Quantitative Analysis Of Anti-Biotics Drug Residues In The Dal Lake, Srinagar, India

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Abstract: This study investigates the occurrence of pharmaceutical residues in Dal Lake, a freshwater lake in Srinagar, Jammu & Kashmir, India, focusing on three targeted compounds: Ofloxacin, Erythromycin, and Amoxicillin. A total of 8 water samples were collected from eight sampling sites along the littoral zone of the lake on a single occasion between 11:00 am and 4:30 pm. The concentrations of Ofloxacin, and Erythromycin were determined through Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Analysis revealed the presence of two out of the three targeted pharmaceuticals. Ofloxacin was the most prevalent, with concentrations ranging from 82.28 µg/L to 133.98 µg/L (mean: 99.49 µg/L, SD: 17.90 µg/L), exhibiting significant variability across sampling sites. Erythromycin was also detected, albeit at lower concentrations, ranging from 1.55 µg/L to 2.00 µg/L (mean: 1.74 µg/L, SD: 0.17 µg/L), showing minimal variability. Amoxicillin was consistently below the quantifiable limit at all sites, suggesting its absence or presence at extremely low concentrations in the lake which could be ascribed to its natural degradation. These findings highlight the concerning levels of certain pharmaceutical residues in Dal Lake, particularly Ofloxacin, and underscore the need for further research on their environmental impact and potential mitigation strategies.

Keywords: Pharmaceutical residues; Dal Lake; Ofloxacin; Erythromycin; Amoxicillin; Water pollution; HPLC; LC-MS/MS;

Introduction:

Pharmaceuticals, representing a significant achievement in scientific progress, have extended human lifespans, eradicated numerous deadly diseases, and enhanced overall quality of life. However, this remarkable success has paradoxically led to their emergence as fast growing environmental contaminants (Zuccato et al. 2000; Daughton 2004; Fent, Weston, and Caminada 2006; Schwarzenbach et al. 2006; Kümmerer 2009; Li 2014; Lapworth et al. 2012). These pollutants are now detectable in various water bodies, including surface waters such as lakes, rivers, streams, estuaries, and seawater (Akhter et al. 2023a, McArdell et al. 2003; Ferdig, Kaleta, and Buchberger 2005; Brown et al. 2006; Kim and Carlson 2006; Cha, Yang, and Carlson 2006), as well as in groundwater (Sacher et al. 2001, Fick et al. 2009). They are also present in wastewater treatment plant (WWTP) effluents, influents, and sludge (Halling-Sørensen et al. 1998; Homem and Santos 2011). The widespread distribution of pharmaceutical contaminants extends to both the geosphere (Yang et al. 2011; da Silva et al. 2011) and biosphere (Ahmed et al. 2014, Kim et al. 2011; Lajeunesse et al. 2011; Du and Liu 2012). Even polar regions, traditionally considered the most pristine environments on Earth, have not escaped this contamination (Kallenborn et al. 2008; Gonzalez-Alonso et al. 2017). Notably, several endocrine disruptors, antimicrobials, and synthetic estrogens have been detected in Northern Antarctica (Esteban et al., 2016), underscoring the global reach of this environmental issue.

The impact of pharmaceutical contaminants on flora and fauna remains poorly understood, and even less is known about their potential long-term effects on humans at environmental concentrations (Lapworth et al. 2012; Bartrons and Peñuelas 2017; Sehonova et al. 2018). This knowledge gap is compounded by a global scarcity of specific guidelines and regulations addressing this issue (Webb et al. 2003; Daughton 2004). To date, Australia stands out as the sole country to have established guidelines for pharmaceuticals in drinking water, to the best of our knowledge (Pomati 2007). The scope of this challenge is vast, given that pharmaceuticals encompass an extensive array of compounds. The U.S. Food and Drug Administration reports that approximately 12,000 prescription pharmaceuticals are currently distributed for human consumption (FDA 2013). This large number of compounds in circulation underscores the complexity of addressing pharmaceutical contamination in the environment.

The recognition of pharmaceuticals as environmental contaminants dates back over three decades. Pioneering studies conducted in the United States during the 1970s first documented the presence of various medications in wastewater, including heart drugs, analgesics, and contraceptives (Tabak & Bunch, 1970; Garrison, Pope & Allen, 1976; Hignite & Azarnoff, 1977). These early investigations laid the groundwork for understanding the environmental fate of pharmaceutical compounds. A significant milestone in this field came with the publication of a comprehensive survey by the United States Geological Survey in 2002. This landmark study examined more than 50 pharmaceutical compounds across 139 streams in 30 states during the period of 1999-2000 (Akhter 2023b, Khan 2022, Kolpin et al., 2002). The breadth and depth of this research have made it the most frequently cited reference in peer-reviewed literature concerning pharmaceutical contamination in surface waters. Its findings have been instrumental in shaping subsequent research and raising awareness about the widespread presence of pharmaceuticals in aquatic environments.
Despite their widespread presence in the environment, pharmaceuticals remain largely unregulated as pollutants (Madhav 2024, Tijani et al. 2016, 27-49; Larsson 2014). Their residues in ecosystems are classified as "compounds of emerging concern" due to their potential for significant impacts on human health and environmental systems (Daughton 2004, 711-732).

Advancements in analytical techniques, such as GC-MS/MS, LC-MS/MS, and UPLC/MS, have revolutionized our ability to detect and quantify pharmaceutical compounds in the environment. These methods have enabled researchers to establish environmental effects of pharmaceuticals at concentrations as low as µg/L and ng/L (Zhang et al., 2019; Angeles et al., 2020; Tran et al., 2019; Trautwein et al., 2014; Kosma et al., 2014; Oguz and Kankya, 2013; Stroski et al., 2020; Petrovic and Barcelo 2006, 1259-1267; Joss et al. 2006, 1686-1696; Drewes et al. 2003, 64-72).The sophistication of these analytical tools has led to the identification and quantification of nearly 3000 biologically active compounds in various environmental matrices (Richardson 2006, 4021-4046; Richardson and Ternes 2014, 2813-2848). This extensive catalog of detected substances underscores the complexity and scale of pharmaceutical contamination in the environment.

Pharmaceutically active compounds exhibit a characteristic known as pseudo-persistence in the environment. This phenomenon occurs due to their continuous introduction into environmental matrices, which counterbalances their ongoing degradation and removal through various natural processes (Daughton 2004). The result is the formation of "a complex pharmaceutical pool" within numerous natural matrices (Stackelberg et al. 2004, 99-113; Kümmerer 2001, 957-969; Ebele, Abdallah, and Harrad 2017, 1-16).The environmental impact of these compounds extends beyond water and soil contamination. Once pharmaceutical residues infiltrate these media, they can be absorbed by plants growing in contaminated soils or waters. This uptake has been documented in a variety of common crops, including cabbages, cucumbers, corn, carrots, lettuces, and green onions (Akhter 2023c, Migliore, Cozzolino, and Fiori 2003, 1233-1244; Kumar et al. 2005, 2082-2085). This bioaccumulation in food crops raises concerns about potential exposure to humans through the food chain, adding another layer of complexity to the issue of pharmaceutical contamination in the environment.

Occurrence and route of pharmaceutical drugs in the environment:
Many different pharmaceuticals have been detected in drinking water. Underground waters can also be polluted with substances of pharmaceutical origin as a result of surface water infiltration and leaching by waste sites. Substances of pharmaceutical origin are monitored in water environments by many research centres. However, the most important problem with monitoring is lack of appropriate analytical procedures for quantitative determination of residues of active pharmaceutical components and their metabolites.

The first of these sources is urban wastewater, which contains a high load of pharmaceuticals from human excrement, and also the inadequate disposal of expired or unused drugs due to the scarce control in their management. Another major source of pharmaceuticals is agricultural and livestock waste, especially the latter, since in large farms for intensive livestock, animals are often fed with feed supplemented containing drugs and excreta are often used in agriculture as soil amendments, reaching groundwater by leaching. Effluents from the pharmaceutical industry are another important source, with high concentrations of pharmaceuticals being found due to discharges from factories in Asia, Europe and America, despite strict regulation of pharmaceutical production in Europe and the United States (Lin et al., 2008)
The compounds may be released into surface waters or enter terrestrial systems when sewage effluent is used for irrigation or where sewage sludge is applied as a fertilizer to agricultural land . Veterinary pharmaceuticals are released to the environment either directly, from use in aquaculture and the treatment of pasture animals, or indirectly during the land application of manure and slurry from livestock feed.

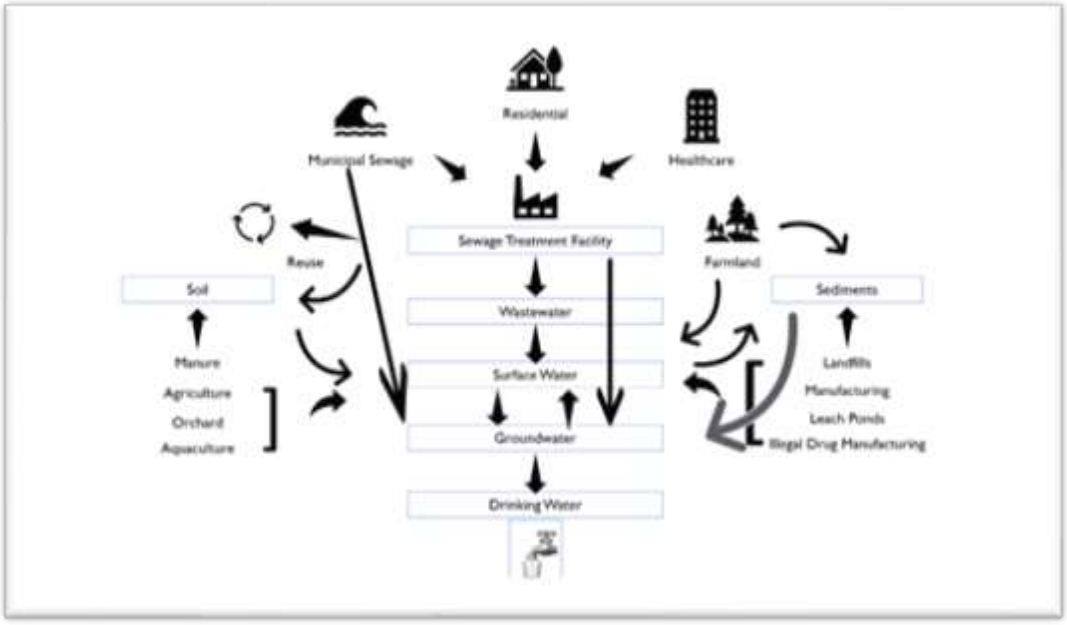

Figure 1: Occurence and paths of pharmaceutical residues in the environment.

Materials and Methods

Study Area Description

Dal Lake is an urban-type lake located in the Kashmir Himalayas in the city of Srinagar, Jammu and Kashmir. It lies between 34°5′ and 34°9′ N to 74°49′ and 74°53′ E at an average altitude of 1583 meters above mean sea level. The lake's catchment area includes mountain ranges to the north and northeast, while flat arable land surrounds the other sides. This catchment area is characterized by rugged terrain with high relief. Dal Lake is a multi-drainage basin covering a total area of 24 km², with its catchment extending over 337 km² (Rashid et al., 2017). The lake has a total water holding capacity of 15.45 million cubic meters (Mm³) and an open water spread of approximately 10.5 km². The shoreline, about 15.5 kilometers long, is lined with Mughal-era gardens, parks, houseboats, and hotels.

Dal Lake has been a cradle of civilization in the Kashmir Valley and plays a crucial role in the region's economy by providing livelihoods for many people in Srinagar. It is a significant tourist destination and an important source of fish, vegetables, and recreation for locals. Additionally, the lake waters form a vital part of the water supply for Srinagar city. The main basin of Dal Lake is a complex of five interconnected basins with causeways: the Nehru Park basin, the Nishat basin, the Hazratbal basin, the Nigeen basin, and the Barari Nambal basin. Navigational channels provide transportation links to all five basins. The shallow, open-drainage lake is fed by the Dachigam-Telbal Nallah (with perennial flow), Dara Nallah ("Nallah" means "stream"), and several other small streams. The lake is classified as 'warm monomictic' under the sub-tropical lake category. Spring sources also contribute to the lake's flow, though specific data on their contribution is unavailable.

The ecosystem of Dal Lake is rich in macrophytes, including submerged, floating, and phytoplankton species. The lake is particularly noted for its Nelumbo nucifera (lotus flowers) which bloom in July and August. The eutrophic zones see prolific growth of Ceratophyllum demersum, with Myriophyllum spicatum and Potamogeton lucens being dominant species. The faunal distribution includes zooplankton, benthos, and fish. Zooplankton species in the lake include Keratella cochlearis, K. serrulata, Polyactis vulgaris, Brachionus plicatilis, Monostyla bulla, Alona monocantha, Cyclops ladakanus, and Mesocyclops leukarti. Benthos include Chironomus sp. and Tubifex sp., while fish species include Cyprinus carpio specularis (economically important), C. carpio communis, Schizothorax niger, S. esocinus, S. curviformis, and Crossocheilus latius.

Sampling Sites

Eight sampling sites were chosen based on their relative location and lake exposure route to urban drainage, such as their proximity to main city and the extent of the anthropogenic entry route from city to the lake, as depicted in Fig. 2 which illustrates the location of Dal Lake, its catchment area, and the sampling sites. This figure was generated using QGIS software.

Based on this, the first site, S-1, is located near a super-speciality healthcare facility on the Jehlum outflow channel of the lake and is close to the commercial area of the city. This site receives both wastewater and healthcare waste, that may contain pharmaceutical residues from the point source (the hospital). It is crucial to evaluate the impact of hospital waste on the lake's water quality at this site. The second site, S-2, also located on the outflow channel and is proximal to a pulmonary healthcare facility. The third sample, S-3, was taken near Ghat no. 9 of the lake which is a tourist hotspot,

bustling with houseboats and hotels, abutted by a road and experiences a substantial volume of drainage, sewage, and stormwater runoff from these sources. It receives all the waste from houseboats and other encroachments . It is worth noting that one million litres (260,000 gallons) of sewage comes from houseboats (Al Jazeera 2021) The presence of pharmaceutical residues from non-point sources in the lake's water quality is of utmost importance at these sites. Site 4 (S4) is located around Nishat which experiences a substantial volume of drainage, sewage, and stormwater runoff from various sources. On the other hand, Site 5 (S-5) and Site 6 (S-6) are located just next to two major sewage treatment plants; Habak STP and Hazratbal STP respectively. Site 7 (S-7) is on the northern basin of the lake at Hazratbal where the primary inflow channel, Telbal nala with other small streams enter the lake. Site 8 (S-8) is the nearest from the influence of human activities in the city as it is located in the middle of a residential area. The selected sampling sites' codes, names, and geographical locations are shown in Table 1.

Table 1: Sampling site code, name, and geographic locations at Dal Lake

Site	Latitude (N)	Longitude (E)	Name
Site 1	34.08044	74.82918	Outflow Channel, proximal to a healthcare facility (Paras Hospital)
Site 2	34.07838	74.82936	Outflow Channel (CD Hospital)
Site 3	34.08533	74.83781	Main Lake Basin (Ghat no.9)
Site 4	34.13295	74.87128	Main Lake Basin, proximal to STP
Site 5	34.14579	74.84315	Near Habbak STP
Site 6	34.13437	74.84148	Near Hazratbal STP
Site 7	34.13081	74.84305	Northern Lake sub-Basin (Hazratbal)
Site 8	34.09732	74.82973	Residental Area (Rainawari)

Fig 2: Map of the study area and sampling sites (Generated through QGIS software)

Sample Collection

A total of 8 samples of water were collected from eight sampling sites on one sampling occasion along the littoral zone of Dal Lake, Jammu and Kashmir from 11:00 am to 4:30 pm

Surface water samples were collected from the uppermost 20 to 30 cm of Dal Lake using 500 mL HDPE bottles by employing grab sampling method. The bottles were filled upto the rim to avoid any bubbles and tightly capped. The bottles were then kept away from sunlight and stored at a cool temperature as soon as possible.

Criteria For Selection Of Pharmaceuticals:

The selection of pharmaceuticals for this study was based on multiple criteria to ensure a comprehensive and relevant analysis. High-priority compounds that are frequently and regularly present in the environment were primarily considered. The selection process was informed by several key factors. Local market intelligence played a crucial role; information on prevalent usage patterns in the region was obtained through consultation with stakeholders in the pharmaceutical supply chain. This approach enabled the analysis to focus on compounds with high local relevance, as determined by reported consumption trends. Additionally, compounds with known or suspected ecological or human health concerns were included to address potential environmental and public health impacts. Bioaccumulation potential, a fundamental risk factor for aquatic life, was also considered an important criterion, as it directly relates to the compound's potential impact

on ecosystems (Jean et al., 2020). By applying these criteria, which encompassed local usage patterns, environmental and health concerns, bioaccumulation potential, and frequency of environmental occurrence, three antibiotics were ultimately selected for the study: Amoxicillin, Ofloxacin, and Erythromycin. These compounds not only met the established criteria but also represented a class of pharmaceuticals (antibiotics) known for its environmental persistence and potential ecological effects

Sample Preparation and Analysis:

The sample preparation protocols were adapted from a previously developed method for pharmaceutical analysis by Waters corporation. The sample pre-treatment including extraction, analyte enrichment, and clean-up was made according to the procedure in Waters application notes (Waters Corporation 2024).

Reagents and Chemicals

Pharmaceutical standards were purchased from CPA Chemicals (Amoxicillin, Ofloxacin) and Sigma-Aldrich (Erythromycin). The study utilized high-performance liquid chromatography (HPLC) grade solvents, specifically acetonitrile and methanol, sourced from Merck, a company located in Darmstadt, Germany. Formic acid, employed as the eluent additive, was acquired from Sigma-Aldrich (Steinheim, Germany). All pharmaceutical standards and labelled standards utilized in the analysis were of analytical grade, exhibiting a purity level of 98-99%.

Sample Analysis

The analysis of the Pharmaceuticals was performed by Liquid chromatography tandem mass spectrometry (Agilent 6495, USA). For the analysis of amoxicillin, the mobile phase consisted of two components: Mobile Phase A, which was composed of water with 0.1% formic acid, and Mobile Phase B, which was acetonitrile with 0.1% formic acid. The chromatographic separation was performed using a C18 column, maintained at a temperature of 35°C. For the analysis of ofloxacin, the Mobile Phase A, which was 1 mM ammonium formate in water, and Mobile Phase B was 1 mM ammonium formate in methanol. The chromatographic separation was conducted using a C18 column, with the column temperature maintained at 35°C. For the analysis of erythromycin, the Mobile Phase A consisted of 5 mM ammonium formate in water with 0.1% formic acid, and Mobile Phase B, was acetonitrile with 0.1% formic acid. The separation was carried out using a T3 column, maintained at a temperature of 35°C. Samples were thoroughly homogenized by mixing. A 50 mL portion of the homogenized sample was subsequently filtered through a 0.45 µm membrane filter paper. The filtered sample was acidified with 1 N H_2SO_4 to reach a pH of 3 and was then loaded onto an Oasis HLB 6 cc Cartridge containing 200 mg of sorbent. The cartridge was activated sequentially with 5 mL of methanol, 5 mL of a 50:50 methanol/water mixture, and 5 mL of acidified water at pH 3. Following activation, the cartridge was washed with 5 mL of acidified water, and the compounds adsorbed onto the cartridge were eluted using 5 mL of 5% trimethylamine in methanol. The eluent was then evaporated to dryness under a gentle stream of nitrogen gas at 50 °C. The resultant residue was reconstituted in a solution of acetonitrile and water (20:80) to a final volume of 1 mL, before being injected into the LC-MS/MS for analysis.

Results And Discussions.

Occurrence of pharmaceutical residues in water samples

Of the 3 targeted pharmaceutical compounds, 2 were detected across eight different sites. Specifically, Ofloxacin and Erythromycin were detected in the water samples, while Amoxicillin was below the quantifiable limit (BQL) at all sites. Here, in the water samples, the most detected pharmaceutical was the ofloxacin. Ofloxacin concentrations ranged from 82.28 µg/L (Site 7) to 133.98 µg/L (Site 1) with an average concentration of 99.49 µg/L and a standard deviation of 17.90 µg/L (Table 4, Fig 3), indicating significant variability. Erythromycin concentrations ranged from 1.55 µg/L (Site 7) to 2.00 µg/L (Site 6), with an average concentration of 1.74 µg/L and a standard deviation of 0.17 µg/L (Table 2, Fig 3), indicating minimal variability. The consistent BQL readings for Amoxicillin across all sites suggest its absence in the sampled locations.

Table 4: Concentration of pharmaceuticals across different sites

	Compound Concentration (µg/L)		
Sample	**Ofloxacin**	**Amoxicillin**	**Erythromycin**
Site 1	133.98	BQL	1.60
Site 2	88.28	BQL	1.91
Site 3	84.07	BQL	1.60
Site 4	118.42	BQL	1.59
Site 5	110.08	BQL	1.87
Site 6	93.93	BQL	2.00
Site 7	82.28	BQL	1.55
Site 8	84.88	BQL	1.83
Average	99.49	BQL	1.74
Std DEV	17.90	BQL	0.17

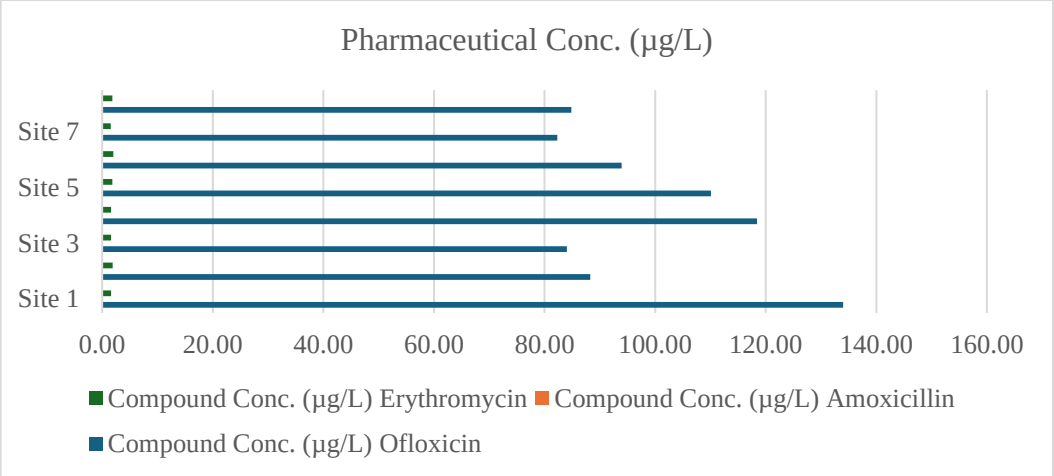

Fig 3: Pharmaceutical concentrations across different sites

Ofloxacin

Ofloxacin was detected in all water samples, with concentrations ranging from 82.28 µg/L at Site 7 to 133.98 µg/L at Site 1. The average concentration was 99.49 µg/L, and the standard deviation was 17.90 µg/L, indicating considerable variability. The limit of quantification (LOQ) for Ofloxacin is 5 ppb (5 µg/L) (Table 4.2), which is well below the detected concentrations, confirming the presence of Ofloxacin as a prevalent contaminant with significant fluctuation in its levels across different sites.

Amoxicillin

Amoxicillin was below the quantifiable limit (BQL) at all sites, with the LOQ for Amoxicillin being 1 ppb (1 µg/L) (Table 4).

Erythromycin

Erythromycin was detected in all water samples, with concentrations ranging from 1.55 µg/L at Site 7 to 2.00 µg/L at Site 6. The average concentration was 1.74 µg/L, and the standard deviation was 0.17 µg/L, indicating low variability. The LOQ for Erythromycin is 1 ppb (1 µg/L (Table 4), which confirms the detected concentrations are above the quantification limit. This suggests that Erythromycin is present in low but relatively consistent concentrations across the sampled areas.

Table 5: Summary of pharmaceutical analysis conducted water samples

Pharmaceutical Drug	Common Use	Quantification Limit (ppb)	Min. concentration (µg/L)	Mean concentration (µg/L)	Max. concentration (µg/L)	Std. Dev
Ofloxacin	Antibiotic	5	82.28	99.49	133.98	17.90
Amoxillin	Antibiotic	1	-	-	-	-
Erythromycin	Antibiotic	1	1.55	1.74	2.00	0.17

Discussion:

In an investigation into drug consumption patterns in the Srinagar area, consultations were conducted with five major pharmaceutical agencies. While none provided exact figures, the accessible data indicated notable trends. The information on the approximate number of strips sold was obtained, with each strip containing 10 tablets. The analysis suggests that Amoxicillin is the most consumed drug, followed by Ofloxacin. In contrast, Erythromycin sales were negligible, corroborating the lower prevalence of this drug in the environment (Table 6, Figure 3).

Table 6: Approximate Annual Sales of pharmaceuticals in Srinagar, 2023

NAME OF THE PHARMACEUTICAL AGENCY	No. of strips sold		
	AMOXICILLIN	OFLOXACIN	ERYTHROMYCIN
New City Pharmacy	1200000	900000	30000
Extensor Pharmaceuticals	1500000	1000000	20000
A R Rizwan Traders	1000000	750000	50000
North Point Pharmacy	700000	560000	80000
New Light Medical Agency	600000	410000	76000

Amoxicillin is a penicillin antibiotic prescribed for the treatment of bacterial infections, including tonsillitis, bronchitis, sinusitis, pneumonia, and infections of the ear, nose, throat, skin, and urinary tract. Amoxicillin clavulanate combines amoxicillin with clavulanate potassium, where the amoxicillin combats bacteria and the clavulanate potassium prevents

certain bacteria from developing resistance to amoxicillin. This drug is utilized to treat various bacterial infections such as middle ear infections, strep throat, pneumonia, skin infections, odontogenic infections, and urinary tract infections. It is typically administered orally, though it can also be given by injection in some cases.

Ofloxacin is a quinolone antibiotic effective in treating various bacterial infections. Administered either orally or via intravenous injection, it is used to treat pneumonia, cellulitis, urinary tract infections, prostatitis, plague, and certain types of infectious diarrhea. Additionally, in combination with other medications, it is employed in the treatment of multidrug-resistant tuberculosis. Ofloxacin eye drops are used for superficial bacterial infections of the eye, and ear drops are indicated for otitis media when a perforation of the eardrum is present.

Erythromycin is an antibiotic prescribed for treating various bacterial infections, such as respiratory tract infections, skin infections, chlamydia infections, pelvic inflammatory disease, and syphilis. It is also used during pregnancy to prevent Group B streptococcal infection in newborns and to facilitate delayed stomach emptying. Erythromycin can be administered intravenously or orally. Additionally, an eye ointment is commonly recommended post-delivery to prevent eye infections in newborns. It is noteworthy that azithromycin has become more commonly used, largely replacing erythromycin in clinical practice.

Of the antibiotics, ofloxacin was the leading in concentration among the detected APIs in this study. This is presumably associated with the wide use of the drug in the study area due to its broad spectrum among the antibiotics of the fluoroquinolone family, used worldwide in human and veterinary medicine (Wilkinson et al., 2016). Kümmerer (2008) also indicated that after use, ofloxacin is generally not completely metabolized in mammals, so this drug and/or its metabolites can reach and contaminate water bodies and other environmental compartments easily. Moreover, ofloxacin is a recalcitrant compound with high stability in the environment (Rodrigues-Silva et al., 2019). The proximity of site 1 to a super-speciality healthcare facility may have led to the high concentration of ofloxacin detected. These could be some of the reasons why we found ofloxacin in high concentrations, with a mean value of 133.98 µg/L and a range of 82.28 µg/L - 133.98 µg/L.

Degradation of Antibiotics:

The amounts of pharmaceuticals reaching surface water are influenced by several factors, including environmental degradation. The degradation rates of many pharmaceuticals in the environment are often unknown and can only be estimated from laboratory data, which are sometimes available in the literature. Table 5 reports the degradation rates of pharmaceuticals in water, as described in various studies.

Table 5: Stability of some pharmaceuticals in water (Zuccato et al. 2004, Andreozzi et al., 2003, 1319–1330)

Pharmaceuticals	Stability in water	Comments
Amoxicillin	$t_{90} < 2d$	Low stability
Erythromycin	$t_{50} \geq 1$ y 11.5 d (20 ◦C)	Prolonged stability
Ofloxacin	t_{50} 10.6 d	Moderate Stability

Amoxicillin

Amoxicillin was below the quantifiable limit (BQL) at all sites, with the LOQ for amoxicillin being 1 ppb (1 µg/L). The consistent BQL readings suggest that amoxicillin is not a significant contaminant in the sampled areas, as its concentrations were too low to be detected by the analytical methods used. This indicates that amoxicillin is unlikely to pose a contamination risk in these environments. A recent study by Rocha et al. investigated the photodegradation of amoxicillin in aquatic environments under simulated solar radiation, providing crucial insights into the antibiotic's environmental fate. The research examined how various factors, including pH, salinity, and dissolved organic matter (DOM), influence amoxicillin breakdown rates. Key findings revealed that :

a) Photodegradation is a significant factor in amoxicillin's environmental persistence, with pH playing a critical role. At pH 8.05, the degradation rate constant (k) was 0.033 ± 0.001 h^{-1} with a half-life ($t_{1/2}$) of 21.0 ± 0.6 h, while at pH 9.00, k increased to 0.087 ± 0.004 h^{-1}, reducing $t_{1/2}$ to 8.0 ± 0.3 h.

b) The presence of DOM, particularly humic substances, enhanced photodegradation through indirect photolysis. For instance, 20 mg L^{-1} of humic acid yielded $k = 0.060 \pm 0.001$ h^{-1} and $t1/2 = 11.5 \pm 0.2$ h, while fulvic acid at the same concentration resulted in $k = 0.077 \pm 0.002$ h^{-1} and $t1/2 = 9.0 \pm 0.3$ h.

c) Conversely, high salinity slowed degradation, with a 20‰ sodium chloride solution showing $k = 0.0276 \pm 0.0007$ h^{-1} and $t1/2 = 25.1 \pm 0.7$ h, and simulated seawater at 20‰ yielding $k = 0.0070 \pm 0.0002$ h^{-1} and $t1/2 = 99 \pm 4$ h.

These findings have significant implications for understanding the below quantification limit findings of amoxicillin in Dal Lake waters. According to LCMA report 2023, the pH of Dal Lake ranges from 7.97 to 8.87, which aligns with the conditions found to promote rapid Amoxicillin degradation in Rocha et al.'s study. The higher end of this pH range is particularly conducive to Amoxicillin breakdown, potentially explaining its low detectability. Furthermore, Kumar and Mahajan (2023) reported a low salinity of 139.0 mg L^{-1} in Dal Lake. This low salinity contrasts with the high-salinity

conditions that were found to inhibit Amoxicillin degradation in the study, suggesting that Dal Lake's low salinity environment would not impede the photodegradation process.
Additionally, Dal Lake is known for its high levels of dissolved organic matter, which further supports the case for rapid Amoxicillin degradation. The BOD of the lake was found to range between 3.00 mg/l to 7.19 mg/l and COD was found to be 22.20 mg/l to 69.10 mg/l (LCMA, 2023) The study demonstrated that the presence of DOM, especially humic substances, significantly accelerates Amoxicillin photodegradation through indirect photolysis. Given the high DOM content in Dal Lake, it is plausible that this factor contributes substantially to the enhanced breakdown of Amoxicillin, potentially leading to its concentrations falling below detection limits.

The study also compared Amoxicillin degradation in different water matrices, finding faster rates in real-world samples compared to synthetic solutions. Brackish water exhibited the highest degradation rate, followed by freshwater, with both surpassing the rate in phosphate-buffered saline. This finding further supports the argument for rapid Amoxicillin degradation in Dal Lake, as it represents a real-world freshwater system with complex environmental factors at play.

Ofloxacin:
In a study conducted by Andreozzi et al., 2002, Ofloxacin, one of the pharmaceuticals studied, has shown varying rates of photodegradation under solar irradiation. The research indicates that ofloxacin degrades relatively slower compared to other pharmaceuticals in certain conditions. Specifically, it was observed that under solar irradiation in bi-distilled water, ofloxacin has a half-life time ($t_{1/2}$) of approximately 10.6 days at high latitudes during winter, which indicates it is persistent in the environment.
The photodegradation of pharmaceutical compounds in aqueous environments is a complex process influenced by various factors, including the presence of nitrate ions and humic acids. This study focuses on the photodegradation of ofloxacin, a widely used antibiotic, and examines the effects of these substances on its degradation rate.
Nitrate ions have been found to enhance the photodegradation rate of most investigated pharmaceutical compounds, including ofloxacin. This enhancement is primarily attributed to the photolysis of nitrate, which generates hydroxyl (HO) radicals. These highly reactive radicals can then interact with ofloxacin, promoting its degradation. Experiments conducted with ofloxacin in the presence of both nitrate and humic acids demonstrated increased degradation rates compared to control runs without these substances. Nitrate acts as a photosensitizer, facilitating the phototransformation of ofloxacin under solar irradiation and leading to a reduced half-life for the compound.
The presence of humic acids affects the photodegradation of ofloxacin through two main mechanisms: the inner filter effect and photosensitization. As an inner filter, humic acids absorb UV radiation across a broad spectrum, potentially reducing the available energy for ofloxacin photodegradation. Conversely, when irradiated by UV light, humic acids can enter an excited state and generate reactive species such as singlet oxygen, which can enhance the phototransformation of ofloxacin. The overall effect of humic acids on the degradation rate depends on the balance between these opposing actions and can vary based on specific environmental conditions.

Environmental factors, particularly the intensity of solar radiation, play a crucial role in the photodegradation of pharmaceuticals like ofloxacin. Studies have shown that degradation rates vary with latitude and seasonal changes, reflecting differences in sunlight intensity and duration. For instance, at 40° N latitude during spring and summer, ofloxacin in bi-distilled water was found to degrade with a half-life of 10.6 days, while other pharmaceuticals such as sulfamethoxazole and diclofenac exhibited half-lives of 2.4 and 5.0 days, respectively.
It is important to note that the impact on degradation rates can vary depending on the concentration of other substances in the solution and specific environmental conditions. The complex interplay between chemical interactions and environmental factors underscores the need for comprehensive studies to fully understand the fate of pharmaceuticals like ofloxacin in aquatic ecosystems.

Erythromycin:
The persistence of erythromycin (ERY) in the environment is significantly influenced by its complex chemical structure. ERY's molecular architecture, characterized by a 14-membered lactone ring (erythronolide), plays a crucial role in its environmental stability. This ring structure exhibits resistance to hydrolysis, thereby reducing its susceptibility to microbial degradation (Kanfer et al., 1998; Lange et al., 2006; Roberts, 2014). The molecule's persistence is further enhanced by two key sugar components. D-desosamine, an amino sugar attached to the C-5 position of the lactone ring, contributes to ERY's alkaline nature, influencing its environmental interactions and solubility. Additionally, L-cladinose, unique to ERY, is bound to the C-3 position via a β-glycosidic bond, which is notably resistant to enzymatic cleavage (Kanfer et al., 1998; Lange et al., 2006; Roberts, 2014).
ERY's polyhydroxylactone framework, featuring multiple hydroxyl groups, forms hydrogen bonds that increase molecular rigidity and reduce accessibility to degradative enzymes (Islas-Espinoza et al., 2018). The molecule's amphiphilic nature, with both hydrophobic and hydrophilic regions, further complicates its degradation. This dual character can lead to association with organic matter in sediments, potentially reducing bioavailability for microbial breakdown (Islas-Espinoza et al., 2018).
The overall structural complexity of ERY, encompassing multiple rings, sugars, and functional groups, presents a significant challenge for biodegradation. Few microbial species possess the enzymatic capability to efficiently degrade

such an intricate molecular structure (Islas-Espinoza et al., 2018). This combination of structural features collectively contributes to ERY's persistence in aquatic environments, underlining the need for advanced treatment methods and continued research into its environmental fate and impact.

Conclusion:

Despite its widespread use, Amoxicillin was detected at concentrations below the quantifiable limit in the water samples. Dal Lake's environmental conditions significantly influence antibiotic degradation and persistence. The lake's alkaline pH, low salinity, and high DOM content create an environment conducive to rapid Amoxicillin degradation, explaining its below quantification limit findings. In contrast, Erythromycin was detected at high concentrations, particularly near the Hazratbal STP effluent discharge point, consistent with its prevalence in sewage treatment plant outflows globally. This suggests that the treatment plant effluent is a significant source of Erythromycin contamination, raising concerns about its impact on aquatic life and potential antibiotic resistance development. Ofloxacin was detected in all water samples, confirming Ofloxacin as a prevalent contaminant in Dal Lake. The significant variability in Ofloxacin concentrations across sampling sites suggests complex distribution patterns and potential multiple sources of contamination. This study emphasizes the importance of considering local environmental factors when interpreting antibiotic persistence data and underscores the need for comprehensive, context-specific assessments in environmental monitoring and management strategies. The varying concentrations and behaviors of Amoxicillin, Erythromycin, and Ofloxacin in Dal Lake highlight the complex interplay between drug properties, environmental conditions, and anthropogenic inputs, necessitating tailored approaches to antibiotic pollution management in aquatic ecosystems.

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