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Occurrences and fate of an antibiotic amoxicillin in extended aeration-based sewage treatment plant in Delhi, India: a case study of emerging pollutant

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ABSTRACT

In recent years, pharmaceutical and personal care products (PPCPs) have been detected in various environmental matrices, including ground water, surface water, and municipal wastewater. In order to evaluate the impact of PPCPs on environment, their distribution must be accurately established in these matrices first, as very limited studies have been carried out especially in the Indian subcontinent. In this study, the occurrence and removal of an antibiotic, Amoxicillin, at a sewage treatment plant (STP) located in Delhi has been studied. Amoxicillin was selected for its widespread use as a prescribed over the counter drug.

Sampling exercises were carried out over a period of six months so as to cover seasonal variations. Sewage samples were collected from the influents and effluents of STP to determine the occurrences and removal of the amoxicillin. Concentration of amoxicillin in the untreated raw sewage at the STP varied from "not detected" (ND) to 172.6 $ng\,L^{-1}$. After treatment, it varied from ND to 62.5 $ng\,L^{-1}$. Average removal efficiency was 49.7%. Presently, there is great focus on the reuse of the treated sewage. So, findings of the study can help to build a better understanding of reuse options for treated effluents and preparation of appropriate water resources management plans.

Keywords: PPCPs; Antibiotics; Amoxicillin; STP

1. Introduction

Publication of *Silent Spring* [1] has attracted the civil society's attention to and concern about chemical pollutants in the environment. Since then, the impact of chemical pollution has focused almost exclusively on persistent organic pollutants. The new category of chemicals that has currently captured the attention of both the scientific community and the public is the pharmaceutical and personal care products (PPCPs). Their occurrence, behavior, and biological impact have become a topical issue which, in turn, has given rise to a new set of water quality issues. Vast ranges of pharmaceuticals, including β-blockers, analgesics, endocrine disruptors, hormones, and antibiotics, have been detected in effluents from municipal wastewater treatment plants (WWTPs) [2,3]. Worldwide data indicate that antibiotics are used indiscriminately, and much of the antibiotics used in humans and animals are not metabolized, thereby adding a significant amount to the environment via excretion. Excessive

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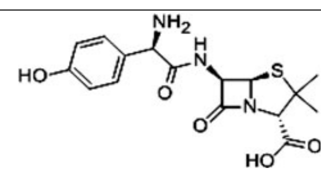
use of antibiotics has resulted in water resource contamination. Now, antibiotic's residues are being detected in different water matrices, including surface water [4], hospital wastewater [5–7], WWTP effluents [5,8], WWTP biosolids [9], ground water [10], and drinking water [11]. The β-lactum antibiotics are reported to account for over 65% of the global antibiotic market [12]. In β-lactum group, amoxicillin is the most widely used antibiotic drug [13], whose widespread occurrence in the aquatic environment including wastewater, has been reported. Important physicochemical properties of amoxicillin are given in Table 1. During its metabolization, it is excreted 80–90% unchanged from human body [14]. With its high recommended daily dose (750–2,250 mg day^{-1}) and its resistance to changes in forms during excretion, amoxicillin is expected to be in greater proportion than other PPCPs in the municipal wastewater, in terms of both levels and frequency. The reported concentration of amoxicillin in sewage varied from not detected (ND) to few μg L^{-1} in different parts of the world (Table 2). Most of these reports on the occurrences of amoxicillin in the treated municipal effluents are from Europe, USA, Japan, and China; hardly, any data are available from Indian subcontinent. Recently, Diwan et al. [7] have found amoxicillin level to be below detection limit in a small hospital wastewater from Ujjain city of India. In the this study, we have focused on a sewage treatment plant (STP) in Delhi, so as to understand the distribution of antibiotics in the sewage and its fate during the sewage treatment.

Table 2
Reported amoxicillin concentration in various STPs

Location	Concentration (ng L^{-1})		References
	Influent	Effluent	
Kumamoto city, Japan	100–3,000	ND	[6]
Queensland, Australia	6,940	50	[21]
Queensland, Australia	280	30	[8]
Brisbane, Australia	317	38	[24]
New York, USA	367	ND	[25]
Varese and Torino, Italy	NM	4.7	[26]
Cagliari and Roma, Italy	NM	120	[26]
Cosenza, Italy	NM	7.40	[26]
Napoli, Italy	NM	15.20	[26]
Wan Chai (E & W) Hong Kong, China	NM	1,660	[27]
Central Hong Kong, China	NM	1,300	[27]
Tai Pao, Hong Kong	NM	66	[27]
Milan, Italy	NM	120	[42]

ND = not detected.
NM = not measured.

Table 1
Important physicochemical properties of amoxicillin

Amoxicillin ($C_{16}H_{19}N_3O_5S$)			
Molecular weight	365.4		
CAS No	26787-78-0		
Solubility	4,000 mg L^{-1}		
pKa_1	2.8		
pKa_2	7.2		
Log K_{OW}	0.87		
K_{OC}	865.5		
Vapor pressure	4.69 E−14 (mmHg)		
Half life in environment	30% degradation after 3 months in laying hen feces		
	34% degradation after eight day broiler feces		
Toxicity (acute) (mg L^{-1})	*Microcystic aeruginosa*	Growth EC_{50}	0.0037
	Oncorhynchus mykiss	Hepatocyte toxicity 24 h (hour) EC_{50}	182.7
	Lemna gibba	Wet weight, chlorophyll seven day LOEC	1
	Vibrio fischeri	IC_{50} 5 min	1,320
	Daphnia magna	EC_{50} 24 h	>1,000
	Moina macrocopa	EC_{50} 24 h	>1,000
	Oryzias latipes	EC_{50} 24 h	>1,000

The properties of amoxicillin have been gleaned from the available literature [34,37–41].

2. Materials and methods

2.1. Sampling location and sample collection

Samples were taken from Vasantkunj STP located in south Delhi representing moderate to high-income group society. The STP is well connected by a sewer network which collects the sewage and transport to the STP. It has an installed capacity of 14 MLD (millions of liters per day) and operates at around 67% of its installed capacity. This STP is based on the extended aeration process; treatment scheme consist of a pre-treatment for the separation of floating matter and grit, an extended aeration tank with surface aerators for biological treatment, a final clarifier for separation of biomass and sludge drying bed for sludge stabilization. Biochemical oxygen demand (BOD), chemical oxygen demand (COD) and turbidity removal were 99.1, 96.6, and 99.5%, respectively [15], making it one of the most well-maintained STPs of Delhi. The operational characteristics of the plant studied are given in Table 3.

Grab samples were taken from the inlet and outlet of the STP representing the raw and treated sewage. Samples were collected around 10 o'clock in the morning. Five sampling exercises were carried out during the entire study period (October–March) and simultaneously two replicated samples from each sampling point were collected. Samples were collected in 2 L of amber-colored, food grade, and virgin plastic bottles. Before sampling, the bottles were washed with tap water and distilled water, and rinsed twice with the sample water. A standard protocol described by American Public Health Association [16] was followed to collect wastewater samples for organic analysis. The collected sample bottles were kept in airtight large plastic ice-cold containers and were transported to the laboratory within one hour of their collection and stored in a refrigerator at 4˚C until further analysis. Extraction of samples was completed on the same day as sampling was.

Table 3
Operational characteristics of Vasantkunj STP

Parameters	Value
Flow (MLD)	14
Hydraulic retention time in the biological process (h)	30
Final clarifier (h)	3.3
MLSS (mg L^{-1})	3,500–3,800
SVI (mL g^{-1})	185
SRT (days)	12–15
F/M	0.08–0.12

2.2. Sample preparation

2.2.1. Extraction of amoxicillin residue

Samples were vacuum-filtered through 0.7 μm glass fiber filter (Millipore, USA) and solid phase extraction (SPE) was performed. The SPE procedure was applied to the wastewater samples using commercial Oasis HLB (divinylbenzene/N-vinylpyrrolidone copolymer) cartridges (200 mg, 6 cc) from Waters (Mildford, MA, USA). A vacuum manifold assembly (Mildford, MA, USA) fitted with an external pump from Gast (Gast, USA) was used for this purpose. The SPE cartridge were pre-conditioned with 5 mL of ethyl acetate, 5 mL of methanol and 5 mL of milli-Q (liquid chromatography) LC-grade water (pH 3) at a flow rate of 1 mL min^{-1}. Wastewater samples (500 mL) were loaded at a flow rate of 5–8 mL min^{-1} followed by a washing step with 5 mL of distilled water. After that, the cartridges were dried by nitrogen stream for approximately 15 min and finally eluted with 3×3 mL of ethyl acetate at 1 mL min^{-1}. The extracts were initially concentrated in Rotavac (Buchi, Switzerland) and finally evaporated to dryness by a gentle nitrogen stream. The residues were re-dissolved in 500 μL methanol for high pressure liquid chromatography (HPLC) analysis.

2.2.2. Reagents and standards

Analytical grade (AR) chemicals (Merck, Darmstadt, Germany) were used throughout the study without any further purification. To prepare all the reagents and calibration standards, milli-Q water and methanol were used. The glassware were washed with dilute nitric acid (1.15 N) followed by several portions to distilled water. Amoxicillin standard (Fluka Biochemika 10039) was procured from Sigma–Aldrich, USA. The working standards of amoxicillin were prepared by dissolving the suitable amount of amoxicillin in methanol.

2.2.3. Chromatographic conditions

Chromatographic separation of amoxicillin was performed on Waters Spherisorb® ODS-2 (250 4.6 mm, 5 μm) HPLC column. Analyses were performed at flow rate of 1.5 mL min^{-1} at the ambient temperature. An isocratic program was used with the mobile phase, containing buffer A (0.45% Na_2HPO_4 in water) and B (MeOH) in the fixed ratio of 2:1. The injection volume was 20 μL and compound eluted at 4.8 + 0.1 min. The photo diode array detector was used for detection and chromatograms were extracted at 228 nm for amoxicillin. Quantification was performed by using external calibration and peak area measurements.

2.2.4. Limit of quantification (LOQ) and recovery

For the concentration to be accepted as limit of quantification (LOQ), the % deviation from the nominal concentration (accuracy) must be ±20%, and the relative standard deviation should be less than 20%. The relative analytical recovery for the method was determined by spiking five different mass of amoxicillin (1 ng–1 μg) to 500 mL of double-distilled water. The spiked samples were extracted by the same SPE method and the residues were re-dissolved in 500 μL methanol. The relative recovery of amoxicillin was calculated by comparing the peak areas for extracted amoxicillin from spiked water and a standard solution of amoxicillin in de-ionized water.

Table 4
Relative recovery of amoxicillin from spiked water samples

Amoxicillin spiked concentration (ng)	Amoxicillin concentration recovered (ng)	Recovery (%)
1	0.64	64.20
20	15.48	77.41
50	39.48	78.96
100	87.79	87.79
1,000	810.97	81.01
Mean recovery		77.87 + 8.61

3. Results and discussion

3.1. Sewage generation in Delhi: potential source of antibiotics

Delhi, the National Capital Territory, is spread over a total area of 1,483 km^2, of which more than 60% is urbanized with a population of 16.8 million (Census 2011, www.censusofindia.gov.in). The custodian agency for water and sewage management in the city is Delhi Jal Board (DJB). DJB produces and distributes around 3,600 MLD of drinking water, around 3,059 MLD of sewage is generated, and have a capacity to treat around 2,780 MLD sewage [17]. The treated effluents from STPs are either reused for the purpose of horticulture/urban landscaping or disposed of in various drains which finally flow into river Yamuna. Presently, only 34% of the total sewage produced is being reused in Delhi, as 33% of the total wastewater produced is used for irrigation purposes, and 1% for industrial purposes [15]. Thus, there is a huge scope of increasing water reuse in the city. It is also planned to treat the sewage to tertiary level for potable reuse as new source of water. (*The Times of India*, March 24, 2012). Presently, most of the STPs treat sewage to the secondary level, so the quality of the treated effluent becomes very essential for safe and acceptable public reuse of wastewater as it could contain the trace levels of the emerging contaminants (PPCPs). The present study focuses on one such compound, amoxicillin, the most used antibiotic world over.

3.2. Measurement of amoxicillin in the influent and the effluent

For amoxicillin standards the calibration curve was linear over the range of 1–1,000 ng mL^{-1} solutions. The correlation coefficient (r^2) obtained for these amoxicillin standards was 0.998. For each point of calibration standard, the concentrations were recalculated from the equation of the linear regression curves. The relative analytical recoveries of amoxicillin was evaluated by spiking five different masses of amoxicillin, that is, 1, 20, 50, 100 and 1,000 ng considering the low, median and high quality control QC concentrations. The recoveries for the method varies from 64 to 88% with the average recovery of 77.87 ± 8.61% ($n = 5$) (Table 4). The LOQ and limit of detection for this method were 10 and 1 ng L^{-1}, respectively. Recovery tests were carried out by extracting the analytes at the QC concentrations from de-ionized water. The average recovery efficiency (77.87%) was found to be much better than that reported (41%) previously by Kasprzyk-Hordern and others [18], but lesser than that reported (88%) by Benito-Pena and others [19]. The higher recovery by Benito-Pena and others [19] may have been due to the use of Oasis MAX SPE cartridge at neutral pH because penicillins are in their basic form and can be retained in the mixed-sorbent phase by anionic exchange and hydrophobic interactions [20].

In HPLC analysis, quantification was performed by comparing the area of chromatogram of spiked de-ionized water sample to that of wastewater sample at same retention time. Unspiked de-ionized water was used as a blank sample. The levels of amoxicillin

Table 5
Amoxicillin concentration in wastewater samples collected from Vasantkunj STP

Sampling	Ranges (ng L^{-1})	
	Influents	Effluents
S1	61.4–65.0	53.1–58.0
S2	88.2–140.8	ND–62.5
S3	53.9–98.1	22.9–23.3
S4	ND–36.7	ND
S5	64.4–172.6	23.0–52.1

detected during the entire sampling campaign are shown in Table 5. Amoxicillin concentrations in the influent and effluent of the STP were found to be in the ranges of ND–172.6 and ND–62.5 $ng\,L^{-1}$, respectively. The detected influent levels were lower than 200 $ng\,L^{-1}$ in all cases, but we should wisely conclude from the findings, as there could be very high annual variation in the amoxicillin concentration in the same STP. For instance, Watkinson and others [21] found the levels of amoxicillin (6,940 $ng\,L^{-1}$) in Australian STP in 2009 to be 30–35 times higher than those in 2007. The minimum and maximum concentrations detected in the influents were respectively 36.7 and 172.6 $ng\,L^{-1}$ which were reported in the S4 and S5 sampling campaigns respectively. The antibiotic concentration in treated effluent ranged from ND to 62.5 $ng\,L^{-1}$. The maximum concentration in treated effluent was measured in S2, while the minimum concentration was recorded in S4 wherein none of the duplicate samples were found positive for amoxicillin residues. Sampling S4 showed the lowest levels of amoxicillin during the entire sampling campaign.

Low F/M ratio (Table 3) in extended aeration imparts good settling characteristics to the biomass in the final clarifier, as the mixed liquor suspended solids (MLSS) are in endogenous phase of growth, thereby responsible for better removal efficiencies. Sorption in the biomass is considered a major mechanism of removal of antibiotics in STP [22,23] and high MLSS in extended aeration ensures have better removal of antibiotics. On an average, during the entire sampling period, 49.7% of amoxicillin was removed. Low Kow value (0.84) for amoxicillin makes it difficult to get sorbed on to the biomass even at high MLSS in STP. Thus, a significant concentration of this compound was present in the treated effluents. Similar ranges of amoxicillin in the domestic wastewater have been reported from Australia [8,24], USA [25], and Italy [26] (Table 2). However, Minh and others [27] reported very high levels of amoxicillin (1,300–1,660 $ng\,L^{-1}$) in the treated effluents of Hong Kong, clearly indicating the unsuitability of the current wastewater treatment units for the complete removal of antibiotics. An STP in Hong Kong has been reported to have 15–20 times higher than normal concentration of amoxicillin. Such high levels and high frequency of detection of amoxicillin residues in sewage from the STP in Hong Kong indicated the presence of heavy loads of β-lactum antibiotic. This highlights its considerable local use. As per the Hospital Authority of the Hong Kong, amoxicillin consumption in 2007 in public hospitals in Hong Kong was 3,998 kg and thus high levels of amoxicillin residues could find their way into wastewater [27]. A Student's *t*-test was applied for the influent and effluent amoxicillin levels to understand the difference in various sampling campaigns and the role of STPs in amoxicillin removal. The statistical test did not show any statistically different concentrations of the amoxicillin ($p > 0.05$) in the sewage during different sampling campaigns. However, amoxicillin levels in the influent and effluent were statistically different ($p < 0.05$). This indicates that STPs play a critical role in the removal of emerging molecules too, but incomplete removal of emerging molecules in STPs leads to the entry of contaminated water in freshwater systems (river, lakes, ponds, and groundwater) which may cause severe ecological and health hazards to the environment.

β-lactum antibiotics have been reported to produce low to moderate toxicity (acute toxicity) in test organisms (Table 1), but chronic exposure to these compounds always gives rise to antibiotic-resistant bacteria. These trace environmental concentrations of the antibiotics brings about significant irreversible changes in the ecological systems as very low concentrations of β-lactum found in STPs are responsible for producing antibiotic resistance in bacteria [28]. The occurrence of drugs in the environment gives rise to various still unanswered questions with regard to their biological potency and impact on flora, fauna, and humans, that is, their endocrine-disruption activity and related adverse effects. Lemus et al. [29] found the high concentration of various antibiotics, including amoxicillin, in vultures and correlated this with their increased mortality. The other mode of antibiotic exposure for vultures could be antibiotic residues in poultry meat and animal tissues [30,31]. Kim et al. [32] and Oetkein et al. [33] reported death and decline in the reproduction of standard test organisms like *Vibrio fischeri*, *Daphnia magna*, *Moina macrocopa*, *Oryzias latipes* and some invertebrates. Kummerer [34] reported other environmental effects like development of various resistant bacterial strains due to the discharge of antibiotics in the drains. Reinthaler et al. [35] found in three Austrian STPs that the resistance rate in *Escherichia coli* was the highest in an STP receiving municipal sewage. But no amoxicillin-resistant *E. coli* was detected in the hospital effluents from India [5]. This sensitivity of *E. coli* to amoxicillin could be attributed to the non-detection of amoxicillin in hospital effluents. Thus, the effluent samples, in which amoxicillin is detected, should also be checked for antibiotic-resistant strain of bacteria. The new resistant strain has the potential to spread diseases, threaten native species and disturb the ecological balance. Thus, it becomes quite necessary to put a check on the points of entry of antibiotics into the aqua matrix, mainly through the effluent of STP, hospital discharge and emissions from medical care units with drugs manufacturing industries in order to make the water reuse policy publically accepted.

4. Conclusion

The presence of pharmaceutical compounds in our environment is undeniable, and their potential to contaminate the water supply, affect human health and ecological balance is of grave concern. The easiest possible way in which these chemicals find their way into the aquatic environment is through sewage. This study was designed to test for amoxicillin at a site where they would most likely occur (i.e., treated sewage effluent outfalls). Considering $3.7\,\mu g\,L^{-1}$, reported acute lowest observed effect concentration (LOEC) for amoxicillin and attenuation factor of 1,000 [36], the predicted no effect concentration (PNEC) for this compound was $3.7\,ng\,L^{-1}$ [37] and the levels more than PNEC could posses the harmful environmental impact. This study has identified that the conventional treatment processes are inefficient in removing antibiotics from the liquid phase, as antibiotics were still detected in final effluents in the $ng\,L^{-1}$ concentrations, much higher than what is considered safe (PNEC = $3.7\,ng\,L^{-1}$). Thus, there is a need to upgrade the wastewater reuse guidelines by considering the antibiotics and other emerging molecules in the treated effluents; to promote the revised water reuse policy; and to secure its acceptance by public. Advanced technologies like nano-filtration, reverse-osmosis [43,44], and membrane bioreactor with powdered active carbon [45] have better removal of organic micro pollutants. It is also not clear how strongly these chemicals are sorbed and how these still remain biologically active after sorption [34]. Thus, for water reuse to become a valuable part of a total water cycle in urban communities, economically viable treatment processes, sensible regulations and improved health risk assessment and management are necessary. This is a preliminary study aimed at understanding the levels of the most consumed β-lactum antibiotic, viz., amoxicillin, in wastewater systems. This study recommends the more detailed studies of the levels of drug residues including non-steroids and antibiotics in different water matrices and resistance strains.

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