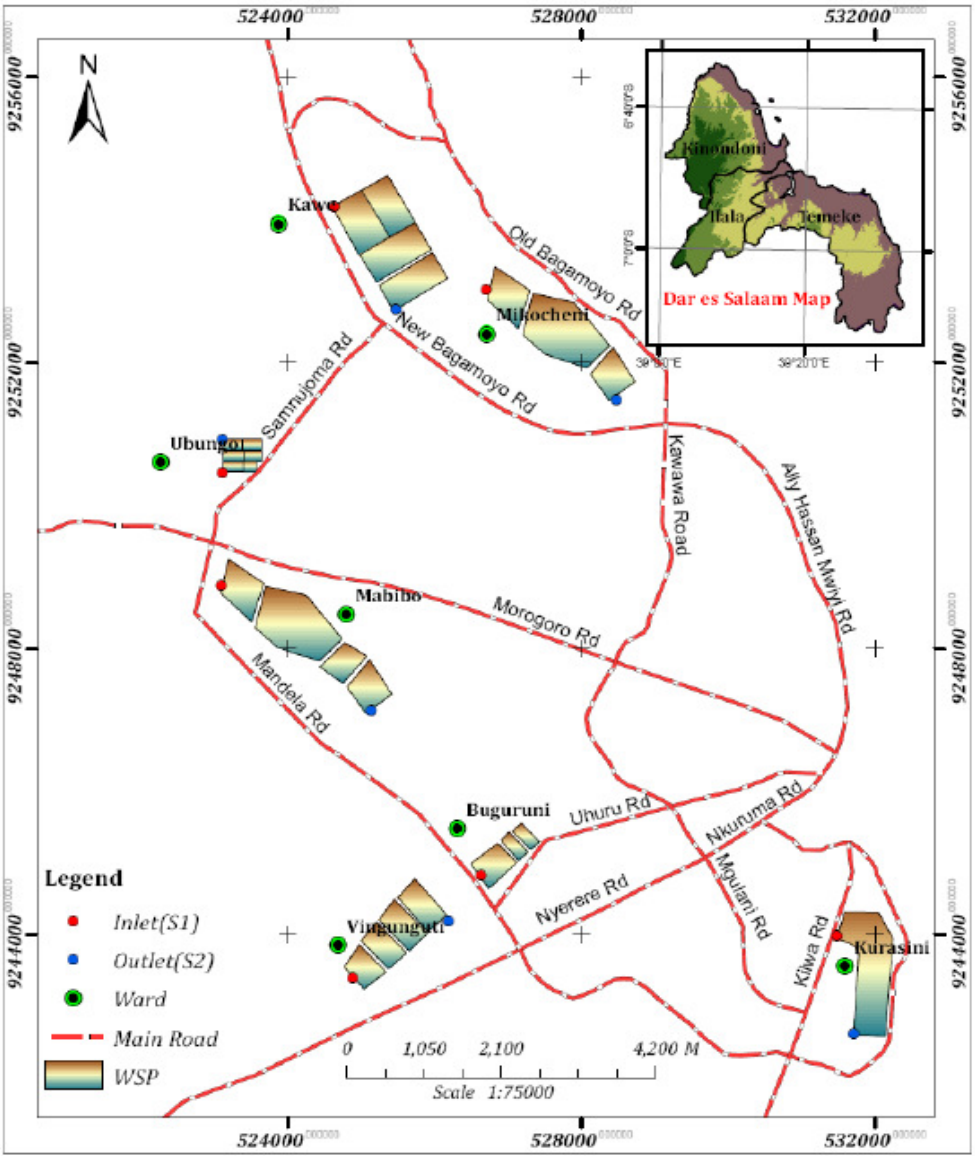

Figure-1
Study are descriptions and sampling positions' distribution

Table-1
Description of the studied Dar es Salaam wastewater treatment plants

Sampling point (WWTP)	Geographical location	Wastewater sources	Significant industrial discharges	River/ Stream
S1 (UDSM)	S 06.77768 E039.21402	Domestic, laboratories, workshops, health centre	70% Institution 30% Residential	Mlalakuwa
S2 (Mabibo)	S06.81151 E039.22733	Industries, institutions, residential	50% Industrial 50% Residential	Msimbazi
S3 (Vingunguti)	S06.83721 E039.23685	Industries	85% Industrial 15% Residential	Msimbazi
S4 (Buguruni)	S06.82560 E039.24585	Residential	75% Residential 15% Industrial	Msimbazi
S5 (Kurasini)	S06.85297 E039.29103	Industrial, residential	80% Residential 20% Industrial	Kurasini
S6 (Mikocheni)	S06.76829 E039.22780	Industrial, residential	85% Industrial 15% Residential	Mikocheni
S7 (Lugalo)	S06.74358 E039.22780	Ammunition, hospitals, schools and laboratories	75% Institution 25% Residential	Mlalakuwa

Results and Discussion

Levels of antibiotics in MWWTPs: Concentrations of three antibiotics compounds amoxicillin, ampicillin and ciprofloxacin measured in wastewater samples from seven MWWTPs are summerised in figure 2. These antibiotic compounds were chosen for investigation because they are frequently used in clinical medicine and no information is available about their fate in the city environment. The three antibiotics belong to two groups of antibiotics, the penicillin and fluoroquinolones. Penicillins or β-lactam antibiotics function by destroying the bacteria cell walls in the reproduction stage. β-lactam antibiotics comprise of ampicillin, penicillin G, cloxacillin, penicillin V, ticarcillin, oxacillin, amoxicillin and nafcillin[8,9]. There is no much information available on sorption and degradation of penicillin in the environment. It is speculated that these compounds hydrolyse rapidly and are not commonly detected in surface waters. This is because the β-lactam ring is of poor stability and can be unlocked by chemical hydrolysis or by βlactamase enzyme [8,9].

Fluoroquinolones are synthetic antibacterial drugs that function by inhibiting bacterial DNA synthesis. Several fluoroquinolone drugs have been removed from the market due to their fatal adverse effects, only six are still recommended for use, these include ciprofloxacin, moxifloxacin, levofloxacin, norfloxacin, gemifloxacin and ofloxacin[10]. Contamination of fluoroquinolones into the water environments originates from human and animal use, and it occurs mainly as the parent compounds, conjugates or as metabolites of the parent compound after undergoing oxidation, hydroxylation, dealkylation or decarboxylation. Most of the primary metabolites still exhibit antibacterial activity[11]. Fluoroquinolones are reported in previous published studies as one among the most prevalent groups of antibiotics found in the environment, and particularly in surface waters[12]. For instance comparatively high concentrations of fluoroqinolones have been reported in effluents from sewage treatment plants in developed countries like USA (19–45 ng/L), Canada (102–506 ng/L), France (330–510 ng/L), Italy (290–580 ng/L), Greece (460 ng/L), and Switzerland (249–405 ng/L)[12].

Results in this study (figure 2) shows that concentrations of antibiotics amoxicillin, ampicillin and ciprofloxacin in influents and effluents samples varied among locations. This variation can be attributed to antibiotics prescription patterns in different locations of Dar es Salaam city, which have an effect on influent concentrations. The influents samples displayed antibiotic concentrations in the ranged of below detection limits (bdl) to 0.367 mg/l while in the effluents samples the respective concentrations ranged bdl to 0.037 mg/l. The highest concentration of ampicillin (0.135 mg/l) and ciprofloxacin (0.031 mg/l) in influents were measured at S5 WWTP, whereas the greatest concentration of amoxicillin (0.367 mg/l) in influent was detected at S7 WWTP. The lowest concentration of antibiotics amoxicillin and ampicillin in wastewater effluents that were below detection limits were measured at S3 and S2 WWTPs, whereas for ciprofloxacin lowest concentration in effluent was detected at S1 WWTP. The distribution patterns of the antibiotics in the effluents are different from that in the influents. Different process and removal of each compound in the WWTPs could be the reason for the observed difference. Among the three compounds amoxicillin showed fairly high concentrations as compared to ampicillin and ciprofloxacin in the influents and effluents samples.

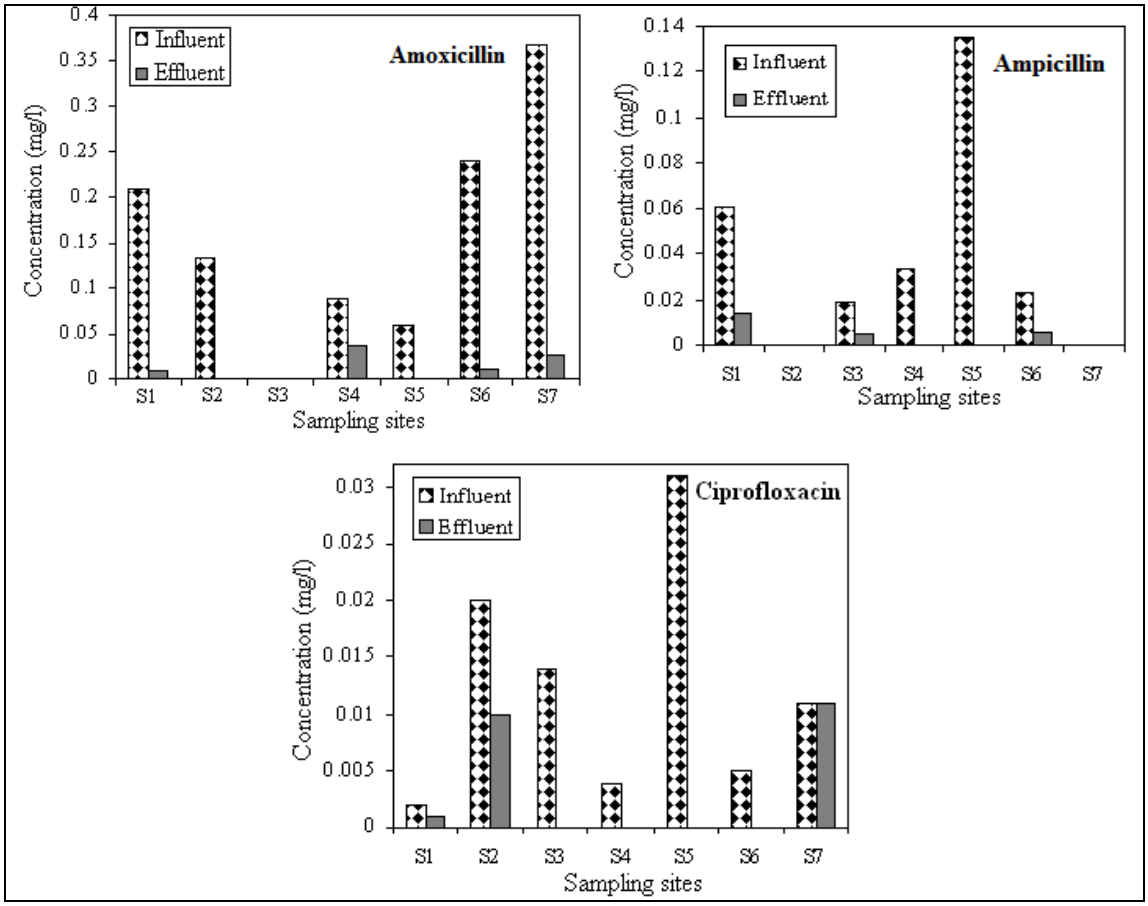

Figure-2
The distribution of selected antibiotics in influents and effluents of investigated WWTPs

Fate of antibiotics in MWWTPs: Wastewater is considered as one of the significant source of water pollution in urban area of Dar es Salam city. About 90% of households in the city use pit latrines and septic tanks, and only 10-15% have access to the sewerage systems. The sewerage systems channels the wastewater into the wastewater treatment plants in which waste stabilization ponds (WSPs) technology is used[13]. WSPs technology involves use of shallow basins where wastewater is treated with the use of anaerobic/aerobic bacteria. The technology consists of three ponds which are anaerobic, facultative and maturation ponds. WSPs can satisfactory remove biodegradable organic matter, suspended solids, nutrients and pathogenic microorganisms[14]. It has however not designed to treat pharmaceutical compounds because of their highly variable physicochemical properties and operational conditions of the biological process as well as substantial variations by which pharmaceuticals are removed[15]. In this study concentrations profile of individual antibiotics detected in the effluent samples differed to the influent concentrations profile (figure 2). All concentrations in the effluents samples were lower than their corresponding influent samples. The highest concentrations of amoxicillin, ampicillin and ciprofloxacin in effluent samples were detected at S4, S1 and S7, while in the influent samples were at S7, S5 and S5, respectively. This variation can be attributed to individual WWTP efficiency on antibiotic removal. Insufficient follow up of operation and maintenance procedures, poor design and mixing of both domestic, hospitals, industries and institutions wastewater can contribute to poor performance of the WSPs on removal of antibiotics and other contaminants[16-18].

The efficiencies of individual WWTP to remove the antibiotics amoxicillin, ampicillin and ciprofloxacin are presented in table 2. The removal efficiency was determined as the percentage of the concentrations difference between influents and effluents samples of each WWTP. Removal efficiencies vary among the WWTP and antibiotic compound. This variation can possibly be associated with specific treatment processes occurring in individual WWTP, residence time of sewage at different WWTP and chemical properties of the antibiotics. Highest removals were for amoxicillin and ampicillin that ranged from 57.95% to 96.17% and lowest was ciprofloxacin ranged from 45% to 50%. The high percentage removal of amoxicillin and ampicillin could be due to β-lactam ring, which is not very stable and can be unlocked by chemical hydrolysis or β-lactamases bacteria. As a result of this, intact βlactams antibiotics are not frequently occurring in the environment[19] and

are reported to be considerably removed under biological process with removal efficiencies higher than 90%[16]. In aqueous medium, fluoroquinolones are reported to be susceptible to photodegradation, involving oxidation, dealkylation and cleavage of the piperazine ring[20]. The polar groups of fluoroquinolones bonded to a lipophilic core provides relatively good solubility in water, strong interaction with natural organic matter and adsorption on soil, due to both hydrophobic and electrostatic interactions and hydrogen bonds. Strong adsorption to sewage sludge during wastewater treatment and stability in sewage sludge could therefore be reason for fluoroquinolones removal[4,11].

Table-2
Antibiotic removal efficiencies in wastewater from Dar es Salaam MWWTPs

WSPs location	Percentage removal		
	Amoxicillin	Ampicillin	Ciprofloxacin
S1 (UDSM)	96.17	77.05	50
S2 (Mabibo)	nd	nd	50
S3 (Vinguguti)	nd	73.68	nd
S4 (Buguruni)	57.95	nd	nd
S5 (Kurasini)	nd	nd	nd
S6 (Mikocheni)	95.38	nd	nd
S7 (Lugalo)	92.92	73.91	45

nd - not determined: influent and/or effluent samples concentrations were below detection limit

Conclusion

Antibiotics drugs have been determined in influents and effluents of WWTPs that are used to treat wastewater originating from residential, industrial, commercial and hospital areas. The findings suggest that the conventional treatment of municipal wastewater by WSPs technology seems to be insufficient for removal of antibiotics from wastewater, this implies that WSPs could be an important source of antibiotics pollution in urban surface water. Information of this study is not enough to reach into conclusion on the significance and impact of potential risks to human health and ecosystems functions. However, if such contamination continues, it may lead to serious health problems particular to community living downstream of WWTPs who are using the water for various domestic activities. This study signifies prerequisite to have addition treatment method within or in series with WSPs that could remove antibiotics before effluents are released into receiving environment.

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