Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.7, No.2, 2017
www.iiste.org

Occurrence and Fate of Antibiotics as Trace Contaminants in Wastewater Collection and Disposal Systems
Case Study: University of Dar es Salaam Wastewater Stabilization Ponds, in Tanzania

Fredrick Salukele* Dionis Rugai Mengiseny. E. Kaseva
Ardhi University, School of Environmental Science and Technology (SEST), Department of Environmental Engineering, P. O. Box 35176, Da es Salaam

Abstract
Antibacterial agents (Antibiotics) are emerging micro-contaminants in institutional and municipal wastewater and the aquatic environment. These substances enter wastewater effluents via urine and faeces and by improper disposal of domestic and or medical waste. An environmental analytical study was conducted at the University of Dar es Salaam (UDSM) Waste Stabilization Ponds (WSP). These ponds receives and treats influents from students' halls of residence and staff quarters (domestic effluents) as well as hospital effluent sources. The study focused on seven (7) antibiotics commonly used in human therapy, which include Ciprofloxacin, Flucloxacillin, Erythromycin, Ampicillin, Penicillin-V, Amoxycillin and Cloxacillin. In hospital wastewater, the detected antibiotics ranged from 0.98 - 7.10 µg/l, while in student's halls of residence, the range was 0.2- 1.47µg/l. In WSP influent and effluent concentrations ranged from 8.89 to 63.75µg/l and 1.51 to 28.01µg/l, respectively. The concentration in the sewage sludge was from 72.12 to 370µg/l. Results of this study indicated that significant amount of antibiotics remain in the treatment plants during wastewater treatment process, through sorption to sewage sludge. This suggests that wastewater treatment options currently applicable in Tanzania including different sanitation options should now be designed taking into account the presence of trace concentrations of antibiotics for the purpose of sanitation and environmental protection at large.
Keywords: Antibacterial Agents and Wastewater Stabilization Ponds

1.0 Introduction

The use of antibiotics globally, has grown tremendously since sulfa drugs were discovered in 1900s. According to a Gouws et.,al (2004) about one third of all people who presented themselves for care at outpatient facilities in Tanzania, 32% were classified as requiring treatment by antibiotics. Among people needing antibiotics, the majority (87%) needed them for pneumonia while others needed them for acute ear infection (7%) and dysentery (10%).

Although antibiotics and other pharmaceuticals are intended to be utilized by the human body, in some instances as much as 50 to 90% of an administered drug may be excreted by the body in biologically active form (Brown 2004). This is because human ability to break down medicine varies widely by individual and by drug. Many drugs are not totally decomposed within the body and as a result a portion of these drugs is excreted in faeces or urine. Halling-Sorensen et al., (1998) have reported the same to be true with domesticated animals. Renew and Huang, (2004) have also reported that very often a high percentage of the administered antibiotics is excreted without metabolism or excreted in conjugated forms that can be readily converted back to the parent compounds. As a result antibiotics are among the important emerging trace contaminants in water and wastewater systems which have potential adverse effects on the ecosystem and on human health. Antibiotics are thus flushed along with all sorts of other chemicals down the toilets and drains. Many are suspected to pass right through wastewater treatment plants into rivers, lakes and aquifers. In wastewater treatment plants the antibiotics are only partially eliminated and residual amounts can reach both surface and groundwater sources. The concern with antibiotic residues in the environment includes the inducement of resistance in bacterial strains. Also antibiotic contaminants may perturb microbial ecology, increase the proliferation of antibiotic-resistant pathogens and thus pose threats to human health (Daughton et. al., 1999).

This paper presents results of a study whose general objective was to assess the presence, levels and fate of human used antibiotics in wastewater systems. The method used was by way of estimation of concentration of selected antibiotics in untreated wastewater and linking them with point source contributions in the case study area with sources such as a University Health Centre (UHC), staff residential areas and from students' halls of residence at the University of Dar es Salaam (UDSM). In this study, the efficiency of a wastewater treatment plant namely the University Waste Stabilization Ponds (UWSP) in the removal of the selected antibiotics was also studied.

2.0 Materials and Methods

2.1 Description of the Case Study Area

The case study for this research work was the UDSM, which is one of the main public Universities in Tanzania. It is situated on the west side of the City of Dar es Salaam, about 13 km from the City Center. The area has a monthly mean maximum and minimum ambient air temperature of 28^{o}C and 23^{o}C, respectively and an annual precipitation ranging from 500-1000mm. Wastewater collection and treatment system at the UDSM consists of sewer network which is connected to Waste Stabilization Ponds (WSP) systems. Wastewater is thus largely of domestic and institutional characteristics. The quantity and quality of wastewater generated is closely dependent on the university academic timetable. Hydraulic and organic loading rates are thus comparatively low when students are on leave and vice-versa when the classes resume.

2.2 Wastewater sampling and sampling locations

In this study, seven sampling points were identified and selected for antibiotics, pH and Chemical Oxygen (COD) analysis. Four of the sampling points (SP_1 to SP_4) were located at the wastewater generation points. The other two (SP_5 and SP_6) were at the inlet and outlet of the UWSP, while SP_7 was for sludge samples from the WSP (anaerobic pond). Figure 1 show the location of sampling points (SP_1: Male hall of residence, SP_2: Female hall of residence, SP_3: UHC, SP_4: Staff houses, SP_5: Inlet point to the anaerobic pond and SP_6: Outlet of maturation pond, S7: sludge from the anaerobic pond).

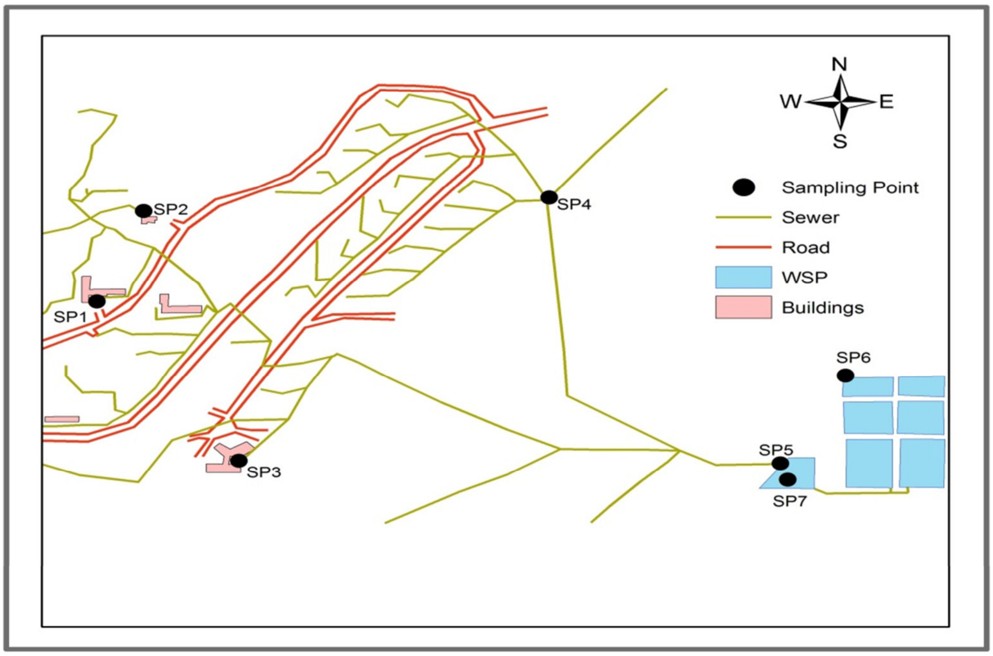

Figure 1: Location of sampling points

2.3 Wastewater Sampling and Sampling Locations

In this study, seven sampling points were identified and selected for antibiotics, pH and Chemical Oxygen (COD) analysis. Four of the sampling points (SP_1 to SP_4) were located at the wastewater generation points. The other two (SP_5 and SP_6) were at the inlet and outlet of the UWSP, while SP_7 was for sludge samples from the WSP (anaerobic pond). Figure 1 show the location of sampling points (SP_1: Male hall of residence, SP_2: Female hall of residence, SP_3: UHC, SP_4: Staff houses, SP_5: Inlet point to the anaerobic pond and SP_6: Outlet of maturation pond, S7: sludge from the anaerobic pond).

2.4 Extraction of Antibiotics from Wastewater

Antibiotics from wastewater samples were extracted by Liquid-Liquid Extraction (LLE) Method using methanol and Cyclohexane as an extracting organic solvents. Since water and methanol form a partial homogeneous mixture that makes a separation line between the two layers (aqueous and organic layer) difficult to be seen, Sodium Chloride-NaCl (Analytical reagent grade) was used in order to increase the density of wastewater sample. The mixing ratio between water and NaCl was on the basis of 1L (1000ml) of the sample with 10 % (volume of sample) being NaCl.

2.5 Questionnaire Survey and Interviews

Questionnaire surveys and interviews were carried out at the UHC. These were aimed at establishing the number of patients visiting the health center daily. Also medical information checklists were prepared in order to establish types of antibiotics that are commonly administered to patients. Data on antibiotic consumption rate was also collected from the UHC in order to establish the concentration of antibiotics in raw sewage. The antibiotics consumption rates were established based on mass of antibiotics (250 mg of antibiotic active ingredient per each tablet and or capsules) administered per day, and the number of patients attending the health center daily.

2.6 Concentration of Antibiotics in Raw Wastewater

A reverse-phase High Performance Liquid Chromatograph (HPLC) system with fluorescence detector was used to analyze all the antibiotics under this study by using external standard technique. The detection was conducted at an excitation wavelength of 278nm and emission wavelength of 450nm.The mobile phases include a solution containing 20mM H_3PO_4 and 20mM NaH_2PO_4 (eluent A, pH-2.4), acetonitrile (eluent B), and methanol (eluent C). The mobile phase begins with 0.5minute isocratic 98% A (2% B) followed by a gradient decrease to 90% A in 0.5 minutes, then a gradient decrease to 75% A in 9 minutes, followed by 5minutes isocratic 75% A. The mobile phase was then switched to 15% A and a column was flushed under these conditions for 5minutes.The mobile phase then shifts to isocratic 100% C for 10minutes to flush the column thoroughly and finally switches back to 98% A and 2%B.

3.0 Results and Discussions

3.1 Patients' Attendance and Antibiotics Human-Use at UHC

The objective of this study was to investigate the occurrence of antibiotics in wastewater collection system at the UDSM and when present, to document their concentrations. Since pharmaceuticals including antibacterial agents are discharged into wastewater systems from hospitals, among other sources, attempt was made to establish the consumption rate of antibiotics at the UHC. This is because according to Brown (2004) hospitals including health centers are generally accepted that they are a significant point source contributor of pharmaceutical active compounds. Huang et al., (2001) have also reported that antibiotics that are likely to be present in municipal wastewater are primarily those used in human therapy.

Based on questionnaire survey, interviews and data from the UHC, the average number of daily inpatients was established to be 50, while that of outpatients was found out to be 250. On average 300 patients including students and staff attend the health center daily. The number of patients as well as antibiotics human-use data were used in the assessment of the potential inputs of these chemicals into wastewater collection and disposal system. Findings of this study revealed that among the seven most popular antibiotics administered to patients, there were five β-lactams (Amoxycillin, Penicillin, Ampicillin, Flucloxacillin and Cloxacillin), one Macrolide (Erythromycin), and one Fluoroquinolone (Ciprofloxacin). Figure 2 indicates that the most commonly administered antibiotics include Amoxycillin (22.6%), Cloxacillin (19.5%) and Penicillin (18.5%), while the least are ciprofloxacin (4.8%) and Flucloxacillin (3.8%). Results obtained in this study indicate that β-lactam antibiotics are the largest fraction of human-use antibiotics.

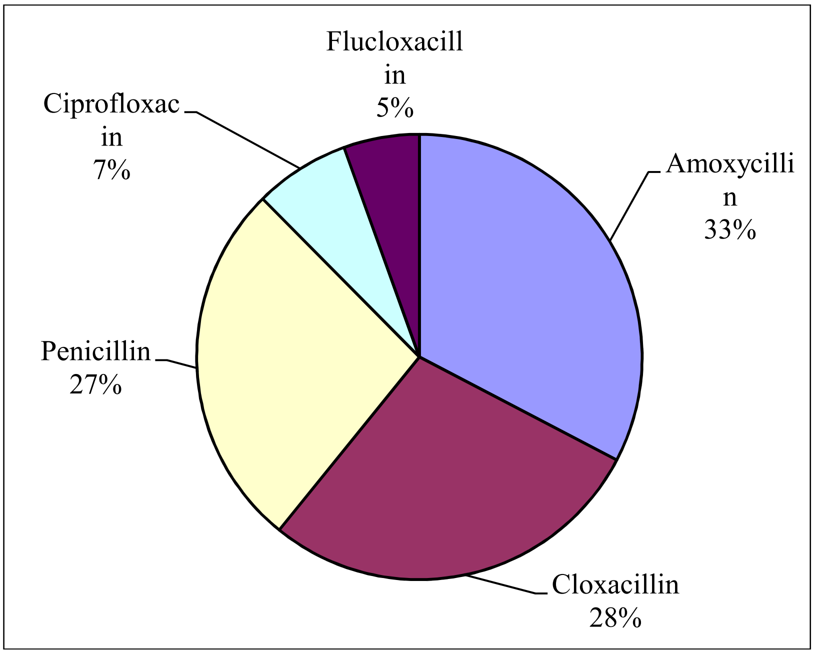

Figure 2: Commonly administered antibiotics

The amount of antibiotics applied daily for human medicinal purposes at the UHC was established to be 810g which is equivalent to approximately 0.3t/year, and that the average human-use varies from 113mg/person/day to 654mg/person/day of individual antibiotics. Figure 3 illustrates the consumption rate of antibiotics as administered at the UHC.

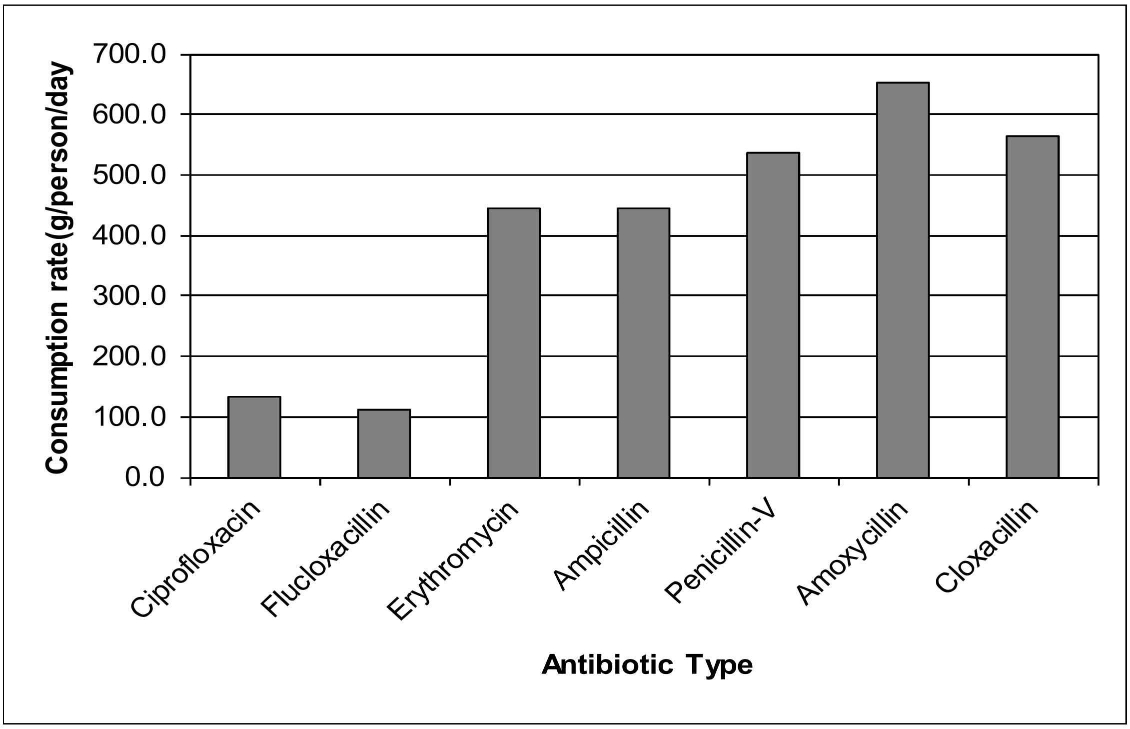

Figure 3: Antibiotics human-use at UHC

3.2 Total Concentration of Antibiotics in the Wastewater Collection System

The seven sampling points from which wastewater samples were collected for antibiotics analysis can be divided into 4 groups. These include:

i) Wastewater generation points - SP1, SP2, SP3 and SP4
ii) WSP inlet point - SP5
iii) WSP outlet - SP6
iv) Sludge from the anaerobic pond – SP7

Figure 4 shows levels of total concentration of antibiotics in wastewater system from the point of generation to the point of disposal to WSP. When Methanol was used as extracting solvent, lower concentration of antibiotics were detected at SP1 (4.39mg/L), SP2 (5.79mg/L) and SP4 (2.45 mg/L), which represents male students hall of residence, female students hall of residence and staff houses, respectively.

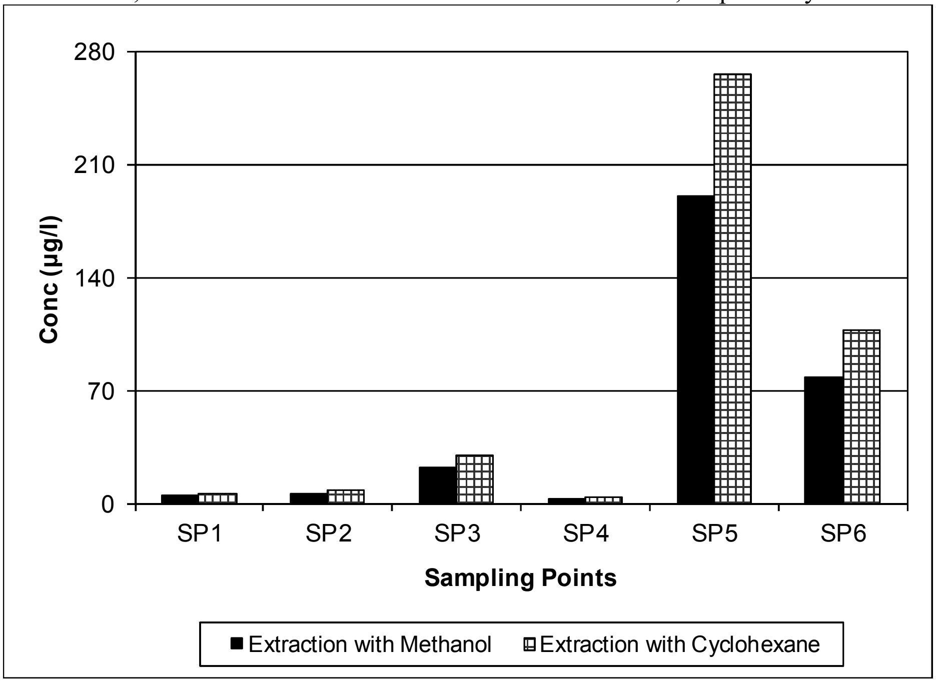

Figure 4: Antibiotics concentration levels in wastewater system

Out of the 4 sampling points considered to be antibiotics generating points, SP3 (located at UHC) had the highest concentration (21.59 µg /L). This can be explained by the fact that wastewater emanating from cleaning and washing of utensils at the UHC was also discharged into wastewater collection system at SP3. A comparable data base on occurrence of some β-lactams in hospital wastewater at concentration of up 30 µg/L has also been reported (Giger et al 2003). Brown, (2004) also found relatively high concentration of antibiotics particularly sulfonamides and fluoroquinolones in hospital wastewater whereas other pharmaceutical active compounds analyzed for, were either absent or found at undetectable concentrations in wastewater. The highest value of antibiotics concentration (189.51mg/L) was observed at entry point to the WSP (SP5). At the outlet point of the WSP (SP6) however, the level of antibiotics concentration was observed to have been reduced to 78.1 µg /L.

A similar trend of antibiotic concentration in wastewater was observed when cyclohexane was used as extracting agent. The antibiotics concentration varied between 6.17 µg /L to 3.43 µg/L in SP1, SP2 and SP4, and it was as high as 29.88 µg /L at SP3 (UHC). At entry point to the WSP the concentration was observed to be 265.96 µg /L/L, but it was reduced to 107.32 µg /L at the outlet point of the WSP. The extremely high value of antibiotics concentration at the inlet point to the WSP i.e. SP5 (compared to other sampling points) might have been due to contribution from other halls of residences which were not included in this study but discharge wastewater in the WSP. The decrease in concentration at the outlet of the WSP indicates that some amount of antibiotics remain in the WSP, suggesting that antibiotics can undergo various transformations during treatment process.

3.3 Concentrations of individual Antibiotics

Figures 5 and 6 present concentrations for all the three antibiotic classes between SP1 and SP4 as extracted by Cyclohexane and Methanol, respectively. When Cyclohexane was used as an extracting solvent, UHC effluent contributed 23.87 µg /L, 5.03 µg /L and 0.98 µg /L of β-lactams, Fluoroquinolone and Macloride antibiotics, respectively while with methanol the concentrations were respectively 17.25 µg/L, 3.63 µg/L and 0.71 µg/L. These results indicate that the discharge of antibiotics by the above sources followed the trend, SP3>SP2>SP1>SP4 suggesting also that the UHC was a significant source contributor of all the antibiotics analyzed as compared to other individual sources.

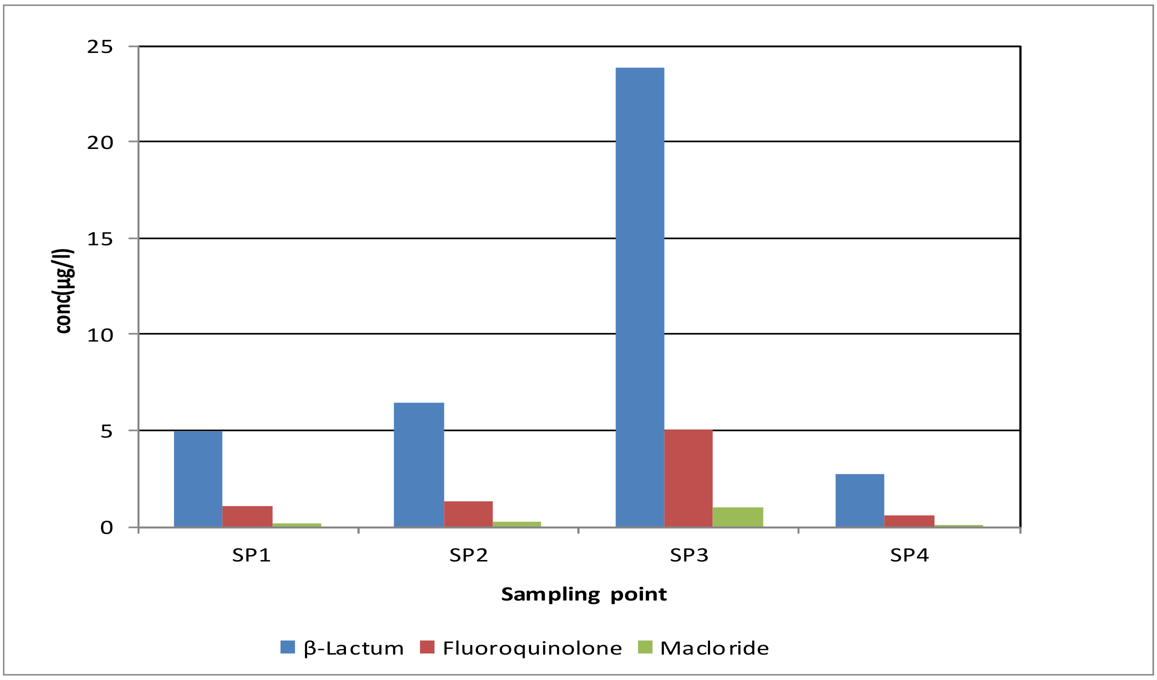

Figure 5: Concentration of Antibiotic at four sources as extracted with Cyclohexane

The results also show the difference in concentration (at all sampling points) between samples extracted with methanol from that extracted with cyclohexane. Higher concentrations of antibiotics were observed in samples extracted with cyclohexane than that with methanol. This is perhaps because cyclohexane is a more polar organic solvent than methanol. Since all the antibiotics used in this study, are polar organic chemicals, then they were more likely to dissolve in a more polar organic solvent "LIKE dissolves LIKES"

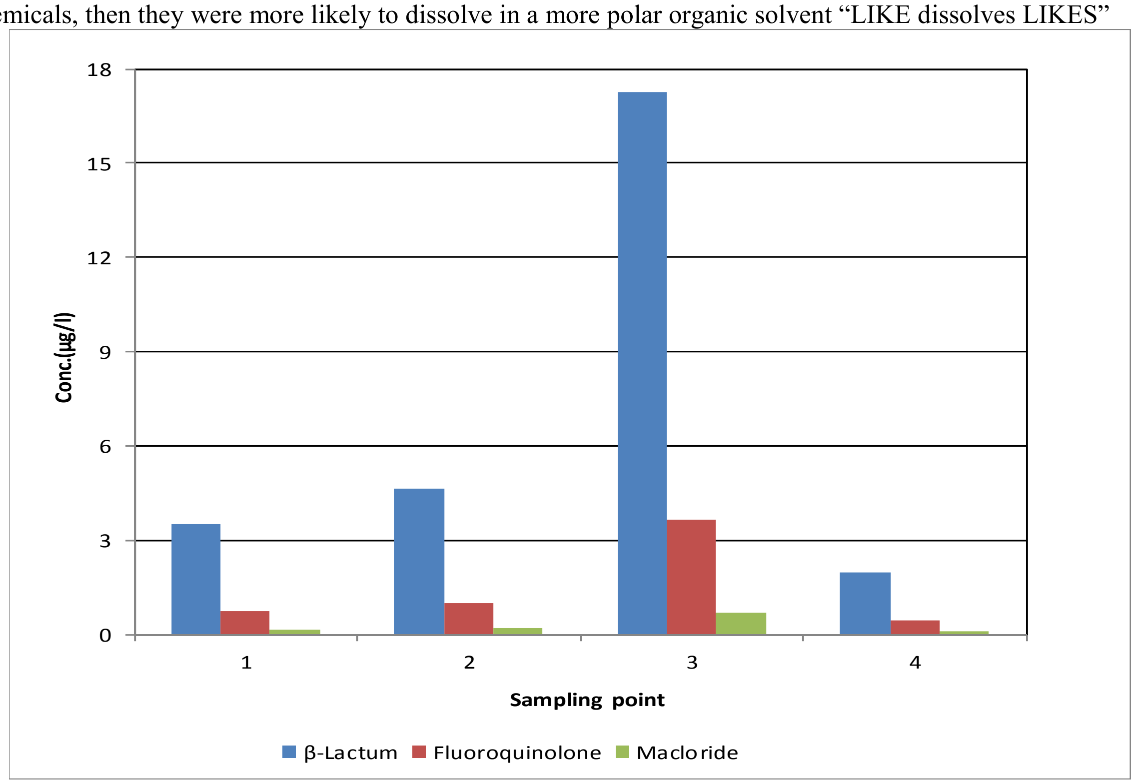

Figure 6: Concentration of Antibiotic at four sources as extracted with methanol

3.4 Performance of WSP in the removal of Antibiotics

WSP is the treatment option available for wastewater at the UDSM. After the treatment at the WSP wastewater is discharged into a nearby river (Mlalakuwa) which eventually drains into the Indian Ocean. An important process for removing the pollutants from sewage wastewater is biodegradation in sewage treatment plants. According to Al-Ahmad et al., (1999), however, currently there is little data available concerning the biodegradability of antibiotics compounds in wastewater treatment plants. In this study, the efficiency for WSP in the removal of antibiotic was investigated based on material balance of each individual antibiotic concentration entering and leaving the WSP system.

Figure 7 indicate that inlet concentration of individual type of antibiotic to the WSP varied from 8.89 µg/L to 63.75 µg/L, while the outlet concentration varied from 1.51 µg/L to 28.01mg/l, when antibiotics was extracted by Cyclohexane. The removal was highest (83%) for Ciprofloxacin followed by Erythromycin (72.07%). The rest of the antibiotics which belongs to the same group (β-lactam) were removed at a range of between 54%-56.5%. Further analysis indicated that the removal of antibiotics in the WSP followed the trend: Fluoroquinolone (Ciprofloxacin)>Macloride (Erythromycin)>β-lactam (Ampicillin, Penicillin-V, Amoxycillin, Cloxacillin, Flucloxacillin). These results generally indicate that WSP is capable of reducing the concentrations of antibiotics in wastewater, and that the extent of reduction observed was dependent on the type of antibiotics.

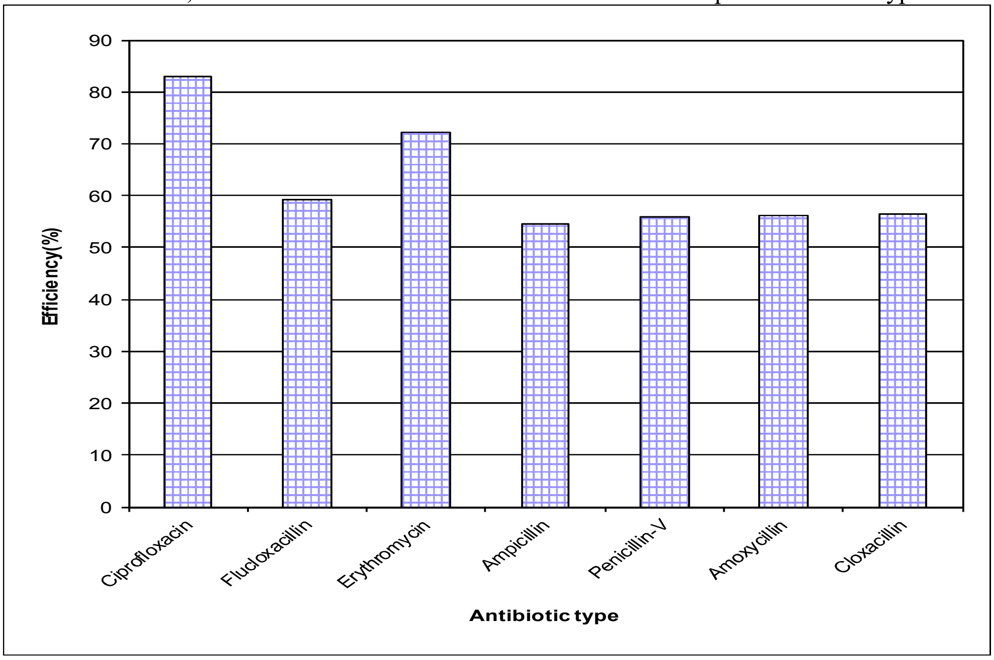

Figure 7: Antibiotic removal efficiencies for samples extracted by Cyclohexane

Results on Figure 8 also shows high percentage removal of Ciprofloxacin (81.56%) followed by Erythromycin (71.34%). The percentage removal for the rest varied from 54% to 59.34%. The removal trend was similar to the results presented in Figure 6. Low removal efficiency for β-lactam may be due to their high polarity and the presence of carboxylic acid functional group. High polarity makes the dissociation of antibiotic compound into free positive and/or negative charges. Positive charge facilitates sorption in a media that typically posses negative charge and vice versa (Huang et al. 2001). Hence the presence of high negative and or positive charge in sewage sludge may probably affect absorption of negative and or positive charge of the dissociated antibiotic group thus affecting their removal efficiency in WSP.

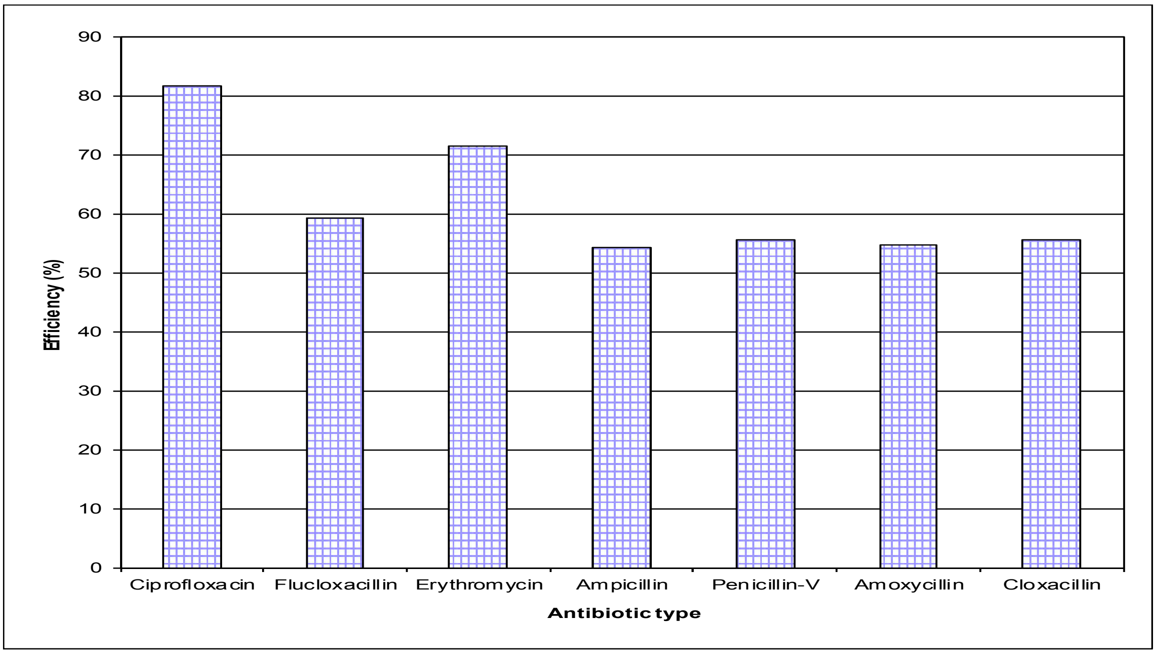

Figure 8: Antibiotic removal efficiencies for samples extracted by Methanol

These results indicate that antibiotics cannot be completely removed during various treatment processes in wastewater treatment plants as also reported by Akmehmet Balcioglu and Otker (2003). This can also be explained by the fact that antibiotics drugs normally disturb the wastewater treatment process and microbial ecology in waters. Also according to Akmehmet Balcioglu and Otker (2003) resistant bacteria may be selected by antibiotic subsistence in the anaerobic digestion process of sewage treatment plants.

3.5 Fate of Antibiotics in Wastewater Treatment Plant

Fate of antibiotics in this study was investigated by comparing the residual amount of antibiotics in the WSP and the actual antibiotic concentration in the sewage sludge as measured in the laboratory. This was carried out because according to Halling-Sorensen et al.,(1998), drugs have the potential to survive sewage treatment. Also some investigations Jacobsen and Berglind, (1988) and Samuelsen et al., (1994) have reported findings of antibiotics in sediments cores originating from medication in fish farms. The residual amount here refers to the difference between the concentration of antibiotics entering and that leaving the WSP. The aim was to investigate whether sorption to sewage sludge could be one of the environmental fates of antibiotics in wastewater treatment plants. Figures 9 and 10 shows the residual and measured concentrations of antibiotics in the sewage sludge as extracted by the two solvents, respectively.

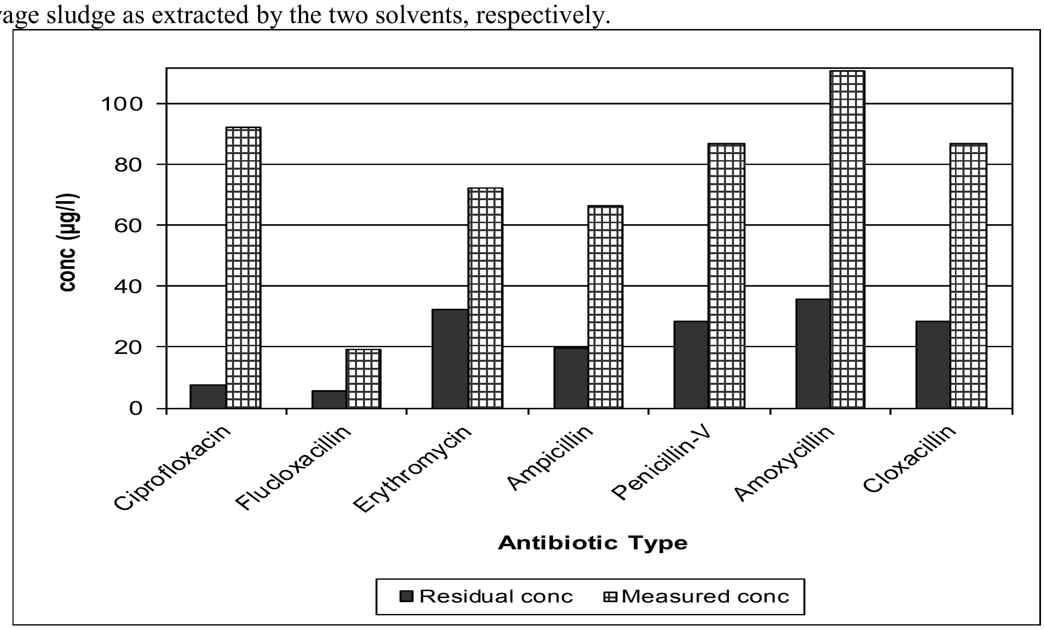

Figure 9: Residual and measured concentration of antibiotics in sewage sludge with cyclohexane used as extracting solvent

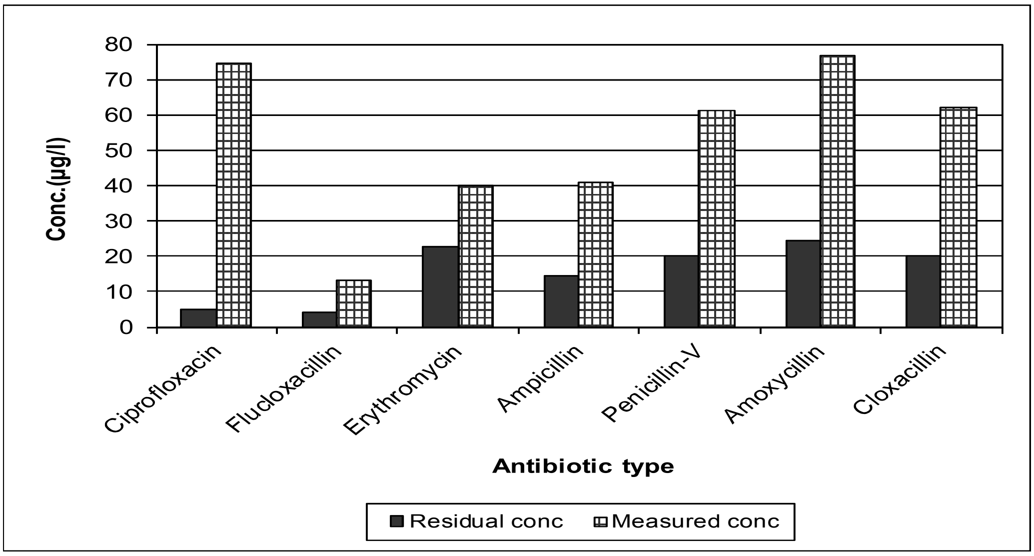

Figure 10: Residual and measured concentration of antibiotics in sewage sludge with methane used as extracting solvent

These results show higher values of measured concentration in the sediments than the residual values. The highest value (111.41 µg /L) was that for Amoxycillin, and the lowest (18.17 µg/L) was Flucloxacillin. The difference between the residual value and the actual concentration measured in the sewage sludge/sediment suggests that antibiotics are accumulated in the sewage sludge. This is also an indication that removal of antibiotics in the WSP is probably due to sorption transfer to sewage sludge. Karthikeyan and Meyer, (2006) have also reported sorption to sewage sludge to be the primary removal mechanism of some antibiotics during secondary wastewater treatment.

3.6 Variation of COD and pH with antibiotic concentration

Chemical Oxygen Demand (COD) and pH were analyzed in order to establish whether or not there was a relationship between them and the concentration of antibiotics. Figure 11 shows the variation of COD and total Antibiotic concentration at each sampling points. Both COD and Antibiotics start increasing gradually from SP1 to SP2 then increase drastically to SP3 (waste stream UHC). High concentration (1408mg/L) at SP3 for COD show that large amount of chemicals are discharged from the health center as compared to effluents concentrations from students halls of residence namely 797.33mg/L, 838.67mg/L and 384mg/L for SP1, SP2 and SP4, respectively.

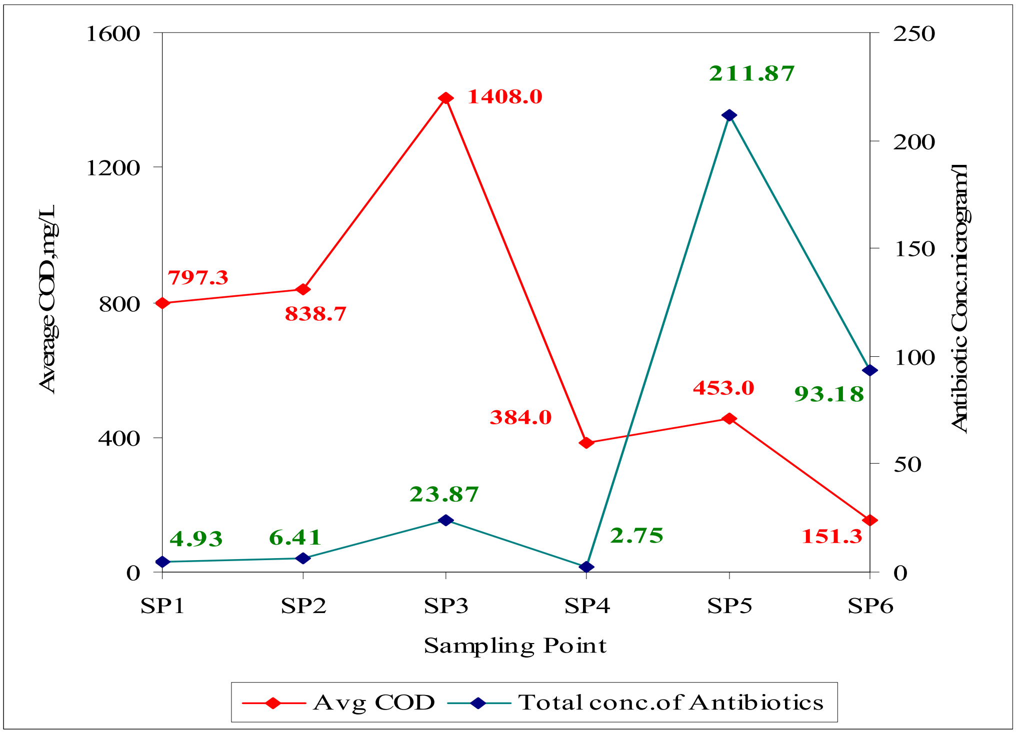

Figure 11: Variation of Total Antibiotic Concentration with COD at various Sampling Points

Figure 12 show that as the concentration of antibiotics increases, pH decrease. This indicates that presence of antibiotics in wastewater systems lowers pH. This may be due to the presence of acidic functional groups (carboxylic acid) in the structure of most of the types of antibiotics thus increasing the acidity in the wastewater system as well as in the receiving water bodies.

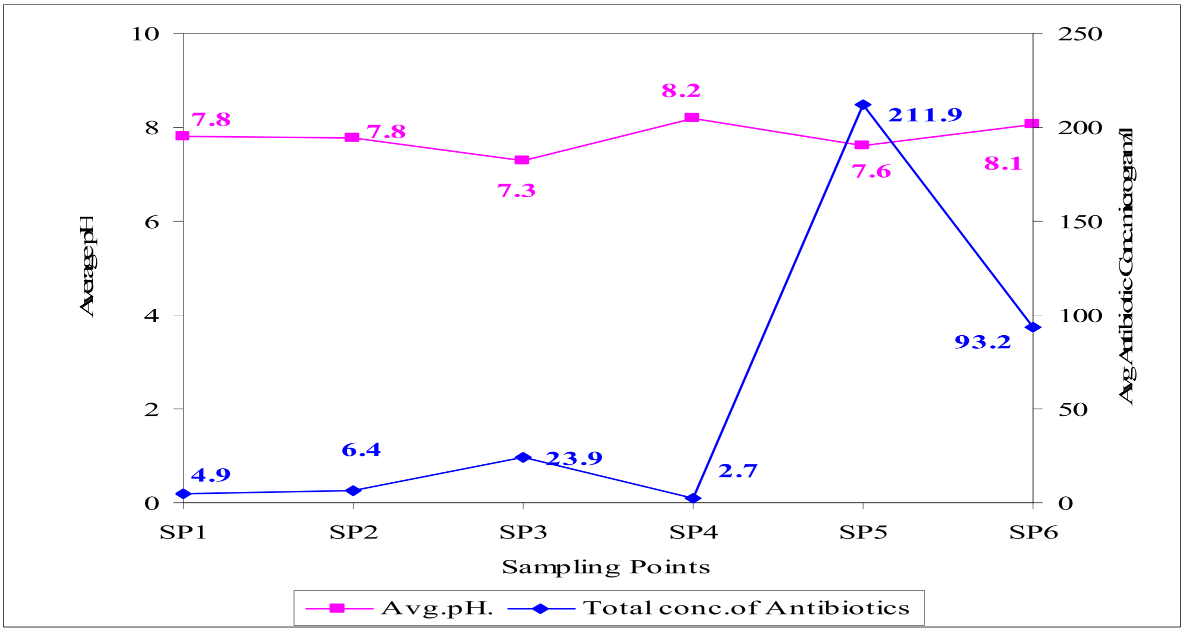

Figure.12: Variation of Total Antibiotic concentration with pH at various sampling points

4.0 Conclusion and Recommendation

Results of this study have shown that UHC is a significant contributor of the antibiotics in UDSM wastewater collection and disposal system and that about 56 to 82% individual types of Antibiotics remain in the Treatment Plant during treatment process, while the rest pass through the treatment plant to the receiving water bodies. This study has also shown that the concentration of the antibiotics in the sewage sludge is higher than that which enters the WSP. This may be due to accumulation of these organic chemicals in the sewage sludge, a phenomenon which suggest that large amount of antibiotics that enter the WWTP are removed through sorption in sewage sludge and thus sorption could be the main environmental fate of antibiotics in the wastewater system. Results obtained in this study points to the need to consider among others antibiotics removal when deciding on the wastewater treatment and disposal options.

REFERENCES

Akmehmet Balcioglu, I. & Otker, M. (2003) Treatment of pharmaceutical wastewater containing antibiotics by O3 and O3/H2O2 processes. *Chemosphere,* 50**,** 85-95.

Al-Ahmad, A., Daschner, F. D. & Kümmerer, K. (1999) Biodegradability of Cefotiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethoxazole and Inhibition of Waste Water Bacteria. *Archives of Environmental Contamination and Toxicology,* 37**,** 158-163.

Brown, K. D. (2004) *Pharmaceutically Actie Compounds in Residential and Hospital Effluent, Municipal Wastewater, and the Rio Grande in* Albuquerque, New Mexico.

Eleanor Gouws, Jennifer Bryce, Jean-Pierre Habicht, João Amaral, George Pariyo, Joanna Armstrong Schellenberg and Olivier Fontaine. Improving Antimicrobial use among Health Workers in First Level Facilities: results from the Multi-Country Evaluation of the Integrated Management of Childhood Illness strategy. *Bulletin of the World Health Organization 2004*; 82:509-515.

Halling-Sorensen, B., Nors Nielsen, S., Lanzky, P. F., Ingerslev, F., Holten Lutzhoft, H. C. & Jorgensen, S. E. (1998) Occurrence, fate and effects of pharmaceutical substances in the environment- A review. *Chemosphere,* 36**,** 357-393.

Huang, C.-H., Renew, J. E., Smeby, K. L., Pinkston, K. & Sedlak, D. L. (2001) Assessment of Potential Antibiotic Contaminants in Water and Preliminary Occurrence Analysis. *2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water.* Minneapolis, Minnesota.

Jacobsen, P. & Berglind, L. (1988) Persistence of oxytetracycline in sediments from fish farms. *Aquaculture,* 70**,** 365-370.

Karthikeyan, K. G. & Meyer, M. T. (2006) Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Science of the Total Environment,* 361**,** 196-207.

Renew, J. E. & Huang, C.-H. (2004) Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A,* 1042**,** 113-121.

Samuelsen, O. B., Lunestad, B. T., Ervik, A. & Fjelde, S. (1994) Stability of antibacterial agents in an artificial marine aquaculture sediment studied under laboratory conditions. *Aquaculture,* 126**,** 283-290.

Walter Giger, Alfredo C. Alder, Eva M. Golet, Hans-Peter E. Kohler, Christa S. McArdell, Eva Molnar, Hansrudolf Siegrist, and Marc J.-F. Suter (2003) Occurrence of Antibiotics in Wastewaters, Sewage Sludges, and Surface Waters. *Chimia* 57 (2003) 485–491© Schweizerische Chemische Gesellschaft ISSN 0009–4293