PRELIMINARY STUDIES ON TEMPORAL VARIATIONS OF ANTIBIOTICS IN SEWAGE TREATMENT PLANTS IN SOUTH INDIA

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Introduction:

Pharmaceutical compounds are referred as an emerging contaminant in water and is detected in various water bodies all over the world [1]. The concentrations of the pharmaceuticals can vary from different areas and countries depending on the usage pattern [2]. The contamination of pharmaceuticals in aquatic environment is due to its large consumption in daily life and their ultimate release from urine and feces to the waste water. These pharmaceuticals after the intake by humans and animals are converted into metabolites and excreted as unchanged. Disposal of unused drugs into water sinks and household wastes are another major contributors to pharmaceuticals in wastewater and landfill leachate [3]. The passage of these pollutants is mainly through the sewage network to wastewater treatment plants (WTPs) and reaches to the surface water. Wastewater treatment plants play a major role in collecting and treating wastewater received from hospitals, households, and industries [4]. The removal efficiency in a WTP can be influenced by the mode of treatment and compound specific properties [5]. However, there is no specifically designed unit for the removal of pharmaceuticals as yet. The concentrations of the pharmaceuticals can vary from region to region and countries. Consequently, antibiotic resistance is spreading extensively because of the high usage of antimicrobial agents. In Indian environment, the WTPs receive antibiotic resistant bacteria directly from the hospitals and discharged to the surface waters or for irrigation [6]. Only 31% of the wastewater generated is treated in India [7]. The present study has carried out to study the occurrence of four selected antibiotics in five sewage treatment plants in South India and to investigate the magnitude of hourly and daily fluctuations of the selected compounds. The selected antibiotics are sulfamethoxazole (SMX), erythromycin (ERY), ofloxacin (OFX), norfloxacin (NOR). A total of 90 samples is collected in five sewage treatment plants (STPs) in three alternative days in a week.

Materials and methods:

Study area: The study was carried out at five STPs from the state of Karnataka. **STP 1** and **STP 2** with a capacity of 2000 and 1500m^3 per day treat wastewater from both domestic and hospital. **STP 3** with a capacity of 2000m^3 treat wastewater from only domestic. **STP 4** with the capacity of 50m^3 treat wastewater exclusively from the hospital. **STP 5** with the capacity of 43.5 MLD treat wastewater from both domestic and hospital.

Sample Collection: Sampling was carried at five wastewater treatment plants in three-time intervals of a day (8:00 am, 1:00 pm and 7:00 pm) for three alternative days from inlet and outlet by grab sampling. Samples are collected in a prewashed polypropylene bottles and measured for physiochemical properties and stored in -20^{o}c until the analysis.

Reagents and standards: Methanol, acetone, EDTA 99.5% Ammonia 5% and Ethyl acetate were purchased

from theWako pure chemical industries ltd). Commercial standards (10mg/l) for Ofloxacin (OFX) Norfloxacin (NOR) Sulfamethoxazole (SMX) and Erythromycin (ERY) were purchased from Kanto Kagaku, Japan. SPE extraction cartridges: Oasis HLB 6cc 150mg (water milford, MA).

Instrument analysis: The analysis was carried out using liquid chromatography coupled with mass spectrometry (LC-MS/MS) system with a mobile phase of Acetonitrile and formic acid. Calibration of three concentrations (0.1ng/ml, 10ng/ml, and 100ng/ml) with a linearity of (r^2 >**0.995)** was established for the quantification of analytes in the samples.

Methodology of SPE extraction for water samples:

Sample Preparation: SPE extraction procedure of wastewater was described by the following methods [8] with some modifications. 250ml of the sample was filtered with Grade GD 1um Whatman filter paper. Before the analysis, the samples are extracted through SPE cartridges by preconditioning with 6ml of methanol followed by 6ml of Milli-Q water, after conditioning the samples were passed through the cartridges with the flow rate of 3ml/min using vacuum. After passing the whole sample the cartridge was washed with 6ml of Milli-Q water, then the cartridge was centrifuged at 2000RPM for 2 min for removing the water particles in the cartridge and dried it for 15 min under the vacuum. The elution was carried out by 6ml of methanol as fraction 1. For the retained compounds in the sample the cartridges are eluted with 3ml of 2:2:1 acetone : methanol : ethyl acetate as fraction 2 and 3ml of 5% ammonia was collected as a fraction 3. Finally, all the three fractions are combined and concentrated until 1ml under the gentle stream of nitrogen at 40°C using turbovap evaporator (EYELA MG 2200). The concentrated samples are filtered with 0.45µm sterile syringe filter and transferred to the amber colored 2ml vials and stored at -20°C before the analysis.

Results and Discussion:

The maximum and minimum concentrations of the antibiotics in all the water samples are as follows: OFX: 9674.4ng/l and 5.6ng/l NOR: 3501.6 ng/l and 4.8ng/l, SFX: 5206.8ng/l and 3.2ng/l, ERY: 33.2ng/l and 2ng/l. The concentration of OFX is high, and ERY is very low compared to the other antibiotics in all the STPs. The fluctuations in the concentrations were observed in three-time intervals as well as day to day basis.

In Fig 1: Day 1: STP 1 and STP 4 shows higher concentrations where as STP 2, STP 3 and STP 5 are showing lower concentrations of the selected compounds except OFX: 6328ng/l and NOR: 1148ng/l in inlets of STP 5. In day 2 the concentrations of the selected componds were observed lesser than day 1 and day 3 except OFX: 9674 ng/l observed in STP 4 at 8 AM inlet.In day 3: STP 2 the concentrations of selected compounds are lesser than other STPs of day 1 and day 2. OFX: 7007ng/l was observed at 7 pm inlet of STP 4.

The observed concentrations of the selected antibiotics were in the order of OFX > SMX > NOR > ERY in the selected STPs. Most of the studies are carried out to understand the occurrence, seasonal variations of the antibiotics. However, the current study is more challenging to understand the distribution of the selected antibiotics. The results of hourly and timely studies can help in optimizing the conditions and removal strategies of sewage treatment plants for the selected antibiotics. These compounds could pose various adverse effects towards the microorganisms which are present in the treatment process.

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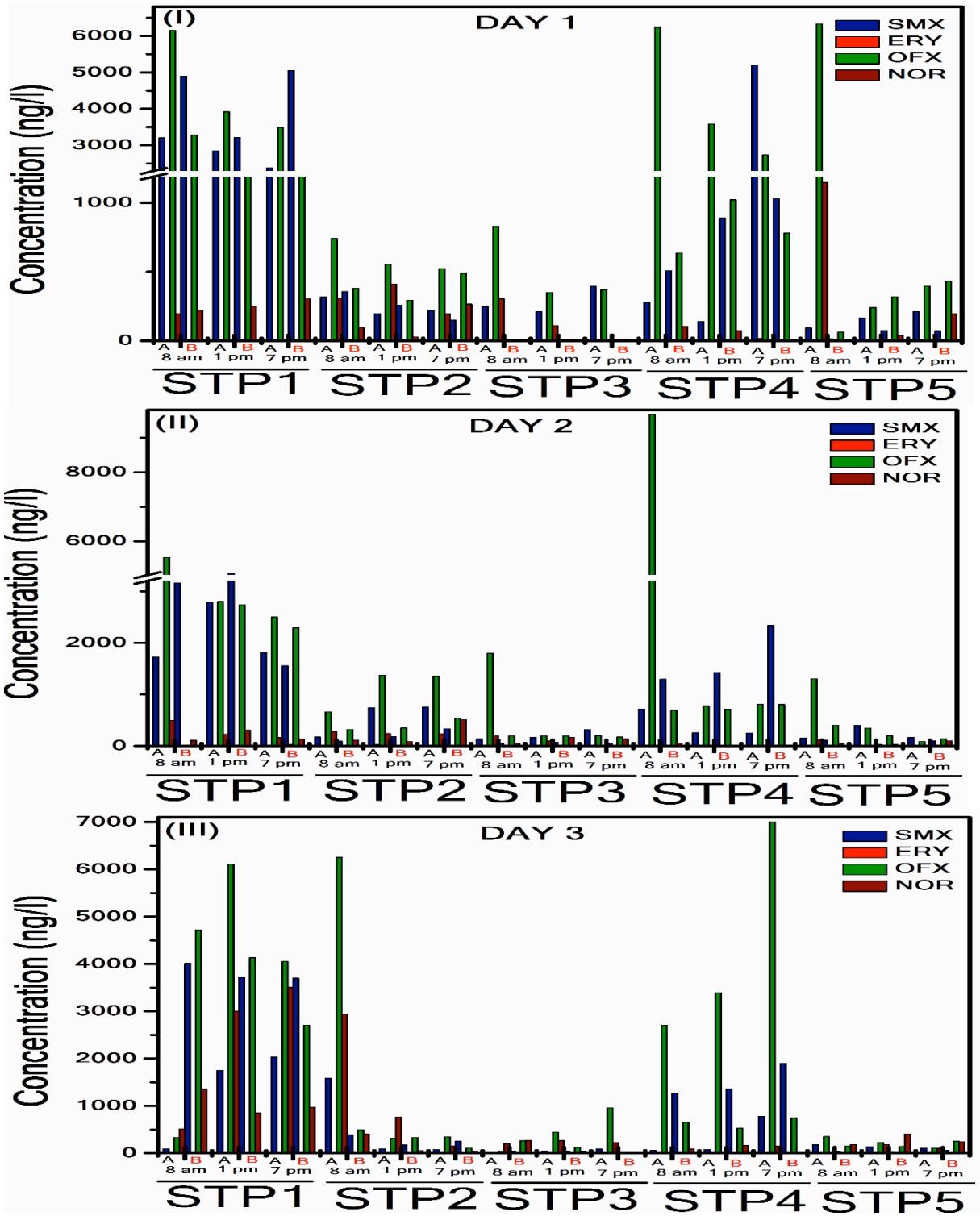

Fig.1. (I) Concentrations antibiotics of Day 1; (II) Concentrations antibiotics of Day 2; (III) Concentrations antibiotics of Day 3; at three-time intervals in five STPs; A: inlet; B: outlet SMX: Sulfamethoxazole; ERY: Erythromycin; OFX: Ofloxacin; NOR: Norfloxacin