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Lateral and vertical variations of pharmaceutical contaminants in natural aquatic systems

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Abstract. The detection of pharmaceuticals and personal care products (PPCPs) in environmental matrices raised concerns over their short- and long-term effects on the ecosystem. Wastewater is the primary carrier of PPCPs to the environment in highly urbanized regions. In the Philippines, Manila Bay serves as a drainage basin for most of the streams of surrounding urban cities, hence, a cumulative drainage basin for effluents transported downstream. Water samples from the surface and the bottom layers of Pasig River mouth and Manila Bay were collected for PPCP analysis. Pharmaceuticals and pharmaceutical-related compounds, such as carbamazepine, clarithromycin, diclofenac, anhydroerythromycin, acetylsulfamethoxazole, trimethoprim were detected, as well as possible untreated wastewater tracers like caffeine and acetaminophen. Total concentrations were higher in Pasig River compared to Manila Bay due to the dilution of river water entering a bigger water body. Vertical variations in PPCP concentrations in Pasig River are not significant. In contrast, the top layer of Manila Bay is more contaminated compared to the bottom potentially due to stratification. Differences in PPCP profiles show how variable contaminants depend on external factors. The presence of PPCPs in natural systems highlights the need for more stringent and targeted wastewater management.

Keywords: emerging contaminants, antibiotics, PPCPs, surface waters, coastal waters

1. Introduction

Pharmaceuticals and personal care products (PPCPs) have been increasingly detected in environmental matrices thus raising awareness on their presence and their potential negative impacts to exposed ecosystems. Several government agencies, such as the United States Environmental Protection Agency and the European Union consider PPCPs as “contaminants of emerging concern (CECs)”, which are compounds that have been detected in surface waters at low levels yet no standard environmental concentration has been established yet. Among the possible deleterious effects of PPCP contamination are antibiotic resistance, endocrine disruption, negative growth effects in and decrease in productivity of primary producers, and sublethal effects on biochemical activities, among others (Kummerer, 2009; Deo, 2014; Davies et al., 2006).

Wastewater is the major source of pharmaceuticals and their metabolites to the environment in highly urbanized regions (Manzetti et al., 2014). Poor treatment of wastewater leads to the release of these compounds into receiving streams and ultimately into the coastal waters. In the past decade, wastewater treatment plants have been constructed within the capital region of the Philippines, Metro Manila, thereby increasing its wastewater treatment capability. However, targeted treatment for PPCP removal remains to be established. The Pasig River is an important water body within the capital region that passes through highly urbanized cities. It has four major tributaries within the region and 43 minor tributaries within the capital city (PRRC, 2014). Owing to the population and infrastructure density traversed by the Pasig River, it contributes about 21 percent of the organic pollution load to Manila Bay, whereas 70 percent of this load is derived from households (PEMSEA, 2021). Manila Bay is a semi-enclosed estuary facing the South China Sea, supporting a multitude of industries and activities, such as fisheries, aquaculture, shipping, manufacturing, and residence. Manila Bay is considered a pollution hotspot, wherein most of the pollutants are derived from land-based human activities: discharge waste from municipal, industrial, and agricultural sectors, runoff and atmospheric deposition.

The current research is a refinement from studies conducted along Pasig River and Manila Bay to determine the assemblage and concentrations of the PPCPs in the water by Shimizu et al. (2013) and Van et al. (2021). In this study, variations in the detection and the lateral and vertical distribution of PPCPs between the freshwater (Pasig River) and the seawater(Manila Bay) parcel it is draining into are examined in depth.

2. Methods

2.1. Sample collection and processing

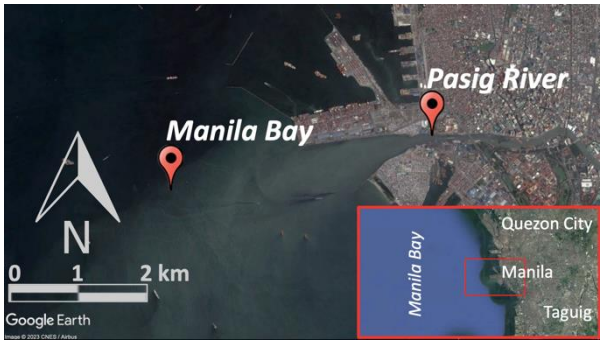

Figure 1. Sampling points along the Pasig River mouth. *(Inset map)* Location of the study site within Manila Bay.

Physicochemical characteristics of the sampling sites (pH, DO, salinity, temperature) were measured using a Hanna HI9829 multiparameter. Following the optimized method by Angeles & Aga (2018), duplicates of 500 mL surface and bottom (1 m above the sediments) water samples were collected along a point in Pasig River(PR) and a point on Manila Bay(MB) (Fig. 1). Samples were stored in pre-acid rinsed high-density polyethylene (HDPE) bottles and were acidified to pH 2-3 with 40% phosphoric acid. Samples were stored in ice chests until arrival in the laboratory. Then, samples were filtered through 0.7 µm glass microfiber filters to remove microorganisms and particulates. A 2 mL sodium diethyldiamine (Na_2EDTA) solution (5% *w/v* in water) was added to prevent the chelation of tetracyclines with metal cations before spiking the filtered sample with 50 uL of the surrogate standard mix.

For cleanup and concentration, the samples were passed through 500 mg OASIS HLB cartridges previously conditioned with 6 mL ultrapure water and 6 mL acetonitrile. The samples were loaded into the cartridges at the rate of 3-5 mL/min, then the cartridges were air-dried on the vacuum manifold. After drying, the cartridges were wrapped in foil and stored in the freezer until its shipment for further processing. Upon arrival in the laboratory, the cartridges were eluted with 6 mL of acetonitrile to obtain the extracts, then dried under nitrogen gas at a temperature not more than 30°C with a turbo evaporator. The extracts were reconstituted with 900 µL of the mobile phase (A, 0.3% formic acid in water; B, 25% acetonitrile: 75% methanol) and was further cleaned up with a 0.45 µm syringe filter. The samples were spiked with 100 ppb of internal standard, d3-diphenyhydramine, to account for possible measurement differences caused by the instrument.

2.2 Materials

Caffeine (CAF), acetylsulfamethoxazole (ASMX), diclofenac (DIC), ciprofloxacin (CIP), and acetaminophen (ACT) standards were purchased from Toronto Research Chemicals. LC-MS grade methanol and acetonitrile were obtained from EMD Millipore Corporation (Billerica, MA), and formic acid (88%) was purchased from Fisher Chemical (Pittsburgh, PA). Oasis HLB solid-phase extraction (SPE) cartridges were purchased from Waters (Milford, MA).

2.3 LCMS

A Waters Cortecs C18+ column (Milford, MA) with dimensions 2.1×150 mm and 2.7 µm particle size was used for the separation of 34 target analytes listed in Table 1. Analysis was conducted using an Agilent 1200 LC system (Palo Alto, CA) and a Thermo Scientific TSQ Quantum Ultra triple quadrupole MS (Waltham, MA) equipped with a heated electrospray ionization (HESI) probe, operated under positive ionization mode.

Table 1. Selected analytes for targeted analysis

	Analytes
Common PPCPs	acetaminophen, caffeine, carbamazepine, diclofenac, iopamidol, metformin, trimethoprim
Macrolides	anhydroerythromycin, azithromycin, clarithromycin, erythromycin, roxithromycin, spiramycin, tilmicosin
Quinolones	ciprofloxacin, enrofloxacin, norfloxacin, oxolinic acid, sarafloxacin
Sulfonamides	acetylsulfamethoxazole, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxydiazine, sulfathiazole
Tetracyclines	anhydrotetracycline, chlortetracycline, demelocycline, oxytetracycline, tetracycline

The total run time for each sample was 45 minutes, with the mobile phase A and B running through the column in a gradient starting from 90% A: 10% B (v/v) to 100% B, then switching back to 90% A: 10% B.

3. Results

Eight (8) analytes were detected for which values were computed through one-point external calibration. The PPCPs were acetaminophen, caffeine, carbamazepine, diclofenac, and antibiotics, such as acetylsulfamethoxazole, clarithromycin, anhydroerythromycin, and trimethoprim (Fig. 2). These PPCPs have been previously detected in Philippine WWTPs within the area of the sampling site (Singh et al., 2019).

Anhydroerythromycin, a degradation product of the macrolide erythromycin, had the highest detection values at an average of 36.05 ng/L. Some studies noted that anhydroerythromycin accompanies erythromycin in the environmental matrix (Voigt et al., 2018), and that it is also the dominant form of erythromycin in aquatic environments (Du et al., 2017), thus suggesting that anhydroerythromycin itself can be used as proxy for erythromycin concentrations. Clarithromycin, another macrolide antibiotic, was detected between 3.15 – 5.59 ng/L. Acetylsulfamethoxazole, a metabolite of the

sulfonamide antibiotic sulfamethoxazole, was detected at a range of 2.02 – 6.18 ng/L. Trimethoprim, an antibiotic which is also commonly used with sulfamethoxazole, was detected between 2.16 – 6.83 ng/L. Macrolides, sulfonamides, and trimethoprim are commonly used in the treatment of a variety of bacterial infections. Other PPCPs detected were caffeine, acetaminophen, carbamazepine, and diclofenac ranging from 0.36 – 19.44 ng/L. Caffeine, acetaminophen, and carbamazepine are commonly used as tracers for untreated or treated sewage (Mizukawa et al., 2019, Dvory et al., 2018).

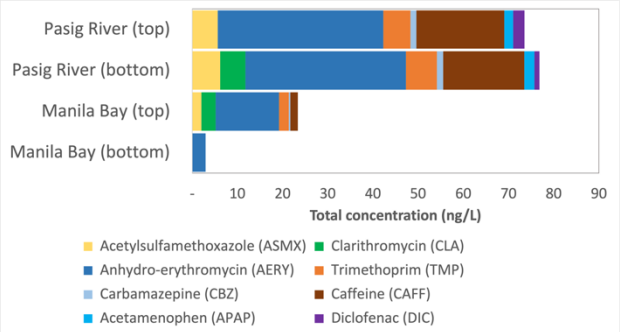

Figure 2. Total assemblage of PPCPs detected in the top and bottom layers of Pasig River and Manila Bay sampling sites.

It was previously observed that sulfonamides dominated the assemblage of PPCPs detected in Pasig River and Manila Bay, with macrolides being the second most dominant, which was attributed to the inexpensiveness of sulfonamide antibiotics (Shimizu et al., 2013). For this study, the contrary was observed wherein macrolide detections in the form of anhydroerythromycin and clarithromycin were 7x to 8x higher than the sulfonamide acetylsulfamethoxaxole in the same study area. It is possible that both classes of antibiotics are used predominantly in the areas draining into Pasig River.

Total PPCP detections in Pasig River were generally higher compared to Manila Bay. Dilution is among the processes during pollutant transport that affects the concentration of contaminants from the river to the sea. Some studies noted that riverine input can both enhance or deplete concentrations of contaminants in the drainage basin depending on several factors such as contaminant flux and dilution factor (Zhu et al., 2019). The difference in the total concentrations between the relatively high river concentration and the lower concentration in seawater samples in this study may indicate a scenario where contaminants from the river are steadily discharged without further input from a nearby point source.

Dissolved oxygen (DO) is an indicator of the predominance of organic matter and bacteria that can degrade it. Lower DO values suggest high amounts of organic matter in the water parcel, thus an abundance in bacteria that consume oxygen. Both top and bottom layers of the Pasig River and the bottom layer of Manila Bay are suboxic with DO values ranging from only 1.88 – 3.45 ppm (Table 2). It has been previously observed in other studies that pollutants can prefer to adsorb to bottom sediments, depleting bottom water concentrations (Fairbairn, et al., 2015). However, the detections on the bottom layer of Pasig River did not have much difference from the top layer, which can be attributed to the river being well-mixed. This is in contrast with the significant difference between the detections on the bottom and top layers of Manila Bay (Fig. 2).

Table 2. Physicochemical characteristics of the sampling sites. PR = Pasig River, MB = Manila Bay

Sites	Depth (m)	pH	DO (ppm)	Salinity (PSU)	Temperature (°C)
PR (top)	1	7.69	1.9	0.42	28.88
PR (bottom)	3	7.39	1.88	0.42	28.87
MB (top)	1	8.4	11.88	23.6	29.51
MB (bottom)	7	7.95	3.45	26.42	29.17

Stratification of the Manila Bay water column as evidenced by the 2.82 PSU difference in the surface and bottom waters may also restrict mixing and lessen the vertical transport of the contaminants, thereby promoting dispersal on the surface waters. Alternatively, it may also indicate that discharge of contaminants to the Pasig River is continuous such that recharge of contaminants occur even before they can be degraded by in situ bacteria or before they can adsorb to the sediments. Further investigation into the levels of contamination in the bottom sediment may help confirm if contaminants have preferential adsorbance in the sediments of Manila Bay.

4. Conclusion

Anhydroerythromycin and caffeine were observed to be predominant in the sewage-impacted Pasig River. Other PPCPs detected were carbamazepine, diclofenac, acetylsulfamethoxazole, clarithromycin, and trimethoprim. Total concentration of contaminants was significantly lower in Manila Bay compared to Pasig River, probably due to dilution effect. PPCP assemblage on the top layer of Manila Bay is more diverse compared to the bottom layer, potentially due to limited mixing between surface and bottom waters due to stratification, bacterial degradation, or adsorption to bottom sediment. Detection of untreated sewage tracers in the study site highlights the need for stricter and more stringent wastewater management. Detection of antibiotics also raise the need to develop targeted wastewater treatment for their efficient removal in order to curb the threat of antibiotic resistance.

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