ANTIBIOTICS AS EMERGING POLLUTANTS- A CASE STUDY OF SEWAGE TREATMENT FACILITIES IN SOUTHWEST INDIA

Prabhasankar VP[1], Joshua DI[1], Iyanee FS[1], Taniyasu S[2], Tsuruta M[2,3], Shimamura K[2,3], Yamashita N[2], Kannan K[4], Guruge KS[5], Akiba M[5], Balakrishna K[1]

[1] Dept. of Civil Engineering, Manipal Institute of Technology, Manipal University, Manipal-576104,India; [2] National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-859, Japan; [3] Department of Chemical Science and Technology, Faculty of Bioscience and Applied Chemistry, Hosei University, Japan; [4] Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences, State University of New York at Albany, New York, USA; [5] National Institute of Animal Health, Kannondai 3-1-5, Tsukuba, Ibaraki 305-0856, Japan

Introduction

Pharmaceuticals and related products have become chemicals of emerging environmental concern in recent years. Antibiotics are pharmaceuticals of primary concern since they are produced in large quantities, biologically active, many of them resist biodegradation. The main source of antibiotics in wastewaters is the result of incomplete metabolism of antibiotics by humans, and discharge of unused antibiotics into water, agricultural runoff, etc. Antibiotics are discharged to water through human and animal excretion (veterinary drugs) and direct disposal. Pharmaceuticals have been frequently detected in sewage treatment plant (STP) effluents [1, 3], since STPs are not designed to remove them. Thus, waste water treatment plants (WWTPs) can be considered an important source of pharmaceuticals in the environment.

No studies have been done in India related to the presence of antibiotics in the waste water treatment facilities. In this study, occurrence and removal of four selected antibiotics are investigated at three sewage treatment plants (STPs) in Manipal (STP 1, STP 2, STP 3) and one STP in Udupi (STP 4), India(Table 1). The antibiotics selected for this study are Sulphamethoxazole (SMX), Trimethoprim (TMP), Chloramphenicol (CAP) and Erythromycin (ERY). The wastewater samples are taken from the inlet and outlet of the STPs. Samples are collected during pre-monsoon, monsoon and post-monsoon seasons to understand seasonal influences in concentrations and removal efficiencies of antibiotics.

Materials and methods

Oasis®hydrophilic-lipophilic balanced (HLB) cartridges used for the solid phase extraction are purchased from Waters (Milford, MA, USA). Milli-Q water is used in this study. Disodium ethylene diaminetetraacetate (Na2EDTA), Sulphamethoxazole (SMX), Trimethoprim (TMP), Chloramphenicol (CAP) and Erythromycin (ERY) are purchased from Wako Pure Chemical Industries Ltd. (Japan).

A multi-step procedure is involved in sample extraction. The procedure starts with pre-conditioning of the sorbent with 5 ml of methanol followed by 5 ml of Milli-Q water. Then the sample is loaded onto the HLB cartridges with a flow rate of 3ml/min and the target analytes were retained on the cartridges. The cartridges then are washed with 5 ml of Milli-Q water. Centrifugation is done at 2000 rpm for 2 min for the removal of residual water present in the cartridges. Elution is done with 5 ml of methanol. Methanol is then evaporated using nitrogen gas at 35°C to near dryness and reconstituted with 1 ml with 25% methanol and is transferred to an auto sampler vial for instrument analysis.

Concentrations of antibiotics in samples were determined using high performance liquid chromatography with tandem mass spectrometry (HPLC-MSMS). The mobile phase used was 2 mM ammonium acetate in methanol and methanol.

The recoveries of target chemicals through the analytical procedure were found to be between 70% and 100% for SMX, TMP and CAP and 60% for ERY. The procedural blank values for pre-monsoon, monsoon, and post-monsoon samples were less than LOQ.

Table 1. Information on the sewage treatment plants studied

STP No.	Location and capacity	latitude/longitude	source of sewage	final discharge	collection point
STP1 [a]	Manipal 2000 KLD[e]	13^{0}22'65.60"N 74^{0}47'17.22"E	hospital and domestic waste water	inland irrigation	influent
					effluent
STP2 [b]	Manipal 1500 KLD	13^{0}22'04.20"N 74^{0}47'18.26"E	hospital and domestic waste water	inland irrigation	influent
					effluent
STP3 [c]	Manipal 2000 KLD	13^{0}20'25.48"N 74^{0}47'44.22"E	domestic waste water	inland irrigation	influent
					effluent
STP4 [d]	Udupi 50 KLD	13^{0}19'57.58"N 74^{0}44'48.26"E	hospital waste water	underground drainage	influent
					effluent

a. Primary treatment includes bar screens followed by equalization for different batches. Surface aeration for aerobic degradation followed by settling in clarifier. Tertiary treatment is done by pressure sand filter and activated carbon filter
b. Tertiary treatment is done by pressure sand filter. Other treatment as same as above.
c. Same to *a.*
d. Tertiary treatment is done by Dual media filter. Other treatment as same as above.
e. Kilolitre per day

Results and discussion

SMX was the predominant compound among all antibiotics analyzed and the highest concentration was observed in STP 1 effluent at 3130 ng/L in monsoon season (Fig 3). Also, the highest concentrations of TMP and ERY were observed in STP 1 effluent with 1050 ng/L and 133 ng/L, respectively. Concentration of antibiotics in monsoon season is high compared to the pre-monsoon(Fig 2) and post-monsoon seasons(Fig1).The higher concentrations in monsoon could be due to the increasing frequency of drug usage on account of sickness due to various water-borne diseases. The CAP concentration was low among all the antibiotics analyzed. The STP 1 and STP 2 showed higher concentrations of antibiotics compared to the other two STPs. STP 3 showed low concentration as it was not receiving any hospital waste. The low concentration for ERY may be because of the degradation of ERY at a faster rate or the limitation of the method for finding out the concentration of ERY.

The removal of antibiotics in STPs was highly variable. The difference between elimination rates of antibiotics in various STPs probably results from many factors, such as the type of the treatment process, the hydraulic retention time (HRT), solids retention time [2], temperature and even the rainwater input [10].

SMX, TMP and CAP sometimes showed an increase in the concentration in the outlet, whose possible explanation could be because of the deconjugation of the conjugated metabolites [8]. Elsewhere higher effluent concentrations of TMP and doxycycline in the outlets were reported in STPs in Sweden [7]. In STPs of lower Tyne catchment, United Kingdom ERY and TMP concentrations were found to be greater in effluents than in influents[9].

A comparison of antibiotics in different Countries in WWTPs

The maximum concentration of the target compounds in different countries has been taken from the literatures and a comparison is made with Indian samples (Fig 4). The maximum concentration of SMX is found in India compared to all other countries. The drug usage in India may be more than other countries or the TMP removal is not good in treatment plants in India. TMP is highest in Mexico[6] followed by US[5] and India.CAP shows very less concentration in all countries. Korea[4] and China[11] shows less concentration of target compounds in the effluent compared to other countries.

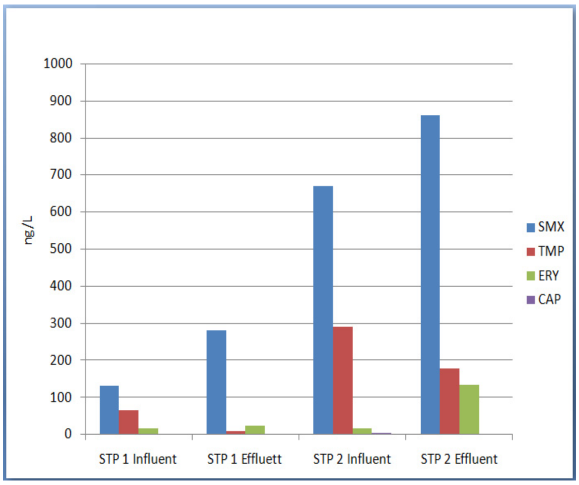

Fig 1. Concentration of antibiotics in WWTPs in post monsoon season

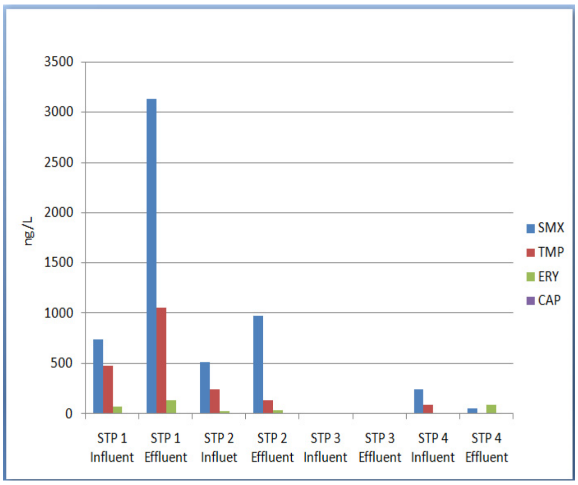

Fig 3. Concentration of antibiotics in WWTPs in monsoon season

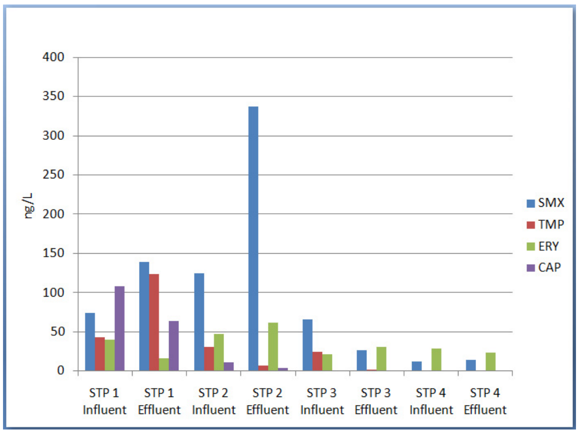

Fig 2. Concentration of antibiotics in WWTPs in pre monsoon season

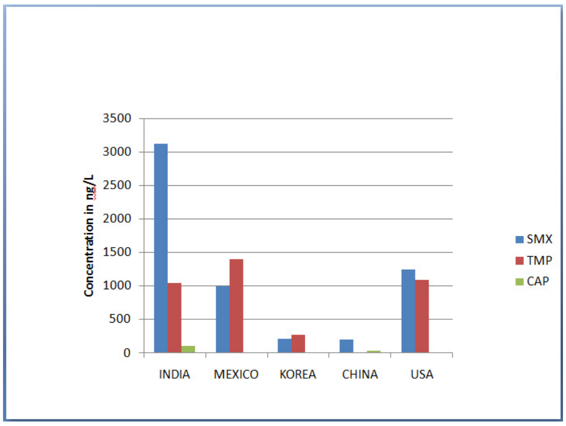

Fig 4. Comparison of concentration of antibiotics reported in different countries in WWTPs

Acknowledgements

This study was partly supported by KAKENHI (project no. 240629 and 24256004) of the Japan Society for the Promotion of Science (JSPS).Analytical facilities were provided at the Department of Civil Engineering, Manipal Institute of Technology, Manipal, India and National Institute of Advanced Science and Technology (AIST), Tsukuba, Japan. We thank to Prof. Murano (Hosei University) for his kind support.

References

1.Ashton, D., Hilton, M., Thomas, K.V.(2004);*Sci. Total Environ.* 333 (1–3):167–184

2.Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H.(2005); *Water Res.* 39 (1):97–106

3.Daughton, C.G., Ternes, T.A.(1999);*Environ. Health Perspect*.107 (6): 907–938

4.Kim.J.W, Yoon.S.M, Lee.S.J, Narumiya.M, Nakada.N, Han.I.S,Tanaka.H, InternationalConférence on Environment and Industrial Innovation, Singapore,2012,57-61.

5. Karthikeyan.K.G, Michael T. Meyer.(2006);*Sci. Total Environ*.361 (1-2): 196–207.

6.Kathryn D. Brown, Jerzy Kulis, Bruce Thomson, Timothy H. Chapman, Douglas B. Mawhinney.(2006);*Sci. Total Environ*.366:772–783.

7.Lindberg, R.H., Wennberg, P., Johansson, M.I.(2005);*Environ. Sci. Technol*. 39 (10):3421–3429

8. Miao, X.S., Koenig, B.G., Metcalfe, C.D.(2002);*J. Chromatogr. A*952 (1–2): 139–147.

9. Roberts, P.H., Thomas, K.V.(2006);*Sci. Total Environ*. 356 (1–3):143–153.
10.Tauxe-Wuersch, A., De Alencastro, L.F., Grandjean, D, Tarradellas, J.(2005);*Water Res*.39 :1761–1772.
11.Weihai Xua,b,d, Gan Zhang, Xiangdong Li, Shichun Zou, Ping Li, Zhaohui Hu, Jun Li,(2007); *Water Res*.41: 4526 – 4534.