water

MDPI

Article

Environmental Monitoring and Risk Assessment of Pharmaceutical Residues Discharged from Large Livestock Complex in the Geum River Basin, South Korea

Hyeri Lee [1,2], Minhee Chae [1] and Seokwon Lee [1,*]

[1] Geum River Environment Research Center, National Institute of Environmental Research, 182-18 Jiyong-ro, Okcheon-gun 29027, Chungbuk, Republic of Korea; hr4848@korea.kr (H.L.); minhiec@korea.kr (M.C.)
[2] Environmental Measurement and Analysis Center, National Institute of Environmental Research, 42 Hwangyeong-ro, Seo-gu, Incheon 22689, Republic of Korea
* Correspondence: swlee23@korea.kr; Tel.: +82-43-730-5642

Abstract: This study aims to collect water samples from two tributaries within the Geum River basin in South Korea, where large-scale livestock complexes are located, to quantify the measured environmental concentration (MEC) of pharmaceutical residues using a multiresidue analytical method developed with liquid chromatography–tandem mass spectrometry (LC-MS/MS), and to evaluate the environmental risks posed by the detected pharmaceuticals to aquatic organisms. The water samples were collected at a total of 17 points, including up-, middle-, and downstream of the Seoksong and Nonsan-Gangkyoung streams connected to the Geum River, from October 2018 to March 2019. A multiresidue analytical method using LC-MS/MS was developed to quantify 49 pharmaceuticals with hydrophilic lipophilic balance using solid phase extraction. The recovery rates varied between 67.23% and 136.98%, while the limits of quantification were from 3.99 to 46.32 ng/L. Ecotoxicological information on acute and chronic effect endpoints (e.g., EC_{50}, NOEC, etc.) was obtained from the U.S. EPA ECOTOX Knowledgebase. Considering the worst-case scenario, the lowest observed effect endpoint (mainly NOEC) of the most sensitive species was selected, and predicted no effect concentration (PNEC) values were calculated by dividing the endpoint by an assessment factor (AF). The mean, minimum, and maximum MECs of pharmaceuticals were divided by PNECs to calculate risk quotient (RQ). Caffeine was detected in all sampling sites with a detection frequency of 100%. High levels of pharmaceuticals (9.212 μg/L of sulfathiazole, 8.479 μg/L of acetaminophen, and 5.885 μg/L of florfenicol) were detected. The RQ values exceeded 1 and reached up to 84.79 (high risk category) for acetaminophen, and were between 0.11 and 0.83 (moderate risk) for carbamazepine, etc. The RQs for the rest of the 15 substances were below 1 (low risk). In the future, further studies should be conducted to monitor other micropollutants, including industrial chemicals, pesticides, etc., at different locations of the Geum River basin, including livestock farms, pharmaceutical manufacturing facilities, wastewater treatment plants, and other facilities, for long-term period.

Keywords: Geum river basin; hydrophilic lipophilic balance; LC-MS/MS; livestock complex; pharmaceuticals; risk assessment

Citation: Lee, H.; Chae, M.; Lee, S. Environmental Monitoring and Risk Assessment of Pharmaceutical Residues Discharged from Large Livestock Complex in the Geum River Basin, South Korea. *Water* **2023**, *15*, 3913. https://doi.org/10.3390/w15223913

Academic Editor: Laura Bulgariu

Received: 12 October 2023
Revised: 31 October 2023
Accepted: 31 October 2023
Published: 9 November 2023

1. Introduction

In recent years, trace levels of various groups of chemicals, typically ranging from ng/L to μg/L, have been found to be persistent in aquatic environments for long extended periods, adversely affecting the water quality and aquatic ecosystems, and have been named "micropollutants" [1–3]. These micropollutants include various substances, such as pharmaceuticals, personal care products (PCPs), pesticides, flame retardants, and perfluorinated compounds (PFCs) [4–6]. Pharmaceutical compounds are mainly discharged into

aquatic environments through a variety of sources, such as municipal wastewater, industrial wastewater, livestock wastewater, and manure application [7]. They are mainly used in humans, animals, and agriculture for the treatment and prevention of diseases. These low concentrations of chemical compounds are directly released into the aquatic environment through various channels, including public sewage treatment plants, hospitals, industrial complexes, and wastewater treatment facilities. Several studies have reported that certain highly persistent micropollutants are not completely broken down and frequently detected at high concentration levels in aquatic environments [7–10].

In previous studies, Jaffrézic et al. [11] reported that animal-specific pharmaceuticals were detected at higher levels of concentrations than those of human-specific pharmaceuticals. Sulfonamides of twelve antibiotics were detected at the maximum concentrations (24–385 ng/L) with maximum detection frequencies (76–100%) [12]. Iopromide, atenolol, TCPP (tris(chloroisopropyl) phosphate), TECP (tris(2-chloroethyl) phosphate), musk ketone, naproxen, DEET (N,N-diethyl-meta-toluamide), carbamazepine, caffeine, and benzophenone are frequently detected in both river and creek samples from the Han River, Republic of Korea [13]. Carbamazepine was detected in the overall water system at representative sites and at the Geum River tributary, whereas tetracycline pharmaceuticals and epimer isomers were detected around livestock farm areas [14]. At a livestock wastewater disposal plant, the highest level of lincomycin detected was 477 μg/L in the Nakdong River [15].

Pesticides and pharmaceutical substances, including atrazine, carbamazepine, and metformin, were detected in the Han River, Nakdong River, and Yeongsan River, which are among the four major river basins in South Korea. The concentration range of these micropollutants was found to be 0.1–58 μg/L. Furthermore, 13 types of perfluorinated compounds, including PFOA, PFOS, PFBS, and PFCAs, were also detected at concentrations ranging from 0.01 to 0.5 μg/L. Consequently, it was observed that the exposure risks of both human health and aquatic environments were relatively high, considering the measured concentrations of these micropollutants [10]. Most pharmaceuticals are polar and non-volatile and are usually analyzed by liquid chromatography–(tandem) mass spectrometry (LC-MS or LC-MS/MS). Various types of MS have been applied, for example triple quadrupole (QqQ) MS [11,16,17], LC-Q-IT (iontrap) MS [8], LC-IT-TOF (time of flight) MS [18], and other techniques. Pharmaceuticals have been extracted using solid phase extraction (SPE) cartridges from water samples, including hydrophilic–lipophilic balance (HLB) [11,16,19], strong anion exchange (SAX) + HLB [12], mixed-mode cation exchange (MCX) [18], and on-line SPE [11].

However, few studies, such as via regular environmental monitoring and measurements of organic substances, including pharmaceutical residues, have investigated detection patterns and the primary pollution routes in the surrounding rivers and streams of the Geum River basin in South Korea. This area is characterized by a high density of livestock farms, organic fertilizer plants, and agricultural industrial complexes. Furthermore, an accurate quantitative analysis method has not been established to measure and analyze various types of pharmaceutical substances. As a result, the concentration levels of these substances, their spatio-temporal patterns, and specific locations of point sources have not been fully identified in major rivers within the Geum River watershed. Consequently, robust legal regulations and environmental pollution management processes have been insufficient due to the lack of environmental monitoring data, toxicological information on their acute and chronic effects, and information on potential exposure risks for humans and the aquatic environment.

The objective of this study is to develop a multiresidue analytical methodology using LC-MS/MS with HLB cartridge pretreatment. This method will enable the simultaneous analysis of surface water samples collected from large livestock and agricultural complexes in two tributaries of the Geum River basin. The present study also aims to determine the measured concentration levels, identify detection patterns of pharmaceutical residues, and finally evaluate the environmental risk posed by the detected substances to aquatic organisms.

2. Materials and Methods

2.1. Selection of Study Sites and Environmental Sampling

After reviewing the data obtained from the 2018 Water Emission Management System [20], field surveys were conducted at five large livestock complexes with substantial emissions related to livestock manure treatment facilities, situated alongside rivers and tributaries in the Geum River basin. Out of these five livestock complexes, the Seokseong and Nonsan-Gangkyoung streams, located near Nonsan city, Chungcheongnam-do, were chosen as they have the highest livestock populations, including pigs and chickens. This is due to their high operational percentages and the direct discharge of significant amounts of livestock excrement into the main stream of the Geum River through livestock manure treatment facilities. Sampling sites (n = 15) in the tributary rivers were selected, covering the upstream, middle, and downstream segments of the Seokseong (S1 to S6) and Nonsan-Gangkyoung (N1 to N9) streams (Figure 1).

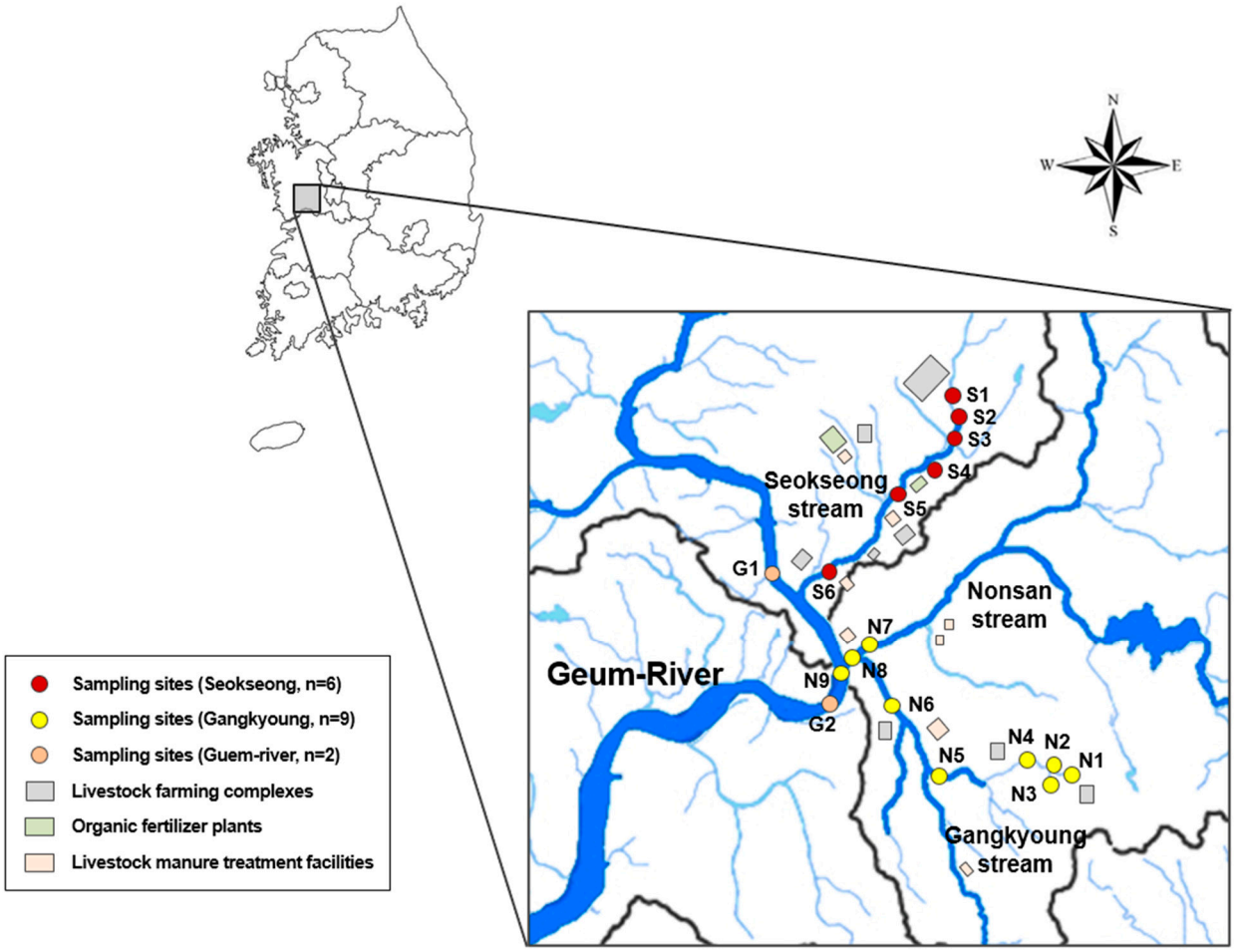


Figure 1. Map of sampling sites used in the study (Seokseong and Nonsan-Gangkyoung streams of the Geum River basin, South Korea).

In October 2018, surface water samples were collected for various water specifications, including water temperature, pH, conductivity, and dissolved oxygen concentrations, using a multiparameter water quality meter (Pro DSS, YSI Inc., Yellow Springs, OH, USA), and the river flow was assessed with a flow meter (Model 002, Valeport Ltd., Devon, UK). For all sites, a total of 1 L was collected as a grab sample using pre-cleaned amber glass containers. Samples were kept on ice during transportation to the laboratory and stored at 4 °C until extraction. All samples were extracted and analyzed within fourteen days from collection. The basic water quality parameters (e.g., BOD, COD, TOC, SS, TN, DTN,

NO_3-N, and NH_3-N) were also analyzed using the Ministry of Environment's water quality testing method.

2.2. Quantitative Analysis and Method Validation

In this study, all pharmaceutical substances were identified through qualitative analysis, and the general information is summarized in Table 1. For the quantitative analysis of these pharmaceutical substances, HPLC-grade high-purity standard substances (>90%) were used (Table 2). These compounds were purchased from Sigma–Aldrich (St. Louis, MO, USA), Acros Organics (Geel, Belgium), and Dr. Ehrenstorfer (Teddinton, UK). Atrazine-d5, used as an internal standard, was purchased from Sigma–Aldrich, and $^{13}C_3$-trimethoprim, $^{13}C_6$-sulfamethazine, $^{13}C_6$-sulfamethoxazole, and thiabendazole (ring-$^{13}C_6$) used as surrogate standards were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). According to Table 2, individual stock standard, isotopically labeled internal standard, and surrogate standard solutions were prepared at the concentrations of 50, 100, 200, 500, and 1000 mg/L with appropriate solvents. After completing preparation, all standards were stored at −20 °C. The working standard solutions, containing all antibiotics, were prepared in methanol.

The cartridges used for SPE were Oasis HLB (200 mg, 3 mL) from Waters Corporation (Milford, MA, USA). GF/C filter papers were purchased from Whatman (UK). HPLC-grade methanol, acetonitrile, and water were supplied by J.T. Baker (Darmstadt, Germany). Ammonium hydroxide, hydrochloric acid (37%), ethylenediaminetetraacetic acid disodium salt solution (Na_2EDTA), and formic acid (98%) were from Sigma–Aldrich. The water samples (500 mL) were spiked with 0.2 mL of surrogate standard solutions (100 µg/L) and filtered through GF/C filters, and the pH was adjusted to 2 with 1 M hydrochloric acid solution. Divalent cations were complexed by the addition of 500 mg of Na_2EDTA to extend the extraction efficiency. Oasis HLB cartridges were employed to clean up and concentrate the samples. The cartridges were pre-conditioned sequentially with 5 mL methanol and 5 mL deionized water. Samples were loaded through the cartridges and afterwards, the target compounds were eluted with methanol (4 mL × 2). Eluates were concentrated with a gentle nitrogen stream at 40 °C and reconstituted with methanol (1 mL) after adding the internal standard solution (100 ng).

LC-MS/MS analysis was performed on a 6470 Triple Quad LC/MS coupled to 1290 Infinity II UHPLC (Agilent Technologies, Inc., Santa Clara, CA, USA) operated in electrospray ionization mode. The analytical column was an HSS T3 column (100 × 2.1 mm i.d., 2.6 µm, Waters Corporation, Milford, MA, USA), and the oven temperature of the column was 40 °C. The injection volume was 5 µL and the mobile phases were eluted at 0.3 mL min^{-1}. Mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). For gradient elution, the initial combination was 95:5 (A:B, v/v), and after 2 min the B solution was increased to 95% for 5 min, and held for 2 min. To establish the scheduled multiple reaction monitoring (MRM) condition on the 6470 Triple Quad LC/MS, precursor ions, product ions, fragmentor voltages, and collision voltages were optimized through the flow injection of each compound standard solution (1 µg/mL) (Table 2). To calculate the method of detection limits (MDLs) and limits of quantification (LOQs), seven replicated samples were prepared by adding fortifying compounds (14 ng/L) to deionized water and analyzed with the established method. For the recovery test, the deionized water sample (500 mL) was placed into a 1000 mL glass bottle and fortified with the standard mixture solution at 100 ng/L levels, except for acetaminophen, clinafloxacin, and nifedipine at 200 ng/L. Subsequently, the sample was treated via the above sample preparation method and quantitatively analyzed by LC–MS/MS.

Table 1. The information on the usage and chemical properties of the 49 pharmaceuticals selected in this study.

Group	Pharmaceuticals	CAS No.	Usage	Chemical Formula	MW *	logKow
Tetracyclines	4-Epichlortetracycline	14297-93-9	Antibacterial	C22H23ClN2O8	478.90	-
Tetracyclines	4-epi-Oxytetracycline	14206-58-7	Antibiotic	C22H24N2O9	460.40	-
Tetracyclines	4-Epianhydrotetracycline	7518-17-4	Antibiotic	C22H22N2O7	426.40	-
Anilines	Acetaminophen	103-90-2	Anti-inflammatory	C8H9NO2	151.16	0.46
Phenicillines	Ampicillin	69-53-4	Antibacterial	C16H19N3O4S	349.11	1.35
Tetracyclines	Anhydrotetracycline	1665-56-1	Antibiotic	C22H22N2O7	426.40	-
Macrolides	Azithromycin	83905-01-5	Antibacterial	C38H72N2O12	748.51	4.02
Methylxanthines	Caffeine	58-08-2	Neuropsychiatric agent	C8H10N4O2	194.19	−0.07
Carboxamides	Carbamazepine	298-46-4	Neuropsychiatric agent	C15H12N2O	236.27	2.45
Tetracyclines	Chlortetracycline	57-62-5	Antibacterial	C22H23ClN2O8	478.90	-
Macrolides	Clarithromycin	81103-11-9	Antibacterial	C38H69NO13	747.48	3.16
Fluoroquinolones	Clinafloxacin	105956-97-6	Antibacterial	C17H17ClFN3O3	365.80	-
Dihydropyridnes	Dehydronifedipine	67035-22-7	Nifedipine metabolite	C17H16N2O6	344.32	-
Digitalis glycosides	Digoxigenin	1672-46-4	Digoxin metabolite	C23H34O5	390.50	1.10
Diphenhydramines	Diphenhydramine	58-73-1	Anti-allergic agent	C17H21NO	255.16	3.27
Tetracyclines	Doxycycline	564-25-0	Antibacterial	C22H24N2O8	444.40	−0.02
Fluoroquinolones	Enrofloxacin	93106-60-6	Antibacterial	C19H22FN3O3	359.40	-
Amphenicols	Florfenicol	73231-34-2	Antibacterial	C12H14Cl2FNO4S	358.20	-
Quinolones	Flumequine	42835-25-6	Antibacterial	C14H12FNO3	261.08	1.6
Others	Fluoxetine	54910-89-3	Neuropsychiatric agent	C17H18F3NO	309.33	4.05
Fluoroquinolones	Lomefloxacin	98079-51-7	Antibacterial	C17H19F2N3O3	351.14	2.8
Fluoroquinolones	Marbofloxacin	115550-35-1	Antibacterial	C17H19FN4O4	362.14	-
Quinolones	Nalidixic acid	389-08-2	Antibacterial	C12H12N2O3	232.09	1.41
Dihydropyridnes	Nifedipine	21829-25-4	Cardiovascular agent	C17H18N2O6	346.12	2.2
Progesterones	Norgestimate	35189-28-7	Progesterone	C23H31NO3	369.23	4.98
Fluoroquinolones	Ofloxacin	82419-36-1	Antibacterial	C18H20FN3O4	361.40	−0.39
Others	Ormetoprim	6981-18-6	Antibacterial	C14H18N4O2	274.14	-
Tetracyclines	Oxytetracycline	79-57-2	Antibacterial	C22H24N2O9	460.15	−0.9
Macrolides	Roxithromycin	80214-83-1	Antibacterial	C41H76N2O15	837.00	1.7
Sulfonamides	Sulfachloropyridazine	80-32-0	Antibacterial	C10H9ClN4O2S	284.72	-
Sulfonamides	Sulfaclozine	102-65-8	Antibacterial	C10H9ClN4O2S	284.72	-
Sulfonamides	Sulfadiazine	68-35-9	Antibacterial	C10H10N4O2S	250.28	−0.09
Sulfonamides	Sulfadimethoxine	122-11-2	Antibacterial	C12H14N4O4S	310.33	1.63
Sulfonamides	Sulfadoxine	2447-57-6	Antibacterial	C12H14N4O4S	310.07	0.7
Sulfonamides	Sulfaethoxypyridazine	963-14-4	Antibacterial	C12H14N4O3S	294.33	-
Sulfonamides	Sulfamerazine	127-79-7	Antibacterial	C11H12N4O2S	264.31	0.14
Sulfonamides	Sulfamethazine	57-68-1	Antibacterial	C12H14N4O2S	278.33	0.14
Sulfonamides	Sulfamethizole	144-82-1	Antibacterial	C9H10N4O2S2	270.03	0.54
Sulfonamides	Sulfamethoxazole	723-46-6	Antibacterial	C10H11N3O3S	253.28	0.89
Sulfonamides	Sulfamethoxypyridazine	80-35-3	Antibacterial	C11H12N4O3S	280.06	-
Sulfonamides	Sulfamonomethoxine	1220-83-3	Antibacterial	C11H12N4O3S	280.06	−0.037
Sulfonamides	Sulfaquinoxaline	59-40-5	Antibacterial	C14H12N4O2S	300.34	1.68
Sulfonamides	Sulfathiazole	72-14-0	Antibacterial	C9H9N3O2S2	255.30	0.05
Sulfonamides	Sulfisoxazole	127-69-5	Antibacterial	C11H13N3O3S	267.07	1.01
Tetracyclines	Tetracycline	60-54-8	Antibiotic	C22H24N2O8	444.40	−1.37
Benzimidazoles	Thiabendazole	148-79-8	Antibiotic	C10H7N3S	201.25	2.47
Others	Trimethoprim	738-70-5	Antibacterial	C14H18N4O3	290.32	0.91
Others	Virginiamycin M1	21411-53-0	Antibiotic	C28H35N3O7	525.6	-
Others	Virginiamycin S1	23152-29-6	Antibiotic	C43H49N7O10	823.9	-

Note: * MW: molecular weight.

Table 2. The concentrations and solvent types of stock solutions and mass spectrometer characteristics of target compounds.

No.	Name	Concentration of Stock Solution (mg/L)	Solvent for Stock Solution	Retention Time (min)	Precursor Ion (Fragmentor, V)	Product Ion (CE, V)
1	4-Epianhydrotetracycline (HCl)	1000	Methanol	5.167	427.2 (126)	410.1 (17), 98.1 (45)
2	4-Epichlortetracycline	1000	Methanol	4.875	479.1 (134)	444.1 (21), 462.1 (17), 98.1 (41)
3	4-Epioxytetracycline	1000	Methanol	4.586	461.2 (132)	426.1 (21), 444.1 (17), 201 (45)
4	Acetaminophen	1000	Methanol	3.954	152.1 (112)	110.1 (17), 93.1 (25), 65.1 (33)
5	Ampicillin	1000	Methanol	4.417	350.1 (120)	106.1 (21), 160 (13), 114 (33)
6	Anhydrotetracycline (HCl)	1000	Methanol	5.423	427.2 (122)	410.1 (17), 97.9 (49), 154 (21)
7	Azithromycin	1000	Methanol	4.81	749.5 (165)	591.4 (29), 158.1 (45), 116.1 (45)
8	Caffeine	1000	20% Methanol	4.472	195.1 (130)	138 (21), 110.1 (25), 83.1 (33)
9	Carbamazepine	1000	Methanol	5.974	237.1 (132)	194 (21), 193 (41), 165 (57)
10	Chlortetracycline (HCl)	1000	Methanol	5.052	479 (85)	444.1 (20), 426 (25), 154 (30)
11	Clarithromycin	1000	Methanol	5.803	748.5 (167)	158.1 (29), 590.4 (17), 83.2 (77)
12	Clinafloxacin	200	50% Methanol	4.653	366.1 (173)	322.1 (17), 279 (25)
13	Dehydronifedipine	200	Methanol	6.576	345.1 (175)	283.8 (29), 151.9 (80), 267.8 (33)
14	Digoxigenin	1000	Methanol	5.184	391.3 (134)	355.2 (13), 105.1 (57), 91.1 (77)
15	Diphenhydramine (HCl)	1000	Methanol	5.448	256.2 (81)	167 (17), 165 (49), 152 (45)
16	Doxycycline (HCl)	1000	Methanol	5.134	445 (130)	428.1 (20), 321.1 (29), 267 (35)
17	Enrofloxacin	1000	Methanol	4.671	360.2 (83)	342.2 (25), 316.2 (17), 245 (29)
18	Florfenicol	1000	Methanol	5.418	355.9 (150)	336 (7), 185 (19)
19	Flumequine	200	Methanol	6.147	262.1 (120)	244 (17), 202 (37), 174 (48)
20	Fluoxetine	500	Methanol	5.809	310.1 (79)	148.1 (5), 91.1 (80), 117.1 (65)
21	Lomefloxacin (HCl)	1000	Methanol	4.608	352.2 (122)	265 (25), 334.1 (21), 308.1 (17)
22	Marbofloxacin	1000	Dimethyl sulfoxide	4.477	362.8 (140)	72 (25), 344.9 (21), 319.8 (15)
23	Nalidixic acid	200	Methanol:acetone (1:1)	6.087	232.8 (110)	214.8 (12), 158.9 (36), 186.8 (27)
24	Nifedipine	1000	Methanol	6.603	347.1 (79)	314.8 (5), 253.8 (17), 167.1 (65)
25	Norgestimate	1000	Methanol	7.644	370.2 (179)	124 (37), 77.1 (77), 91.1 (61)
26	Ofloxacin	200	Methanol	4.526	362.2 (134)	318.1 (21), 261 (29), 205 (45)
27	Ormetoprim	1000	Methanol	4.596	275.2 (169)	259.1 (29), 123 (25), 81.1 (53)
28	Oxytetracycline (HCl)	1000	Methanol	4.57	461 (130)	426 (20), 321.1 (29), 267 (35)
29	Roxithromycin	1000	Methanol	5.817	837.5 (155)	679.5 (21), 116 (41), 158.2 (37)
30	Sulfachloropyridazine	1000	Methanol	5.267	285 (110)	156 (13), 92.1 (33), 108 (29)
31	Sulfaclozine sodium	1000	Dimethyl sulfoxide	5.668	285 (120)	92.1 (33), 108 (25), 156 (17)
32	Sulfadiazine	200	Methanol	4.389	251.1 (118)	156 (15), 65.1 (53), 92.1 (29)
33	Sulfadimethoxine	1000	Methanol	5.697	311.1 (126)	156 (21), 92.1 (41), 65.1 (61)

Table 2. *Cont.*

No.	Name	Concentration of Stock Solution (mg/L)	Solvent for Stock Solution	Retention Time (min)	Precursor Ion (Fragmentor, V)	Product Ion (CE, V)
34	Sulfadoxine	1000	Methanol	5.377	310.8 (140)	156 (18), 107.9 (30), 92 (36)
35	Sulfaethoxypyridazine	1000	Methanol	5.378	294.8 (140)	155.8 (17), 139.9 (19), 107.9 (30)
36	Sulfamerazine	1000	Methanol	4.727	265.1 (122)	92.1 (33), 65.1 (61), 156 (17)
37	Sulfamethazine	1000	Methanol	4.95	279.1 (128)	186 (17), 92.1 (33), 156 (19)
38	Sulfamethizole	1000	Methanol	4.904	271 (79)	156 (13), 92.1 (29), 65.1 (57)
39	Sulfamethoxazole	1000	Methanol	5.385	254.1 (110)	156 (15), 65.1 (53), 92.1 (29)
40	Sulfamethoxypyridazine	1000	Dimethyl sulfoxide	4.949	281 (130)	156 (17), 108 (27), 92.1 (31)
41	Sulfamonomethoxine	1000	Methanol	5.141	280.8 (80)	156 (19), 107.9 (28), 92 (31)
42	Sulfaquinoxaline	1000	Acetone	5.684	300.8 (80)	155.8 (17), 107.9 (25), 91.9 (31)
43	Sulfathiazole	1000	Methanol	4.501	256 (112)	155.9 (13), 92.1 (25), 65.1 (53)
44	Sulfisoxazole	1000	Methanol	5.478	267.8 (70)	155.8 (11), 112.9 (15), 92 (29)
45	Tetracycline (HCl)	1000	Methanol	4.701	445 (95)	410 (15), 154 (30)
46	Thiabendazole	1000	Methanol	4.423	202 (167)	175 (29), 131 (37), 65.1 (53)
47	Trimethoprim	1000	Methanol	4.498	291.2 (169)	230 (25), 261 (29), 123 (29)
48	Virginiamycin M1	1000	Methanol	6.247	526.3 (116)	354.9 (15), 507.8 (11), 108.9 (37)
49	Virginiamycin S1	500	Methanol	6.82	823.8 (230)	204.9 (54), 289.9 (36), 565.7 (32)
-	$^{13}C_3$-Trimethoprim	50	Methanol	4.497	293.8 (170)	125.9 (27), 232.8 (29), 263.9 (29)
-	$^{13}C_6$ Sulfamethazine	100	Acetonitrile	4.948	285.1 (132)	185.8 (16), 161.8 (18), 113.9 (29)
-	$^{13}C_6$-Sulfamethoxazole	100	Acetonitrile	5.383	260.1 (122)	98 (29), 113.9 (25), 161.8 (14)
-	Thiabendazole (ring-$^{13}C_6$)	100	Acetonitrile	4.422	208 (171)	180.8 (29), 70 (53), 136.9 (41)
-	Atrazine-d_5	1000	Methanol	-	221.1 (124)	179 (21), 69.1 (45), 101.1 (29)

2.3. Environmental Risk Assessment

Ecotoxicological data and information on 49 pharmaceutical substances were collected from the US EPA ECOTOX Knowledgebase (https://cfpub.epa.gov/ecotox/) (accessed on 23 July 2023), which is one of the largest databases to have been validated by previous studies [21,22] regarding the accuracy and reliability of the test methods, species, and results. The ecotoxicological effects of each pharmaceutical were assessed based on toxicity values, such as EC50, LC50, LOEC, and NOEC, which covered acute and chronic endpoints (e.g., survival, growth, behavior, reproduction, etc.). These tests were conducted on standard test species in freshwater, including algae, crustaceans, and fish, with high accuracy and reliability. Considering for the worst-case scenario, the lowest toxicity values were selected from the most sensitive test species. Assessment factors (AF) were also chosen in the range of 10 to 1000, taking into consideration the different nutrition stages of each species (US EPA, http://www.epa.gov/risk_assessment/glossary.htm) (accessed on 1 August 2023), as guided by reference guidelines [23] and the European Commission [24]. Subsequently, we

calculated the predicted no-effect concentration (PNEC) values by dividing the acute or chronic toxicity value by the selected AF, following Equation (1).

$$PNEC = \frac{Lowest\ NOEC\ or\ EC_{50}}{AF} \quad (1)$$

Risk quotient (RQ) was determined by dividing the mean, minimum, and maximum values of the measured environmental concentration (MEC) for pharmaceuticals in the surface water samples collected from the upstream to the downstream of the Seokseong and Nonsan-Gangkyoung streams in the Geum River basin area by PNEC at a screening level.

$$Risk\ Quotient\ (RQ) = \frac{MEC}{PNEC} \quad (2)$$

Based on the criteria suggested by several previous studies [3,25–28], the risk category was determined by classifying the calculated RQ into three categories. The three risk categories are defined as follows: RQ < 0.1 (low risk), 0.1 ≤ RQ < 1 (moderate risk), and RQ ≥ 1 (high risk), with RQ exceeding 1 indicating a high level of risk. All statistical analyses were conducted using IBM SPSS Statistics for Windows version 23.0 (IBM Corp., Armonk, NY, USA) and R statistical software version 4.2.2 with Rstudio version 2023.03.1+446 (Rstudio Inc., Boston, MA, USA). A *p*-value less than 0.05 in a two-sided test was considered statistically significant.

3. Results and Discussion

The performance of the analytical method was evaluated through the estimation of the linearity, recoveries, MDLs, and LOQs. Quantification was based on linear regression calibration curves. The calibration curves provided good fits ($r^2 > 0.99$) over the established concentrations, ranging from 0.5, 1, 2, 5, 10, 20, 25, 50, 75, and 100 ng/L, depending on the compounds (Table 3). The concentrations of surrogate and internal standards were set at 50 ng/L and 100 ng/L, respectively. MDLs were calculated based on the standard deviations of seven surface water samples spiked with target analytes at concentrations of 100 ng/L, except for acetaminophen, clinafloxacin, and nifedipine, at concentrations of 200 ng/L. The MDLs of target compounds were within the range of 2.39 ng/L to 14.54 ng/L, while LOQs ranged from 7.60 ng/L to 46.32 ng/L (Table 3).

The recovery rates were calculated to verify the accuracy and precision of the measurements. The recoveries of water samples ranged from 67.2% to 137.0% and the relative standard deviations (%RSD) were satisfactory, ranging from 3.2% to 17.6%. Acetaminophen, clinafloxacin, and nifedipine, with lower sensitivities than the others, were fortified at 200 ng/L (Figure 2). The water quality parameters (e.g., water temperature, pH, biochemical oxygen demand, chemical oxygen demand, dissolved oxygen, total organic carbon, total nitrogen, total phosphorus, electrical conductivity, and suspended solids) of samples were measured in the field using a water quality multiprobe. The values of electrical conductivities at the upstream points, such as S1 and N1, were 1896 µS/cm and 950 µS/cm, respectively, which are higher than those taken at the rest of the sampling sites (Figure 3).

The validated methodology developed in this study was applied to all water samples collected from the Seokseong and Nonsan-Gangkyoung streams of the Geum River watershed. The summary statistics for the measured environmental concentrations of 49 pharmaceutical substances are presented in Table 4. The overall arithmetic mean (AM) and standard deviation (SD) were calculated as 0.017 ± 0.74 µg/L, with concentrations ranging from 0.001 to 9.212 µg/L. The six highest concentrations detected were as follows: 9.212 µg/L (sulfathiazole at S1), 8.479 µg/L (acetaminophen at S1), 8.036 µg/L (marbofloxacin at N1), 5.885 µg/L (florfenicol at N1), 1.591 µg/L (4-epichlortetracycline at N1), and 1.487 µg/L (chlortetracycline at N1) (Figure 4). Out of the 49 pharmaceuticals, the overall detection frequency (%) was 25.5%, with the highest detection frequency being 100% for caffeine. Some pharmaceuticals, including acetaminophen, azithromycin, carbamazepine, florfenicol, sulfamerazine, sulfamethazine, sulfamethoxazole, and sul-

fathiazole, were also detected at a frequency of ≥50%. While higher concentrations and a greater variety of pharmaceutical compounds were observed in the Seokseong stream (AM ± SD: 0.20 ± 0.89 μg/L) compared to the Nonsan-Gangkyoung stream (AM ± SD: 0.17 ± 0.69 μg/L), there was no significant difference between the two streams (Table 4).

Table 3. Regression equations, coefficients of determination (r^2), linear ranges, MDLs, and LOQs of 49 pharmaceuticals analyzed by LC-MS/MS.

No.	Name	Regression Equation	r^2	Linear Range (ng/mL)	MDL (ng/L)	LOQ (ng/L)
1	4-Epianhydrotetracycline	$y = 0.001121x - 0.001526$	0.9970	2–50	6.25	19.90
2	4-Epichlortetracycline	$y = 1.558725^{-4}x - 2.440391^{-4}$	0.9989	5–50	9.77	31.12
3	4-Epioxytetracycline	$y = 0.001002x - 0.001425$	0.9998	2–75	9.72	30.95
4	Acetaminophen	$y = 1.749150^{-4}x + 1.243825^{-4}$	0.9984	5–100	4.78	15.21
5	Ampicillin	$y = 2.354800^{-4}x - 0.001163$	0.9945	5–75	5.29	16.85
6	Anhydrotetracycline	$y = 0.002668x - 0.001453$	0.9926	5–50	10.45	33.29
7	Azithromycin	$y = 0.002338x - 0.007140$	0.9988	5–75	3.68	11.71
8	Caffeine	$y = 4.009872^{-4}x + 4.980610^{-4}$	0.9980	5–100	4.85	15.44
9	Carbamazepine	$y = 0.002668x - 3.984711^{-4}$	0.9991	0.5–10	7.76	24.71
10	Chlortetracycline	$y = 3.231145^{-4}x - 0.001658$	0.9919	5–50	5.60	17.82
11	Clarithromycin	$y = 0.018081x - 5.018711^{-4}$	0.9995	0.5–10	3.96	12.61
12	Clinafloxacin	$y = 6.727147^{-4}x - 0.010709$	0.9995	20–100	14.53	46.27
13	Dehydro nifedipine	$y = 0.006114x + 9.052315^{-5}$	0.9997	0.5–25	3.85	12.27
14	Digoxigenin	$y = 2.547734^{-4}x + 2.013892^{-4}$	0.9982	5–75	5.06	16.10
15	Diphenhydramine	$y = 0.062808x - 0.007092$	0.9993	0.5–10	3.16	10.05
16	Doxycycline	$y = 6.306953^{-4}x - 0.001976$	0.9918	5–50	8.95	28.52
17	Enrofloxacin	$y = 8.330491^{-4}x - 0.001309$	0.9975	2–50	7.69	24.47
18	Florfenicol	$y = 3.093383^{-4}x - 1.114011^{-4}$	0.9919	2–20	7.19	22.89
19	Flumequine	$y = 0.002215x + 4.140469^{-5}$	0.9998	0.5–50	2.72	8.67
20	Fluoxetine	$y = 0.001533x + 3.075431^{-4}$	0.9971	0.5–10	5.57	17.73
21	Lomefloxacin	$y = 0.001862x - 0.007739$	0.9965	5–50	14.34	45.66
22	Marbofloxacin	$y = 7.051740^{-4}x - 0.002732$	0.9931	5–75	14.54	46.32
23	Nalidixic acid	$y = 0.002525x - 1.533324^{-4}$	0.9979	0.5–20	2.67	8.49
24	Nifedipine	$y = 7.860068^{-4}x - 4.214378^{-6}$	0.9987	0.5–20	7.88	25.11
25	Norgestimate	$y = 0.001300x - 9.673252^{-5}$	0.9996	0.5–20	3.44	10.94
26	Ofloxacin	$y = 0.001558x - 0.006164$	0.9976	5–50	11.66	37.14
27	Ormetoprim	$y = 0.012046x + 0.001497$	0.9969	0.5–10	4.76	15.17
28	Oxytetracycline	$y = 4.831707^{-4}x - 0.001026$	0.9962	5–50	8.19	26.07
29	Roxithromycin	$y = 0.005459x - 1.874623^{-4}$	0.9991	0.5–10	2.69	8.55
30	Sulfachloropyridazine	$y = 0.002094x + 9.687999^{-4}$	0.9995	0.5–100	6.44	20.52
31	Sulfaclozine	$y = 3.860528^{-4}x - 8.547179^{-6}$	0.9928	1–25	4.71	15.00
32	Sulfadiazine	$y = 6.807058^{-4}x - 6.161956^{-5}$	0.9994	1–100	5.06	16.11
33	Sulfadimethoxine	$y = 0.002052x + 7.023815^{-4}$	0.9989	0.5–50	5.45	17.37
34	Sulfadoxine	$y = 0.001233x + 1.072618^{-4}$	0.9988	1–20	5.26	16.76
35	Sulfaethoxypyridazine	$y = 0.001003x - 6.420953^{-5}$	0.9992	1–50	5.07	16.16
36	Sulfamerazine	$y = 7.798603^{-4}x + 5.484095^{-5}$	0.9989	0.5–25	6.05	19.28
37	Sulfamethazine	$y = 0.001340x - 2.742459^{-6}$	0.9995	0.5–20	6.08	19.35
38	Sulfamethizole	$y = 0.001298x - 1.781222^{-4}$	0.9996	0.5–10	3.63	11.56
39	Sulfamethoxazole	$y = 8.795675^{-4}x - 1.933642^{-4}$	0.9977	1–20	3.90	12.42
40	Sulfamethoxypyridazine	$y = 0.001079x + 3.132912^{-4}$	0.9996	2–50	4.41	14.04
41	Sulfamonomethoxine	$y = 4.505160^{-4}x - 9.963152^{-5}$	0.9963	2–75	5.64	17.95
42	Sulfaquinoxaline	$y = 6.615775^{-4}x + 1.406531^{-4}$	0.9982	0.5–50	3.40	10.82
43	Sulfathiazole	$y = 9.418226^{-4}x + 6.183180^{-5}$	0.9997	0.5–20	4.23	13.46
44	Sulfisoxazole	$y = 7.569998^{-4}x - 9.792676^{-5}$	0.9983	1–20	4.64	14.77
45	Tetracycline	$y = 2.871229^{-4}x - 0.001264$	0.9923	5–50	6.38	20.31
46	Thiabendazole	$y = 0.005822x + 4.603184^{-4}$	0.9967	0.5–10	2.39	7.60
47	Trimethoprim	$y = 0.005269x + 0.001399$	0.9988	0.5–10	5.69	18.12
48	Virginiamycin M1	$y = 1.333264^{-4}x + 5.226565^{-5}$	0.9998	2–75	10.14	32.29
49	Virginiamycin S1	$y = 1.754436^{-5}x - 2.779775^{-5}$	0.9937	10–75	3.68	11.72

Nevertheless, we observed a similar pattern in the concentration levels of pharmaceuticals measured in the Seokseong and Nonsan-Gangkyoung streams, as well as the Geum River, with the highest MEC at the upstream locations, which values gradually decreased into the downstream areas (Figure 5). The concentrations of six pharmaceuticals, including azithromycin, carbamazepine, clarithromycin, florfenicol, roxithromycin, and trimethoprim, were higher in the middle points of both streams (S4 and S5 of Seokseong stream, N5 of Nonsan-Gangkyoung stream) compared to the upper and downstream locations. For example, carbamazepine, which is used for human diseases such as seizure disorders and neuropathic pain [29], was highly detected, with concentrations of 0.065 μg/L (at S4) and 0.055 μg/L (at N5) in the middle points of both streams. This suggests that the elevated concentration levels at these middle points might be attributed to the significant amounts of human activities and sewage treatment plants, rather than the upstream livestock farms.

Carbamazepine was also detected in five rivers in the Busan area, including the Nakdong and Maekdo Rivers, at concentrations ranging from 0.012 to 0.095 μg/L, with an average concentration of 0.037 ± 0.030 μg/L. In rivers in the Ulsan watershed, such as the Taehwagang and Dongcheongang Rivers, concentrations of carbamazepine were also detected, with the concentrations reaching up to 0.146 μg/L [30]. Within the Nakdong River watershed, the maximum observed level of carbamazepine was 0.089 μg/L in the middle reaches and 0.177 μg/L in the lower reaches, while the mean concentration was relatively lower, at 0.0016 μg/L in the upper reaches [31]. Moreover, in the surface water collected from rivers near a large pharmaceutical industrial complex area, the concentrations of acetaminophen were found to be high, reaching levels of 341 μg/L in the middle and 127 μg/L in the lower reaches [32]. Most of the detected pharmaceuticals, such as acetaminophen, anhydrotetracycline, caffeine, and chlortetracycline, were detected at higher concentrations in the upstream areas compared to the downstream areas. This could be attributed to the proximity of these upstream sites to livestock farming complexes. A group of tetracyclines, including anhydrotetracycline and chlortetracycline, and a group of sulfonamides, including sulfamerazine, sulfamethazine, sulfamethoxazole, and sulfathiazole, were also detected in the study area. This finding aligns with those of Lim et al. [33], who reported that antimicrobials of tetracycline, penicillin and sulfonamide have higher rates of sales than others.

Kim et al. [14] reported 2.91 μg/L, 3.52 μg/L, 0.73 μg/L, and 1.23 μg/L as the maximum concentrations for tetracycline, 4-epitetracycline, anhydrotetracycline, and 4-epianhydrotetracycline, respectively, in river samples from the livestock complex area. Lee et al. [31] also reported that clarithromycin was detected at 0.0316 μg/L, with mean concentrations of 20–65%, in the Nakdong River Basin, whereas it was detected at 10.07 to 45.12 ng/L in the Nonsan-Gangkyoung stream in this study. The highest detection frequency was observed for sulfamethazine (75%) at 0.03 to 211 μg/L, followed by oxytetracycline (64%) ranging from 0.07 to 72.9 μg/L in animal wastewater and surface water around farms in Jiangsu Province, China (Wei et al. 2011) [17]. In this study, sulfamethazine and oxytetracycline were detected at levels ranging from 5.76 to 385.47 ng/L and 14.22 to 21.22 ng/L, respectively.

Table 5 gives detailed information on the test species, type, duration, toxicological effects, endpoints, lowest concentrations, AF, and PNEC values for each pharmaceutical. Among the 49 pharmaceuticals, only 30, including acetaminophen, carbamazepine, florfenicol, and others, had acute or chronic aquatic toxicity data available in the US EPA ECOTOX Knowledgebase (https://cfpub.epa.gov/ecotox/) (accessed on 23 July 2023). The most sensitive endpoints in these toxicity data were mainly chronic effects (LOEC or NOEC) on the population, and the growth and mortality of algae, fish, and crustaceans. The lowest PNEC was estimated as 0.01 μg/L for fluoxetine and diphenhydramine, and the highest one was 1000 μg/L for sulfadimethoxine.

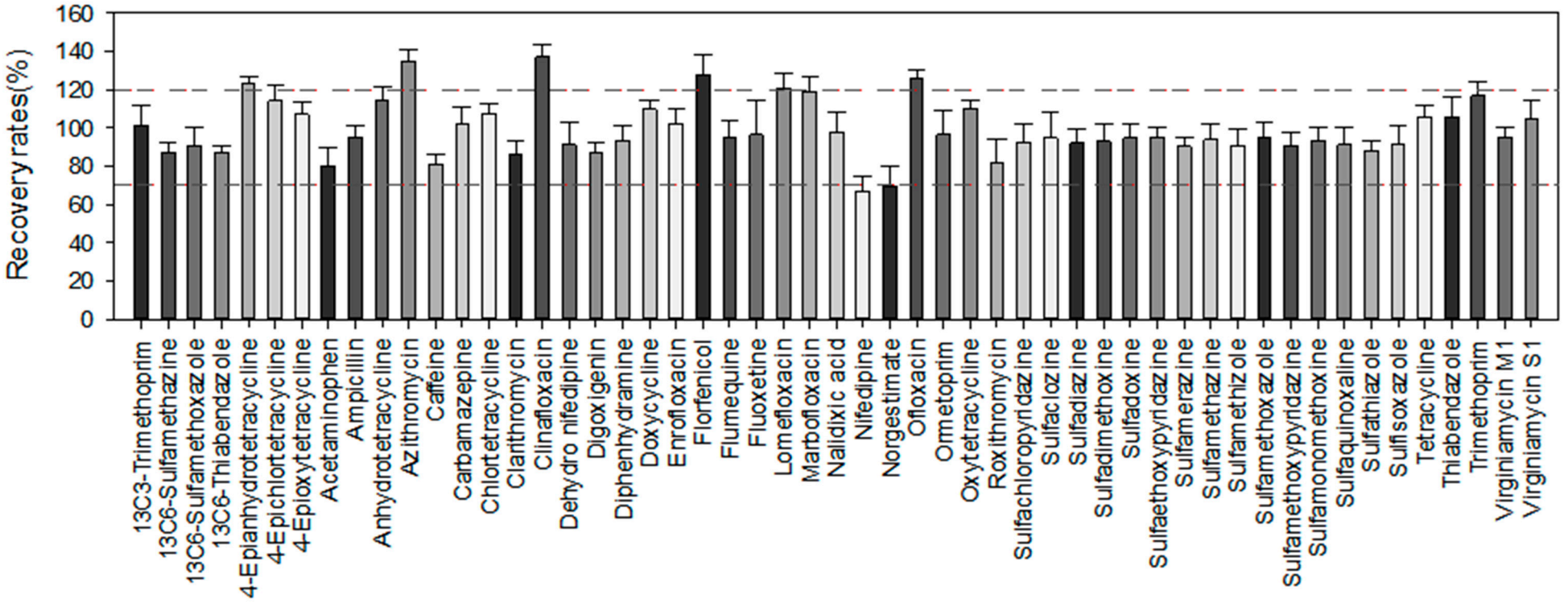


Figure 2. Recovery rates of 49 pharmaceuticals selected in this study.

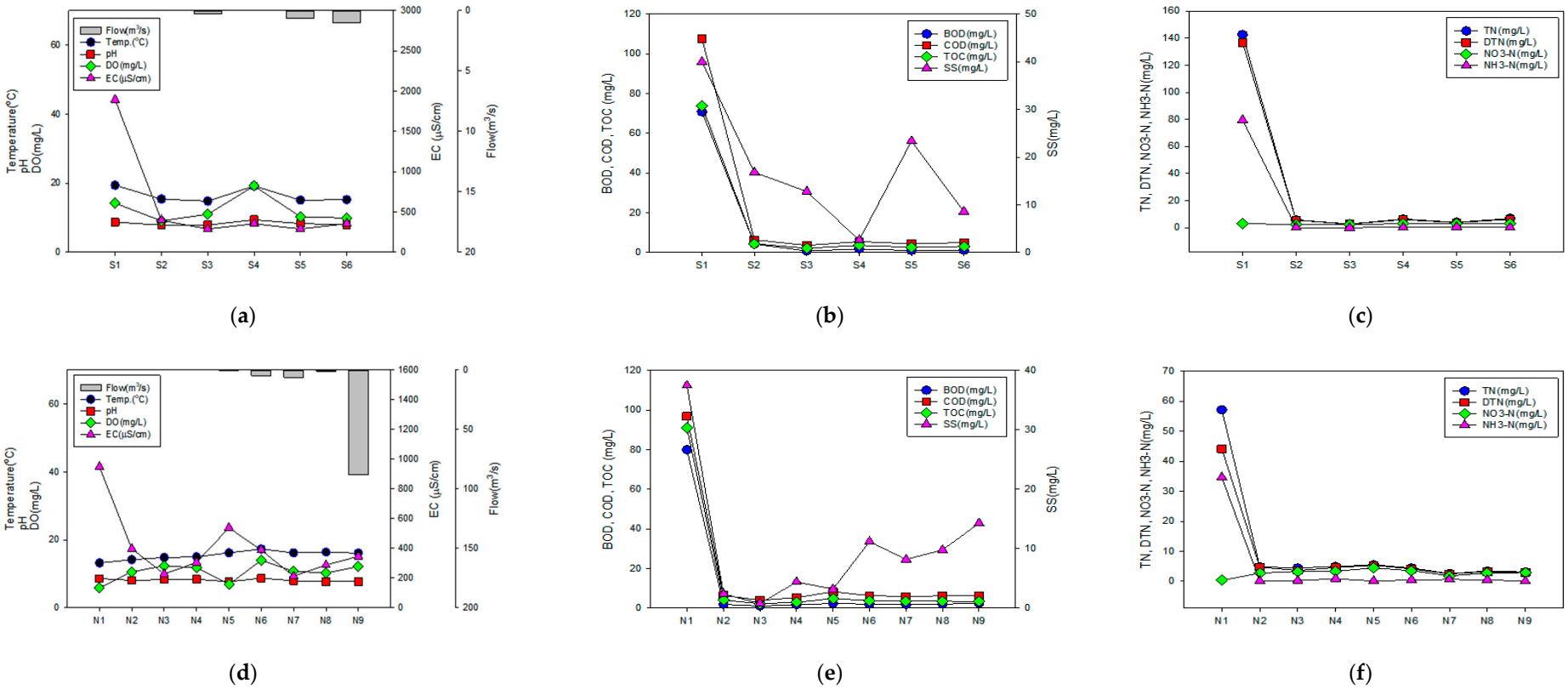


Figure 3. Water quality parameters measured in Seokseong (**a**–**c**) and Nonsan-Gangkyoung (**d**–**f**) streams of the Geum River basin (abbreviations: BOD, biochemical oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; Flow, discharge flow (m^3/s); Temp, water temperature (°C); TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; pH, hydrogen ion concentration; EC, electrical conductivity; SS, suspended solids).

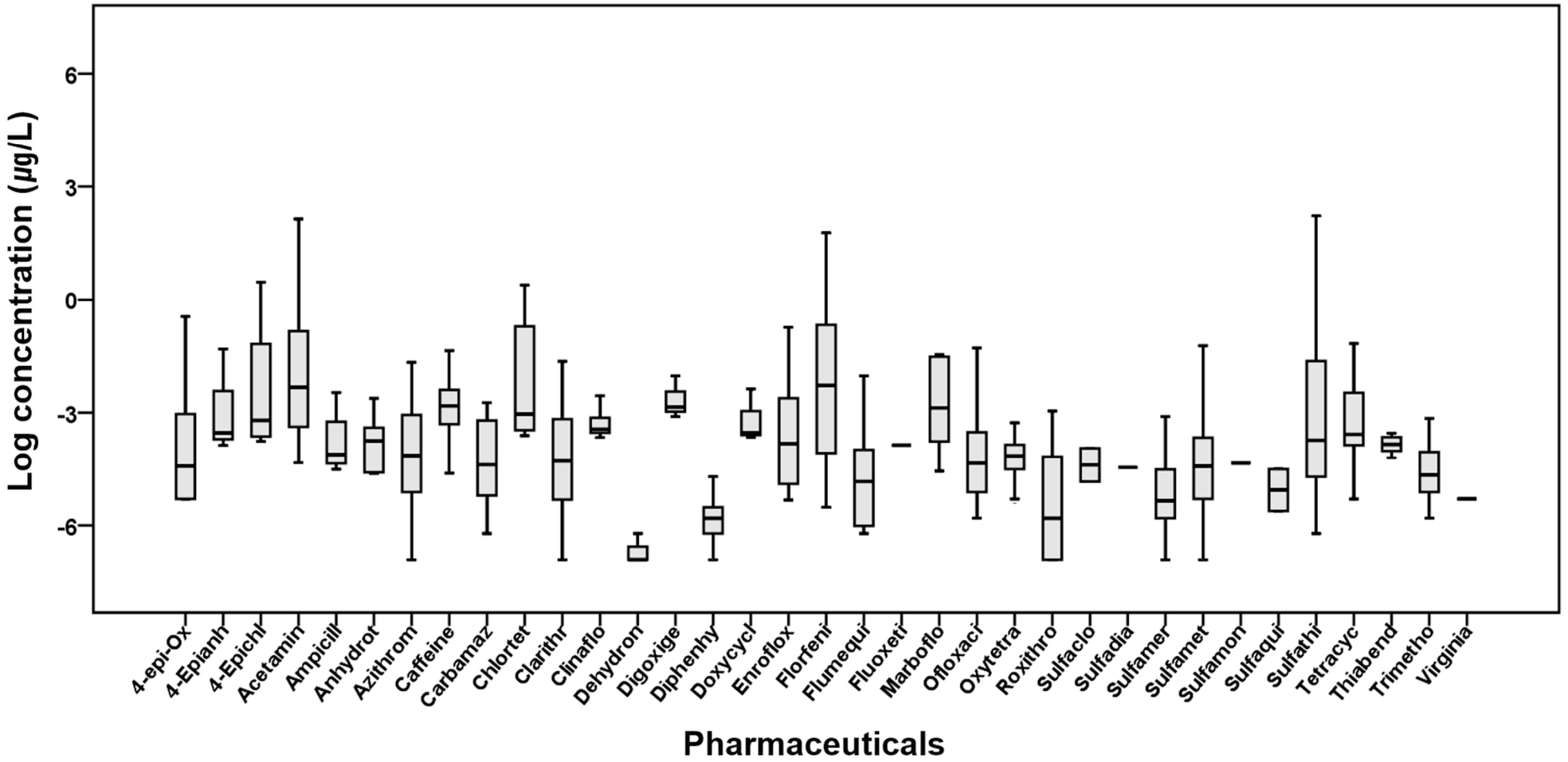
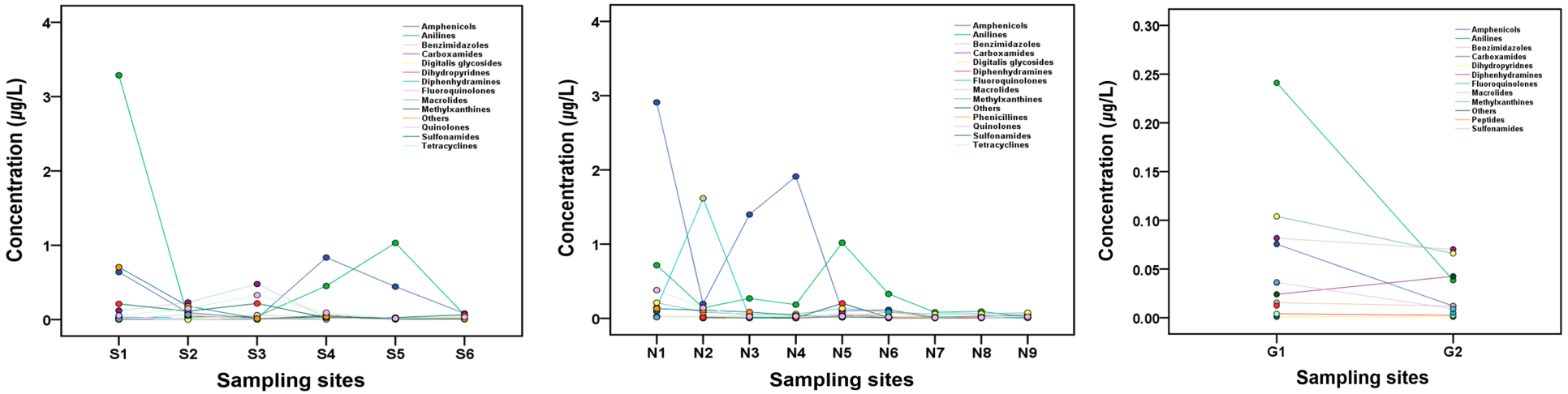


Figure 4. The log-transformed measured environment concentrations (MECs) of 49 pharmaceuticals analyzed using LC-MS/MS.



(**a**) (**b**) (**c**)

Figure 5. The mean concentration levels of 49 pharmaceutical residues collected from October 2018 to March 2019 at each site: (**a**) Seokseong stream, (**b**) Nonsan-Gangkyoung stream, (**c**) Geum River.

Table 4. Summary statistics of the measured concentration levels of 49 pharmaceuticals in the Seokseong and Nonsan-Gangkyoung streams of the Geum River basin.

Group	Pharmaceuticals	CAS no.	N (Total)	N (Detected)	Detection Frequency (%)	AM * (μg/L)	SD *	Median (μg/L)	Min (μg/L)	Max (μg/L)
Total			2315	591	25.5	0.017	0.738	0.020	0.001	9.212
Tetracyclines	4-Epichlortetracycline	14297-93-9	51	12	23.5	0.271	0.453	0.041	0.023	1.591
Tetracyclines	4-epi-Oxytetracycline	14206-58-7	48	5	10.4	0.140	0.275	0.012	0.005	0.632
Tetracyclines	4-Epianhydrotetracycline	7518-17-4	48	3	6.3	0.106	0.141	0.029	0.021	0.269
Anilines	Acetaminophen	103-90-2	49	44	89.8	0.527	1.335	0.098	0.013	8.479
Phenicillines	Ampicillin	69-53-4	47	4	8.5	0.032	0.035	0.017	0.011	0.085
Tetracyclines	Anhydrotetracycline	1665-56-1	47	6	12.8	0.029	0.023	0.024	0.010	0.073
Macrolides	Azithromycin	83905-01-5	48	29	60.4	0.040	0.053	0.016	0.001	0.188
Methylxanthines	Caffeine	58-08-2	51	51	100.0	0.100	0.131	0.060	0.010	0.781
Carboxamides	Carbamazepine	298-46-4	47	36	76.6	0.022	0.019	0.013	0.002	0.065
Tetracyclines	Chlortetracycline	57-62-5	51	14	27.5	0.275	0.409	0.048	0.027	1.487
Macrolides	Clarithromycin	81103-11-9	47	31	66.0	0.037	0.053	0.014	0.001	0.193
Fluoroquinolones	Clinafloxacin	105956-97-6	47	5	10.6	0.042	0.021	0.032	0.026	0.078
Dihydropyridnes	Dehydronifedipine	67035-22-7	47	3	6.4	0.001	0.001	0.001	0.001	0.002
Digitalis glycosides	Digoxigenin	1672-46-4	48	3	6.3	0.078	0.046	0.058	0.045	0.131
Diphenhydramines	Diphenhydramine	58-73-1	47	21	44.7	0.010	0.021	0.003	0.001	0.095
Tetracyclines	Doxycycline	564-25-0	47	3	6.4	0.049	0.038	0.029	0.026	0.093
Fluoroquinolones	Enrofloxacin	93106-60-6	49	17	34.7	0.086	0.137	0.022	0.005	0.478
Amphenicols	Florfenicol	73231-34-2	50	41	82.0	0.633	1.311	0.102	0.004	5.885
Quinolones	Flumequine	42835-25-6	51	8	15.7	0.025	0.044	0.008	0.002	0.131
Others	Fluoxetine	54910-89-3	47	1	2.1	0.021	-	-	-	-
Fluoroquinolones	Lomefloxacin	98079-51-7	47	0	0.0	-	-	-	-	-
Fluoroquinolones	Marbofloxacin	115550-35-1	49	8	16.3	1.082	2.811	0.057	0.010	8.036
Quinolones	Nalidixic acid	389-08-2	47	0	0.0	-	-	-	-	-
Dihydropyridnes	Nifedipine	21829-25-4	47	0	0.0	-	-	-	-	-
Progesterones	Norgestimate	35189-28-7	47	0	0.0	-	-	-	-	-
Fluoroquinolones	Ofloxacin	82419-36-1	48	15	31.3	0.045	0.078	0.013	0.003	0.277
Others	Ormetoprim	6981-18-6	47	0	0.0	-	-	-	-	-
Tetracyclines	Oxytetracycline	79-57-2	48	9	18.8	0.062	0.139	0.016	0.004	0.431
Macrolides	Roxithromycin	80214-83-1	48	23	47.9	0.011	0.014	0.003	0.001	0.052
Sulfonamides	Sulfachloropyridazine	80-32-0	47	0	0.0	-	-	-	-	-
Sulfonamides	Sulfaclozine	102-65-8	47	2	4.3	0.014	0.008	0.014	0.008	0.019
Sulfonamides	Sulfadiazine	68-35-9	47	1	2.1	0.011	-	-	-	-
Sulfonamides	Sulfadimethoxine	122-11-2	47	0	0.0	-	-	-	-	-
Sulfonamides	Sulfadoxine	2447-57-6	47	0	0.0	-	-	-	-	-
Sulfonamides	Sulfaethoxypyridazine	963-14-4	47	0	0.0	-	-	-	-	-
Sulfonamides	Sulfamerazine	127-79-7	47	31	66.0	0.015	0.028	0.005	0.001	0.133
Sulfonamides	Sulfamethazine	57-68-1	49	38	77.6	0.053	0.095	0.013	0.001	0.385
Sulfonamides	Sulfamethizole	144-82-1	47	1	2.1	0.008	-	-	-	-
Sulfonamides	Sulfamethoxazole	723-46-6	47	32	68.1	0.023	0.040	0.012	0.002	0.167
Sulfonamides	Sulfamethoxypyridazine	80-35-3	47	1	2.1	0.005	-	-	-	-
Sulfonamides	Sulfamonomethoxine	1220-83-3	47	1	2.1	0.013	-	-	-	-
Sulfonamides	Sulfaquinoxaline	59-40-5	47	2	4.3	0.007	0.005	0.007	0.004	0.011
Sulfonamides	Sulfathiazole	72-14-0	50	30	60.0	0.476	1.683	0.024	0.002	9.212
Sulfonamides	Sulfisoxazole	127-69-5	47	0	0.0	-	-	-	-	-
Tetracyclines	Tetracycline	60-54-8	50	9	18.0	0.071	0.095	0.028	0.005	0.310
Benzimidazoles	Thiabendazole	148-79-8	49	22	44.9	0.023	0.018	0.022	0.001	0.098
Others	Trimethoprim	738-70-5	47	28	59.6	0.037	0.111	0.010	0.001	0.593
Peptides	Virginiamycin M1	21411-53-0	32	0	0.0	-	-	-	-	-
Peptides	Virginiamycin S1	23152-29-6	32	1	3.1	0.005	-	-	-	-

Note: * AM—arithmetic mean, SD—standard deviation.

Table 5. The information on test species (algae, crustaceans, fish), test type, duration, toxicological effects, endpoints, concentrations, assessment factors (AF), and predicted no effect concentrations (PNEC) of pharmaceuticals in the aquatic environment (collected from the US EPA ECOTOX Knowledgebase).

Pharmaceuticals	Species	Class	Effect	Test Type	Duration (Days)	Endpoint	Concentration(µg/L)	AF	PNEC (µg/L)	Reference
Acetaminophen	*Danio rerio*	Fish	Mortality	Chronic	7	NOEC	1.00	10	0.10	David and Pancharatna [34]
Ampicillin	*Microcystis aeruginosa*	Algae	Genetics	Chronic	4	NOEC	10.00	100	0.10	Qian et al. [35]
Azithromycin	*Daphnia magna*	Crustaceans	Behavior	Chronic	4	LOEC	48.00	50	0.96	Li et al. [36]
Caffeine	*Raphidocelis subcapitata*	Algae	Population	Chronic	56	LOEC	5.00	10	0.50	Lawrence and Zhu [37]
Carbamazepine	*Gobiocypris rarus*	Fish	Biochemistry	Chronic	28	NOEC	0.91	10	0.09	Yan et al. [25]
Chlortetracycline	*Oreochromis niloticus*	Fish	Growth	Chronic	48	NOEC	12.00	50	0.24	Koeypudsa et al. [38]
Clarithromycin	*Pseudokirchneriella subcapitata*	Algae	Growth	Chronic	3	NOEC	2.45	10	0.25	Watanabe et al. [39]
Diphenhydramine	*Ceriodaphnia dubia*	Crustaceans	Reproduction	Chronic	21	NOEC	0.12	10	0.01	Meinertz et al. [40]
Doxycycline	*Danio rerio*	Fish	Genetics	Chronic	10	NOEC	20000.0	100	200.0	Zhu et al. [41]
Enrofloxacin	*Microcystis aeruginosa*	Algae	Population	Chronic	5	NOEC	49.00	10	4.90	Robinson et al. [42]
Florfenicol	*Isochrysis galbana*	Algae	Biochemistry	Chronic	3	NOEC	1.00	10	0.10	Zhang et al. [43]
Flumequine	*Microcystis aeruginosa*	Algae	Population	Acute	7	EC_{50}	159.0	10	15.90	Lützhøft et al. [44]
Fluoxetine	*Danio rerio*	Fish	Genetics	Chronic	9	LOEC	0.09	10	0.01	Chai et al. [45]
Lomefloxacin	*Microcystis aeruginosa*	Algae	Population	Acute	7	EC_{50}	186.0	50	3.72	Robinson et al. [42]
Marbofloxacin	*Ceriodaphnia dubia*	Crustaceans	Mortality	Chronic	21	NOEC	2500.0	100	25.00	Kergaravat et al. [46]
Nifedipine	*Danio rerio*	Fish	Physiology	Chronic	2	NOEC	346.3	100	3.46	Meng et al. [47]
Ofloxacin	*Microcystis aeruginosa*	Algae	Population	Acute	5	EC_{50}	21.00	50	0.42	Robinson et al. [42]
Oxytetracycline	*Chlamydomonas reinhardtii*	Algae	Population	Chronic	7	NOEC	100.00	50	2.00	Garcia et al. [48]
Roxithromycin	*Raphidocelis subcapitata*	Algae	Population	Chronic	7	NOEC	6.60	50	0.13	Guo et al. [49]
Sulfachloropyridazine	*Chlorella fusca var. vacuolata*	Algae	Population	Acute	1	EC_{50}	32250.0	100	322.5	Bialk-Bielinska et al. [50]
Sulfadiazine	*Daphnia magna*	Crustaceans	Mortality	Chronic	4	NOEC	50.00	50	1.00	Bundschuh et al. [51]
Sulfadimethoxine	*Oryzias latipes*	Fish	Mortality	Acute	4	LC_{50}	100000.0	100	1000.0	Kim et al. [52]
Sulfamerazine	*Chlorella fusca var. vacuolata*	Algae	Population	Acute	2	EC_{50}	11900.0	100	119.0	Bialk-Bielinska et al. [50]
Sulfamethazine	*Gammarus pulex*	Crustaceans	Mortality	Chronic	4	NOEC	100.0	10	10.00	Bundschuh et al. [51]
Sulfamethoxazole	*Daphnia magna*	Crustaceans	Growth	Chronic	21	NOEC	120.0	10	12.00	Lu et al. [53]
Sulfaquinoxaline	*Daphnia magna*	Crustaceans	Intoxication	Acute	2	EC_{50}	131000.0	1000	131.0	De Liguoro et al. [54]
Sulfathiazole	*Daphnia magna*	Crustaceans	Reproduction	Chronic	21	NOEC	11000.0	100	110.0	Park and Choi [55]
Tetracycline	*Microcystis aeruginosa*	Algae	Population	Chronic	7	NOEC	50.00	10	5.00	Yang et al. [56]
Thiabendazole	*Oncorhynchus mykiss*	Fish	Growth	Chronic	21	NOEC	12.00	50	0.24	U.S. EPA [57]
Trimethoprim	*Danio rerio*	Fish	Mortality	Chronic	21	NOEC	157.0	100	1.57	Madureira et al. [58]

Table 6 presents the results of the risk assessment, including risk quotients (RQ) and three risk categories (low, medium, and high). The RQ values are categorized as follows: The values of 6.33 (Min–Max: 0.04–58.85) for florfenicol, 5.27 (0.13–84.79) for acetaminophen, 2.22 for fluoxetine, 1.15 (0.11–6.20) for chlortetracycline were classified into the high-risk category. RQ values of 0.83 (0.08–7.92) for diphenhydramine, 0.32 (0.11–0.85) for ampicillin, 0.24 (0.02–0.71) for carbamazepine, 0.20 (0.02–1.56) for caffeine, 0.15 (0.001–0.79) for clarithromycin, 0.11 (0.01–0.66) for ofloxacin, and 0.10 (0.001–0.41) for thiabendazole were classified into the moderate-risk category. The remaining pharmaceuticals, including roxithromycin (RQ = 0.08), azithromycin and marbofloxacin (0.04), oxytetracycline (0.03), and the rest of the substances, had lower RQ values (below 0.1), and these were categorized as low-risk (Figure 6). In a previous study, Wang et al. [59] found that chlortetracycline's (137.59 mg/L at 48 h-EC_{50}) toxicity against *Daphnia magna* was significantly higher than that of tetracycline (617.2 mg/L at 48 h-EC_{50}). In another study, Kim et al. [52] reported that the hazard quotients calculated for carbamazepine and trimethoprim were 0.0044 and 0.0017, respectively. However, the hazard quotients for sulfamethoxazole and acetaminophen were 6.3 and 1.8, respectively. The hazard quotient (HQ) values reported by Lee et al. [31] for carbamazepine, clarithromycin, sulfathiazole, and trimethoprim in the Nakdong River watershed, with values of 0.001, 0.14, 0.00003, and 0.00004, respectively, are quite similar to those found in our study.

Table 6. Results of risk assessment using the measured concentrations of pharmaceuticals and three risk categories classified as low, moderate, and high.

Group	Pharmaceuticals	CAS No.	RQ *			Risk Category
			AM	Min	Max	
Anilines	Acetaminophen	103-90-2	5.27	0.13	84.79	High
Phenicillines	Ampicillin	69-53-4	0.32	0.11	0.85	Moderate
Macrolides	Azithromycin	83905-01-5	0.04	0.00	0.20	Low
Methylxanthines	Caffeine	58-08-2	0.20	0.02	1.56	Moderate
Carboxamides	Carbamazepine	298-46-4	0.24	0.02	0.71	Moderate
Tetracyclines	Chlortetracycline	57-62-5	1.15	0.11	6.20	High
Macrolides	Clarithromycin	81103-11-9	0.15	0.00	0.79	Moderate
Diphenhydramines	Diphenhydramine	58-73-1	0.83	0.08	7.92	Moderate
Tetracyclines	Doxycycline	564-25-0	0.00	0.00	0.00	Low
Fluoroquinolones	Enrofloxacin	93106-60-6	0.02	0.00	0.10	Low
Amphenicols	Florfenicol	73231-34-2	6.33	0.04	58.85	High
Quinolones	Flumequine	42835-25-6	0.00	0.00	0.01	Low
Others	Fluoxetine	54910-89-3	2.22 **	-	-	High
Fluoroquinolones	Marbofloxacin	115550-35-1	0.04	0.00	0.32	Low
Fluoroquinolones	Ofloxacin	82419-36-1	0.11	0.01	0.66	Moderate
Tetracyclines	Oxytetracycline	79-57-2	0.03	0.00	0.22	Low
Macrolides	Roxithromycin	80214-83-1	0.08	0.01	0.39	Low
Sulfonamides	Sulfadiazine	68-35-9	0.01 **	-	-	Low
Sulfonamides	Sulfamerazine	127-79-7	0.00	0.00	0.00	Low
Sulfonamides	Sulfamethazine	57-68-1	0.01	0.00	0.04	Low
Sulfonamides	Sulfamethoxazole	723-46-6	0.00	0.00	0.01	Low
Sulfonamides	Sulfaquinoxaline	59-40-5	0.00	0.00	0.00	Low
Sulfonamides	Sulfathiazole	72-14-0	0.00	0.00	0.08	Low
Tetracyclines	Tetracycline	60-54-8	0.01	0.00	0.06	Low
Benzimidazoles	Thiabendazole	148-79-8	0.10	0.00	0.41	Moderate
Others	Trimethoprim	738-70-5	0.02	0.00	0.38	Low

Notes: * Risk quotients (RQ) were calculated to divide the arithmetic mean, minimum and maximum levels of measured environmental concentrations (MEC) of pharmaceuticals by PNECs. ** Only one sample of pharmaceuticals was detected, thus no other values exist.

It is worth noting that the RQ values for acetaminophen, carbamazepine, and sulfathiazole were significantly lower than 0.1, indicating a low risk. On the other hand, two pharmaceuticals, clarithromycin and sulfamethazine, were found to pose higher potential risks to the aquatic environment, with RQ values exceeding 1 during the spring, summer, and autumn seasons in a study that identified temporal–spatial variations and environmental risks [27]. In another study conducted by Kim et al. [10], the RQ values for pharmaceuticals and personal care products (PPCPs) detected in the surface water of the four major rivers (Han River, Nakdong River, Geum River, and Yeongsan River) in Korea were high in several pharmaceutical substances, with values of 17.34 for clotrimazole, 2.54 for azithromycin, 1.66 for Imidacloprid, 1.61 for dichlorovos, and 1.00 for lincomycin. However, the RQ values of 19 PPCPs, including clarithromycin, albendazole, and sulfapyridine, were observed to be lower than 0.1. Similar to previous study results, we found that most of the detected pharmaceutical substances had low risks of less than 0.1, but the risks of several pharmaceuticals, such as azithromycin and clarithromycin, exceeded 1.

On the other hand, high concentrations of pharmaceuticals, including carbamazepine (ranging from 0.4 to 35.0 ng/L), sulfamethoxazole (ranging from 0.1 to 4.2 ng/L), ketoprofen (ranging from 55.4 to 888.4 ng/L), gemfibrozil (ranging from 16.16 to 17.1 ng/L), and ibuprofen (ranging from 22.6 to 8330.9 ng/L), were detected in surface waters directly discharged from wastewater treatment plants in the Gwangju area of South Korea. The RQ values for these substances exceeded 1, indicating high potential risks to aquatic environments [26]. In another study conducted within the metropolitan area of South Korea, high concentrations of pharmaceuticals were also detected in surface water samples collected from two large concentrated animal feeding operations (CAFO) facilities. These phar-

maceuticals included acetaminophen (ranging from 0.53 to 38.8 μg/L), chlortetracycline (from 0.28 to 3.33 μg/L), oxytetracycline (from 0.10 to 16.9 μg/L), sulfachlorpyridazine (from 0.003 to 6.13 μg/L), sulfamethazine (from 0.20 to 21.30 μg/L), and sulfamethoxazole (from 0.11 to 3.91 μg/L). These pharmaceuticals were analyzed using LC-MS/MS, and the calculated RQ values ranged from 1 to a maximum level of 3,880 [60].

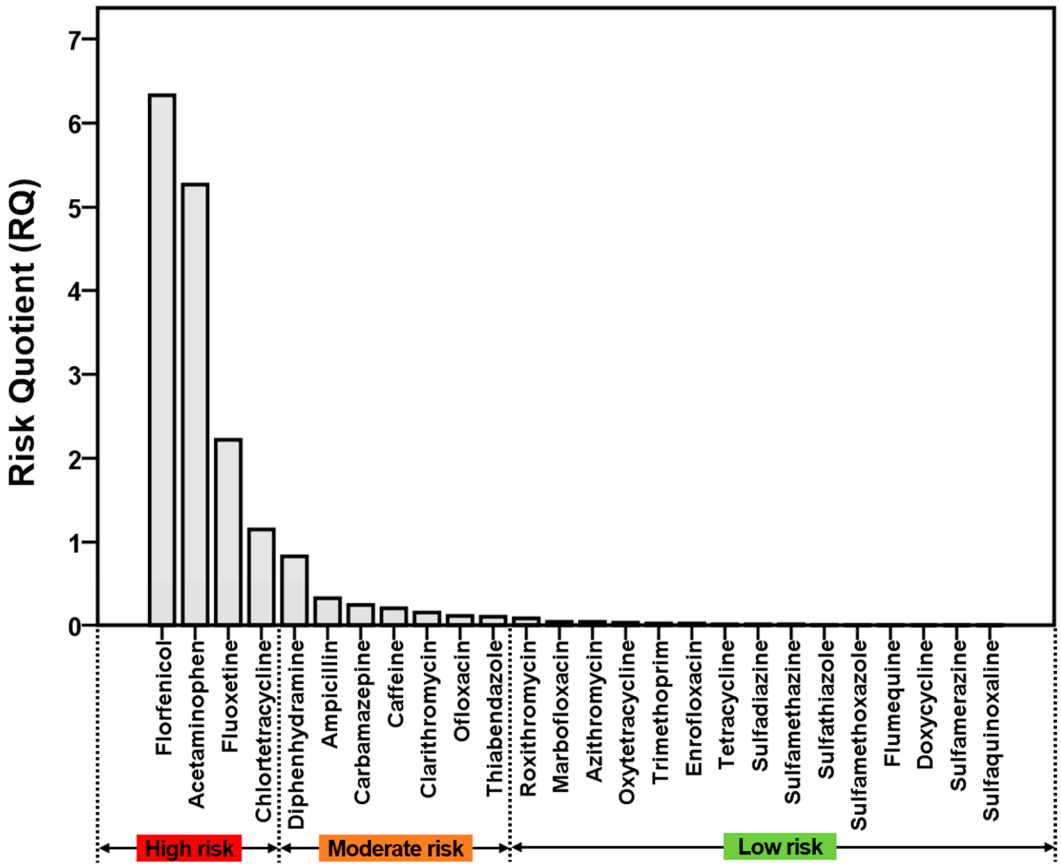


Figure 6. Risk ranking of pharmaceuticals based on the mean effect levels of acute or chronic aquatic toxicity for the most sensitive species (algae, crustaceans, or fish). Risk quotients were calculated and classified into one of three categories (red: high risk; orange: moderate risk; green: low risk).

In 2020, high concentrations of pharmaceuticals, including acetaminophen (341 μg/L), clarithromycin (4.97 μg/L), diclofenac (34.5 μg/L), ibuprofen (86 μg/L), and mefenamic acid (44.2 μg/L), were found in the surface water collected from the effluents of industrial complexes with pharmaceutical manufacturing facilities producing various pharmaceuticals and sanitary products. These complexes were located in the Korean metropolitan area, and the waterways were directly connected to discharge ports of wastewater treatment plants. For most of the pharmaceuticals, the RQ values exceeded 1, ranging from 0.01 to 221.0. Notably, the RQ values were relatively high at the upstream points, and significantly decreased toward the downstream. This suggests a potential environmental risk associated with the discharge of these pharmaceuticals into the water systems connected to the wastewater treatment plants [32].

In a study conducted by Park et al. [61], a similar pattern to that seen in several previous studies was observed [26,32,60,61]. In the upstream regions of Nakdong River, pharmaceuticals, pesticides, and industrial chemical complexes were detected at high concentrations in the effluents, leading to the increase in environmental risks. However, as these substances flowed toward the downstream area from the discharge points, there were significant reductions in both measured concentration levels and associated environmental risks. Similarly, in the present study, we observed that in the upper reaches of the

Seokseong and Nonsan-Gangkyoung streams, the measured concentrations and RQ values for pharmaceuticals were also high. As the water moved downstream, both pharmaceutical concentrations and RQ values significantly decreased. These recent studies, which collected indicator water samples from pollution sources such as wastewater treatment facilities, pharmaceutical and hygiene product manufacturing facilities, and livestock facilities, detected high concentrations of pharmaceuticals like acetaminophen. This encourages further studies, with the additional monitoring and evaluation of environmental risks and investigations, in the future.

To the best of our knowledge, this is the first study performing environmental monitoring, field surveys, risk assessments, and literature reviews for large-scale livestock complexes where pharmaceutical residues are being generated in the Geum River basin. The study subjects were selected considering various environmental factors, including livestock manure production, the operation status and final discharge routes of wastewater treatment facilities, the confluence with the Geum River, and the feasibility of collecting surface water samples on site. Subsequently, the water samples were collected from the Seokseong and Nonsan-Gangkyoung stream areas located in the western part of the Geum River basin, where the target subjects were identified. After the pre-treatment of the HLB (hydrophilic-lipophilic balance), the diluted standard solutions were prepared using solvents, such as methanol. Furthermore, we established an effective analytical method using LC-MS/MS under multiple reaction monitoring (MRM) conditions. That is, we established optimal analytical conditions by systematically considering sensitivity and selectivity through full and product ion scans. We also validated the analytical conditions through a robust QA/QC process, including recovery rates, accuracy, and precision calculations. Using this validated method, we conducted quantitative analyses of the 49 pharmaceuticals present in the collected water samples.

Next, the PNEC values were used to evaluate the environmental risks of pharmaceuticals using chronic toxicity data, mostly NOEC, collected from the latest reliable ecological toxicity database (i.e., US EPA ECOTOX Knowledgebase). Several studies have reported various types of pharmaceuticals in the four major river basins of South Korea, including the Han River basin, Nakdong River basin, and Yeongsan River basin. The environmental risks of these detected pharmaceutical substances have been quantitatively evaluated in different river basins and time periods. The authors found that some RQ values exceeded 1, while others fell below it. In the present study, the concentration levels of detected pharmaceuticals and their environmental risks were consistent with the previous study results. Therefore, our findings suggest that the measured concentrations of pharmaceuticals shown in this study can be used as baseline information and standards for future environmental monitoring and risk assessments related to various pharmaceuticals or other types of micropollutants in other rivers or tributaries within the Geum River basin in the future.

However, there are limitations in this study. Since the water quality samples were collected in autumn and winter (some in early spring) from two tributaries in the Geum River basin, the sample size was relatively small and thus unable to yield results related to seasonal and spatial variations, and the study results are insufficient to represent environmental concentrations and risks for all pharmaceutical substances in the entire Geum River watershed. Similarly to a study conducted by Im et al. [27], which investigated the seasonal variation of pharmaceuticals in the Han River basin from spring to autumn in the mid-2010s, it is necessary to conduct additional environmental monitoring over the course of a year or even longer, in the same study area, in order to accurately analyze and infer a temporal trend or tendency. Furthermore, expanding the environmental monitoring and risk assessment of pharmaceuticals to other rivers, streams, or tributaries with large livestock complexes within the Geum River basin, which may have different point sources of water pollution from industrial, pharmaceutical, and agricultural manufacturing complexes, including main rivers and tributaries (e.g., the Miho River and Gapcheon stream, etc.), is also needed.

Therefore, further studies should include long-term monitoring for various pharmaceuticals considering environmental factors, such as season, location, and pollution sources. This will allow us to build large-scale monitoring datasets of pharmaceuticals of interest, identify temporal–spatial patterns and variations, and comprehensively assess human health and environmental risks in aquatic environments. Based on these efforts, it is anticipated that the characteristics and trends of micropollutants (i.e., pharmaceuticals, etc.) discharged from point sources within the Geum River basin can be fully understood and evaluated in the future.

4. Conclusions

In summary, this study involved the environmental monitoring and analysis of surface water samples collected from both the Seokseong and Nonsan-Gangkyoung streams near large-scale livestock complexes in the Geum River basin in order to assess the measured concentration levels and environmental risks of 49 pharmaceutical residues. We established a multiresidue analytical method using the LC-MS/MS instrument after pretreatment with HLB cartridges. Using the established method, we successfully quantified the concentration levels of 49 pharmaceuticals, and the maximum concentrations of individual pharmaceuticals were detected as 9.212 μg/L of sulfathiazole, 8.479 μg/L of acetaminophen, 8.036 μg/L of marbofloxacin, and 5.885 μg/L of florfenicol in the aquatic environment. Moreover, the RQ values were calculated to be in the range of 1.15–84.79 (high risk) for four pharmaceuticals including acetaminophen, 0.11–0.83 (moderate risk) for seven substances including carbamazepine, and below 0.1 (low risk) for the rest of the substances. Our study findings emphasize that there may be high exposure potential and environmental risks associated with pharmaceuticals that impact human health, aquatic environments, and various species of organisms in the Geum River basin. In the future, further longitudinal studies should be conducted for the long-term monitoring of various pharmaceuticals in the Geum River basin. It is also important to build a large-scale monitoring database based not only on the Seokseong and Nonsan-Gangkyoung streams, but also on other main rivers and tributaries within the Geum River basin. This will help us characterize spatial and temporal patterns of detected pharmaceuticals and identify exact point sources of pollution; such investigations will be beneficial to evaluations of human health and environmental risks.

Author Contributions: Conceptualization, H.L., S.L. and M.C.; methodology, H.L. and S.L.; software, H.L. and S.L.; validation, H.L., S.L. and M.C.; formal analysis, H.L. and S.L.; investigation, H.L.; resources, S.L.; data curation, H.L. and S.L.; writing—original draft preparation, H.L. and S.L.; writing—review and editing, H.L., S.L. and M.C.; visualization, H.L. and S.L.; supervision, M.C.; project administration, H.L. and S.L.; funding acquisition, H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from the National Institute of Environment Research (NIER), funded by the Ministry of Environment (ME) of Republic of Korea (no. NIER-2018-01-01-076).

Data Availability Statement: The data presented in this study are available on request from the corresponding author. Requests should be sent to swlee23@korea.kr and are subject to approval by all named authors participating in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Luo, Y.; Guo, W.; Ngo, H.H.; Nghiem, L.D.; Hai, F.I.; Zhang, J.; Liang, S.; Wang, X.C. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci. Total Environ.* **2014**, *473–474*, 619–641. [CrossRef] [PubMed]
2. Ebele, A.J.; Abou-Elwafa Abdallah, M.; Harrad, S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg. Contam.* **2017**, *3*, 1–16. [CrossRef]
3. Yang, Y.; Zhang, X.; Jiang, J.; Han, J.; Li, W.; Li, X.; Yee Leung, K.M.; Snyder, S.A.; Alvarez, P.J.J. Which Micropollutants in Water Environments Deserve More Attention Globally? *Environ. Sci. Technol.* **2022**, *56*, 13–29. [CrossRef]

4. Nikolaou, A.; Meric, S.; Fatta, D. Occurrence patterns of pharmaceuticals in water and wastewater environments. *Anal. Bioanal. Chem.* **2007**, *387*, 1225–1234. [CrossRef] [PubMed]
5. Daughton, C.G. Chapter 1. Pharmaceuticals in the environment: Sources and their management. In *Comprehensive Analytical Chemistry*; Petrović, M., Barceló, D., Eds.; Elsevier: Amsterdam, The Netherlands, 2007; Volume 50, pp. 1–58.
6. Venkatesan, A.K.; Halden, R.U. Wastewater treatment plants as chemical observatories to forecast ecological and human health risks of manmade chemicals. *Sci. Rep.* **2014**, *4*, 3731. [CrossRef]
7. Andersson, D.I.; Hughes, D. Microbiological effects of sublethal levels of antibiotics. *Nat. Rev. Microbiol.* **2014**, *12*, 465–478. [CrossRef] [PubMed]
8. Gros, M.; Rodríguez-Mozaz, S.; Barceló, D. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *J. Chromatogr. A* **2013**, *1292*, 173–188. [CrossRef]
9. Jeong, D.-H.; Ham, S.-Y.; Lee, W.; Chung, H.; Kim, H. Study on occurrence and management of organic micropollutants in sewer systems. *J. Korean Soc. Water Wastewater* **2017**, *31*, 551–566. [CrossRef]
10. Kim, J.Y.; Jeon, J.; Kim, S.D. Prioritization of pharmaceuticals and personal care products in the surface waters of Korea: Application of an optimized risk-based methods. *Ecotoxicol. Environ. Saf.* **2023**, *259*, 115024. [CrossRef] [PubMed]
11. Jaffrézic, A.; Jardé, E.; Soulier, A.; Carrera, L.; Marengue, E.; Cailleau, A.; Le Bot, B. Veterinary pharmaceutical contamination in mixed land use watersheds: From agricultural headwater to water monitoring watershed. *Sci. Total Environ.* **2017**, *609*, 992–1000. [CrossRef]
12. Luo, Y.; Xu, L.; Rysz, M.; Wang, Y.; Zhang, H.; Alvarez, P.J. Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. *Environ. Sci. Technol.* **2011**, *45*, 1827–1833. [CrossRef] [PubMed]
13. Yoon, Y.; Ryu, J.; Oh, J.; Choi, B.-G.; Snyder, S.A. Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). *Sci. Total Environ.* **2010**, *408*, 636–643. [CrossRef] [PubMed]
14. Kim, I.; Park, Y.; Kim, S.; Sim, K.; Chung, I.; Suk, K.; Hwang, S. Analysis of tetracycline antibiotics and their metabolites samples from streams near concentrated livestock operations using LC/MS/MS. *J. Korean Soc. Environ. Anal.* **2016**, *19*, 199–208.
15. Lee, S.-H.; Jung, H.-W.; Jung, J.-Y.; Min, H.-J.; Kim, B.-R.; Park, C.-G.; Oh, J.-E.; Onoda, Y.; Satou, N. Characteristics of occurrence of pharmaceuticals in the Nakdong River. *J. Korean Soc. Environ. Eng.* **2013**, *35*, 45–56. [CrossRef]
16. Vanderford, B.J.; Snyder, S.A. Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. *Environ. Sci. Technol.* **2006**, *40*, 7312–7320. [CrossRef] [PubMed]
17. Wei, R.; Ge, F.; Huang, S.; Chen, M.; Wang, R. Occurrence of veterinary antibiotics in animal wastewater and surface water around farms in Jiangsu Province, China. *Chemosphere* **2011**, *82*, 1408–1414. [CrossRef] [PubMed]
18. Kim, H.; Hong, Y.; Park, J.-e.; Sharma, V.K.; Cho, S.-i. Sulfonamides and tetracyclines in livestock wastewater. *Chemosphere* **2013**, *91*, 888–894. [CrossRef] [PubMed]
19. US Environmental Protection Agency (EPA). *EPA Method: 1694, Pharmaceuticals and Personal Care Products in Water, Soil, Sediment and Biosolids by HPLC/M/M*; EPA-821-R-08-002; USEPA: Washington, DC, USA, 2007.
20. NIER. Water Emission Management System. Available online: https//wems.nier.go.kr (accessed on 17 October 2018).
21. Moermond, C.T.; Kase, R.; Korkaric, M.; Ågerstrand, M. CRED: Criteria for reporting and evaluating ecotoxicity data. *Environ. Toxicol. Chem.* **2016**, *35*, 1297–1309. [CrossRef]
22. Olker, J.H.; Elonen, C.M.; Pilli, A.; Anderson, A.; Kinziger, B.; Erickson, S.; Skopinski, M.; Pomplun, A.; LaLone, C.A.; Russom, C.L.; et al. The ECOTOXicology Knowledgebase: A Curated Database of Ecologically Relevant Toxicity Tests to Support Environmental Research and Risk Assessment. *Environ. Toxicol. Chem.* **2022**, *41*, 1520–1539. [CrossRef] [PubMed]
23. NIER. *Regulations on Specific Methods of Chemical Risk Assessment*; NIER: Incheon, Republic of Korea, 2021.
24. EC. *Revised Technical Guidance for Deriving Environmental Quality Standards. Common Implementation Strategy for the Water Framework Directive Guidance Document No. 27*; European Commission: Luxembourg, 2018.
25. Yan, S.; Chen, R.; Wang, M.; Zha, J. Carbamazepine at environmentally relevant concentrations caused DNA damage and apoptosis in the liver of Chinese rare minnows (*Gobiocypris rarus*) by the Ras/Raf/ERK/p53 signaling pathway. *Environ. Pollut.* **2021**, *270*, 116245. [CrossRef] [PubMed]
26. Offiong, N.; Lema, E.L.; Kang, S.; Inam, E.I.; Kang, S.; Kim, K. Risk evaluation of pharmaceutical residues in waste water from selected treatment plants in Gwangju, South Korea. *J. Chem. Soc. Niger.* **2019**, *44*, 504–514.
27. Im, J.K.; Hwang, M.Y.; Lee, E.H.; Noh, H.R.; Yu, S.J. Pharmaceutical compounds in tributaries of the Han River watershed, South Korea. *Environ. Res.* **2020**, *188*, 109758. [CrossRef] [PubMed]
28. Anagnostopoulpou, K.; Nannou, C.; Aschonitis, V.G.; Lambropoulou, D.A. Screening of pesticides and emerging contaminants in eighteen Greek lakes by using target and non-target HRMS approaches: Occurrence and ecological risk assessment. *Sci. Total Environ.* **2022**, *849*, 157887. [CrossRef]
29. Gianturco, S.L.; Pavlech, L.L.; Storm, K.D.; Yoon, S.; Yuen, M.V.; Mattingly, A.N. *Carbamazepine: Summary Report*; University of Maryland: Baltimore, MD, USA, 2020.
30. Kwon, H.; Sim, W.; Kim, H.; Oh, J.; Choi, S. Distribution of pharmaceuticals and personal care products (PPCPs) in main rivers of Ulsan. *Korea. J. Korea Soc. Environ. Anal.* **2011**, *14*, 158–164.
31. Lee, H.-J.; Kim, H.-Y.; Kim, K.Y.; Yang, D.-S.; Lee, I.; Lim, Y.-K.; Kim, J.-H.; Oh, J.-E. Characteristic occurrence and distributions of pharmaceuticals in the Nakdong River. *J. Korean Soc. Environ. Eng.* **2017**, *39*, 403–411. [CrossRef]

32. Park, S.; Kang, H.; Shin, H.; Ryoo, I.; Choi, K.; Kho, Y.; Park, K.; Kim, K.; Ji, K. Ecological Risk Assessment of Pharmaceuticals in the Surface Water Near a Pharmaceutical Manufacturing Complex in Korea. *J. Environ. Health Sci.* **2020**, *46*, 45–64.
33. Lim, S.-K.; Lee, J.-E.; Lee, H.-S.; Nam, H.-M.; Moon, D.-C.; Jang, G.-C.; Park, Y.-J.; Jung, Y.-G.; Jung, S.-C.; Wee, S.-H. Trends in antimicrobial sales for livestock and fisheries in Korea during 2003–2012. *Korean J. Vet. Res.* **2014**, *54*, 81–86. [CrossRef]
34. David, A.; Pancharatna, K. Effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, Danio rerio. *J. Appl. Toxicol.* **2009**, *29*, 597–602. [CrossRef]
35. Qian, H.; Pan, X.; Chen, J.; Zhou, D.; Chen, Z.; Zhang, L.; Fu, Z. Analyses of gene expression and physiological changes in Microcystis aeruginosa reveal the phytotoxicities of three environmental pollutants. *Ecotoxicology* **2012**, *21*, 847–859. [CrossRef]
36. Li, Y.; Ma, Y.; Yang, L.; Duan, S.; Zhou, F.; Chen, J.; Liu, Y.; Zhang, B. Effects of azithromycin on feeding behavior and nutrition accumulation of Daphnia magna under the different exposure pathways. *Ecotoxicol. Environ. Saf.* **2020**, *197*, 110573. [CrossRef]
37. Lawrence, J.R.; Zhu, B.; Swerhone, G.D.; Roy, J.; Tumber, V.; Waiser, M.J.; Topp, E.; Korber, D.R. Molecular and microscopic assessment of the effects of caffeine, acetaminophen, diclofenac, and their mixtures on river biofilm communities. *Environ. Toxicol. Chem.* **2012**, *31*, 508–517. [CrossRef] [PubMed]
38. Koeypudsa, W.; Yakupitiyage, A.; Tangtrongpiros, J. The fate of chlortetracycline residues in a simulated chicken–fish integrated farming systems. *Aquac. Res.* **2005**, *36*, 570–577. [CrossRef]
39. Watanabe, H.; Tamura, I.; Abe, R.; Takanobu, H.; Nakamura, A.; Suzuki, T.; Hirose, A.; Nishimura, T.; Tatarazako, N. Chronic toxicity of an environmentally relevant mixture of pharmaceuticals to three aquatic organisms (alga, daphnid, and fish). *Environ. Toxicol. Chem.* **2016**, *35*, 996–1006. [CrossRef] [PubMed]
40. Meinertz, J.R.; Schreier, T.M.; Bernardy, J.A.; Franz, J.L. Chronic toxicity of diphenhydramine hydrochloride and erythromycin thiocyanate to daphnia, Daphnia magna, in a continuous exposure test system. *Bull. Environ. Contam. Toxicol.* **2010**, *85*, 447–451. [CrossRef] [PubMed]
41. Zhu, Y.; Yang, D.; Duan, X.; Zhang, Y.; Chen, D.; Gong, Z.; Liu, C. Perfluorooctane sulfonate promotes doxycycline-induced liver tumor progression in male Krasv12 transgenic zebrafish. *Environ. Res.* **2021**, *196*, 110962. [CrossRef]
42. Robinson, A.A.; Belden, J.B.; Lydy, M.J. Toxicity of fluoroquinolone antibiotics to aquatic organisms. *Environ. Toxicol. Chem. Int. J.* **2005**, *24*, 423–430. [CrossRef]
43. Zhang, Y.; Zhang, X.; Guo, R.; Zhang, Q.; Cao, X.; Suranjana, M.; Liu, Y. Effects of florfenicol on growth, photosynthesis and antioxidant system of the non-target organism Isochrysis galbana. *Comp. Biochem. Physiol. Part. C Toxicol. Pharmacol.* **2020**, *233*, 108764. [CrossRef] [PubMed]
44. Lützhøft, H.-C.H.; Halling-Sørensen, B.; Jørgensen, S. Algal toxicity of antibacterial agents applied in Danish fish farming. *Arch. Environ. Contam. Toxicol.* **1999**, *36*, 1–6. [CrossRef]
45. Chai, T.; Cui, F.; Di, S.; Wu, S.; Zhang, Y.; Wang, X. New insights into cardiotoxicity induced by chiral fluoxetine at environmental-level: Enantioselective arrhythmia in developmental zebrafish (Danio rerio). *Environ. Pollut.* **2021**, *270*, 116182. [CrossRef]
46. Kergaravat, S.V.; Hernandez, S.R.; Gagneten, A.M. Second-, third-and fourth-generation quinolones: Ecotoxicity effects on Daphnia and Ceriodaphnia species. *Chemosphere* **2021**, *262*, 127823. [CrossRef]
47. Meng, H.; Liang, J.; Zheng, X.; Zhang, K.; Zhao, Y. Using a high-throughput zebrafish embryo screening approach to support environmental hazard ranking for cardiovascular agents. *Sci. Total Environ.* **2020**, *702*, 134703. [CrossRef] [PubMed]
48. Garcia, R.J.; Kane, A.S.; Petullo, D.; Reimschuessel, R. Localization of Oxytetracycline in Chlamydomonas Reinhardtii (*Chlorophyceae*)[1]. *J. Phycol.* **2008**, *44*, 1282–1289. [CrossRef] [PubMed]
49. Guo, J.; Bai, Y.; Chen, Z.; Mo, J.; Li, Q.; Sun, H.; Zhang, Q. Transcriptomic analysis suggests the inhibition of DNA damage repair in green alga Raphidocelis subcapitata exposed to roxithromycin. *Ecotoxicol. Environ. Saf.* **2020**, *201*, 110737. [CrossRef] [PubMed]
50. Białk-Bielińska, A.; Stolte, S.; Arning, J.; Uebers, U.; Böschen, A.; Stepnowski, P.; Matzke, M. Ecotoxicity evaluation of selected sulfonamides. *Chemosphere* **2011**, *85*, 928–933. [CrossRef] [PubMed]
51. Bundschuh, M.; Hahn, T.; Ehrlich, B.; Höltge, S.; Kreuzig, R.; Schulz, R. Acute toxicity and environmental risks of five veterinary pharmaceuticals for aquatic macroinvertebrates. *Bull. Environ. Contam. Toxicol.* **2016**, *96*, 139–143. [CrossRef]
52. Kim, Y.; Choi, K.; Jung, J.; Park, S.; Kim, P.-G.; Park, J. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environ. Int.* **2007**, *33*, 370–375. [CrossRef] [PubMed]
53. Lu, G.; Li, Z.; Liu, J. Effects of selected pharmaceuticals on growth, reproduction and feeding of Daphnia Magna. *Fresenius Environ. Bull.* **2013**, *22*, 2588–2594.
54. De Liguoro, M.; Fioretto, B.; Poltronieri, C.; Gallina, G. The toxicity of sulfamethazine to Daphnia magna and its additivity to other veterinary sulfonamides and trimethoprim. *Chemosphere* **2009**, *75*, 1519–1524. [CrossRef] [PubMed]
55. Park, S.; Choi, K. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. *Ecotoxicology* **2008**, *17*, 526–538. [CrossRef]
56. Yang, W.; Tang, Z.; Zhou, F.; Zhang, W.; Song, L. Toxicity studies of tetracycline on Microcystis aeruginosa and Selenastrum capricornutum. *Environ. Toxicol. Pharmacol.* **2013**, *35*, 320–324. [CrossRef]
57. US Environmental Protection Agency (EPA). *Framework for Ecological Risk Assessment*; EPA/630/R-92/001; US Environmental Protection Agency (EPA): Washington, DC, USA, 1992.

58. Madureira, T.V.; Rocha, M.J.; Cruzeiro, C.; Rodrigues, I.; Monteiro, R.A.; Rocha, E. The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal): Evaluation of impacts on fish liver, by histopathology, stereology, vitellogenin and CYP1A immunohistochemistry, after sub-acute exposures of the zebrafish model. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 34–45. [CrossRef] [PubMed]
59. Wang, H.; Luo, Y.; Xu, W.; Zhou, Q.; Tang, B.; Wang, Y. Ecotoxic effects of tetracycline and chlortetracycline on aquatic organisms. *J. Agro-Environ. Sci.* **2008**, *27*, 1536–1539.
60. Kim, B.; Ji, K.; Kim, C.; Kang, H.; Lee, S.; Kwon, B.; Kho, Y.; Park, K.; Kim, K.; Choi, K. Pharmaceutical residues in streams near concentrated animal feeding operations of Korea—Occurrences and associated ecological risks. *Sci. Total Environ.* **2019**, *655*, 408–413. [CrossRef] [PubMed]
61. Park, N.; Kang, D.; Jeon, J. Occurrence and Concentration of Micropollutants in the Middle-and Down-stream of Nakdong River. *J. Environ. Anal. Health Toxicol.* **2021**, *24*, 1–12. [CrossRef]