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Suspect Screening of Emerging Micropollutants in Wastewater Treatment Plant Influent in Kuantan, Pahang using LC-QTOF-MS

Kaishi Loh, Noor Azuwa Mahidin, Mohammad Azimuddin Izani, Nurul `Azyyati Sabri and Joo Hui Tay*
Faculty of Industrial Sciences and Technology, University Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, 26300 Kuantan, Pahang, Malaysia
*Corresponding author (e-mail: tayjoohui@umpsa.edu.my)

Emerging micropollutants (EMPs) are contaminants released into the environment at trace levels and have recently become a focus of global research. A suspect screening analysis was conducted to investigate EMPs originating from pharmaceuticals, personal care products (PCPs), plasticizers, and flame retardants in 14 raw wastewater samples collected from wastewater treatment plants (WWTPs) in Kuantan using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). A total of 27 EMPs were identified from the chromatograms through comparison with a MS library and manual verification using a prepared in-house database. At least eight different compounds had been determined from each raw wastewater sample. Results showed that all samples were dominated by anti-inflammatory compounds such as triamcinolone, flurbiprofen, and ibuprofen were monitored in all WWTPs, followed by three PCPs, one plasticizer, and one flame retardants. No food additives were detected in this study. This finding highlighted that the pharmaceutical was predominant and the effluent should be investigated in future to assess the removal efficiency of WWTPs.

Keywords: Pharmaceuticals, raw wastewater, suspect screening analysis, LC-QTOF-MS, wastewater treatment plant

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Wastewater is a complex matrix that contains thousands of compounds derived from human metabolism, personal care products, household appliances, industrial processes and environmental runoff. Chemical analysis of wastewater influents entering wastewater treatment plants (WWTPs) can provide valuable information about the health and lifestyle of the community connected to the sewer system [1]. The composition of raw wastewater also informs the selection of appropriate treatment methods to remove or reduce contaminants and prevent their release into the environment [2]. Historically, WWTPs have been designed to abate nutrients (nitrogen and phosphorus), particles, and carbonaceous substances [3]. Several research studies were conducted to investigate the emerging micropollutants and removal efficiency of WWTPs in Malaysia [4, 5]. Pharmaceutical residues, perfluoroalkyl substances (PFAS) and heavy metals had been discovered from wastewater in Johor, Penang, and Kedah, respectively [6, 7, 8]. However, many studies have shown that the removal of emerging contaminants by conventional WWTPs remains very poor [9, 10]. As a result, these contaminants are continuously released into receiving watercourses in trace amounts (typically ranging from ng/L to μg/L).

Emerging micropollutants (EMPs) are biological or chemical contaminants, including both natural and anthropogenic organic substances, that enter aquatic environments through various pathways in trace amounts (<μg/L) as a result of human activities [11]. Chavoshani et al. hypothesized that EMPs may have been released into the environment for a long time but remained undetected until the development of advanced analytical techniques [12]. EMPs have been classified into various categories, including pharmaceuticals, personal care products (PCPs), organic dyes, pesticides, flame retardants, plasticizers, steroid hormones, polycyclic aromatic hydrocarbons (PAHs), surfactants, heavy metals, industrial chemicals [12, 13]. Researchers have reported that EMPs can cause adverse consequences to human and aquatic organisms, including reproductive system of fish, increase the risk of breast cancer, kidney and liver damage [14, 15, 16].

Pharmaceuticals are biologically active compounds manufactured for disease treatment in human. However, they are often not fully metabolized in the body, leading to the excretion of active pharmaceutical ingredients (APIs) into the environment [17]. These APIs are resistant to degradation and can persist in water bodies for extended periods [18]. Analgesic, anti-inflammatory drugs, and antibiotics are among the major pharmaceutical groups frequently detected in aquatic environments [19]. The global

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demand for pharmaceuticals has increased significantly over the past few decades, particularly during the COVID-19 pandemic. Even at low concentrations, these compounds can induce physiological effects in human and ecosystem, such as promoting bacterial resistance to antibiotics, making pharmaceuticals one of the most hazardous contaminants in water bodies [20, 21, 22].

The availability of clean water resources is gradually becoming insufficient due to rising demand from domestic and non-domestic activities, agricultural and industrial sectors [23]. The reuse of treated wastewater has emerged as an alternative water resource to promote water circularity in agriculture and industrial manufacturing [24]. Numerous studies have investigated the occurrence, removal, and environmental risks of pharmaceuticals and other emerging micropollutants (EMPs) in wastewater treatment plants (WWTPs) [25, 26]. Understanding EMPs profiles in wastewater is crucial for improving the design and operation of existing treatment facilities to enhance contaminant removal [27].

Targeted analysis is the primary approach for detecting specific contaminants in environmental samples. However, due to the growing number of existing and newly introduced chemicals, it is impractical to rely solely on targeted approach, which required individual analytical standards for each compound [28]. Suspect screening approaches enable qualitative analysis by utilising information such as occurrence frequency and concentration-relevant indices (e.g., peak area) without the need for reference standards. This approach allows for the simultaneous detection of a large number of compounds in a single injection, offering a significant reduction of time and costs. This approach has become a widely adopted strategy in environmental analytical chemistry and has been applied to identify various classes of emerging contaminants such as pharmaceuticals, pesticides, flame retardants, personal care products, polyfluoroalkyl substances (PFASs), new psychoactive substances and their degradation products/ metabolites in wastewaters [29-33].

Since very few data are available for the occurrence of emerging contaminants in Malaysia's aquatic environment, particularly on the east coast of Peninsular Malaysia [34-36] a wide-scope qualitative screening of wastewater is essential to assess its potential impact on aquatic environment. This study aimed to identify EMPs present in raw wastewater samples collected from WWTPs serving residence areas in Kuantan, the largest city in Pahang.

EXPERIMENTAL

Chemicals and Materials

Formic acid 98-100% for HPLC LiChropur, ethanol and acetonitrile (both LiChrosolv) were purchased from Merck. Oasis HLB SPE cartridges (6 mL, 500 mg) were purchased from Waters, whereas 0.7 µm glass fibre filters were purchased from Whatman.

Characterization Methods

Sampling

24-hour composite influent wastewater samples were collected from 14 largest WWTPs in Kuantan, Pahang between August – November 2021 in conjunction with a sampling campaign under a separate research project. For each station, only one 24-h sample was collected. The exact sampling dates and flow rates for each station are shown in **Table 1**. After sampling, raw wastewater samples were filtered through 0.7 µm glass fibre filter and kept in pre-cleaned high density polyethylene (HDPE) bottles until analysis.

Table 1. Sampling date, WWTP capacity, and flow rate of influent wastewater in Kuantan, Pahang.

WWTP	Capacity (population equivalent, P.E.)	Sampling date	Flow rate (m^3/day)
MMJ	14500	08 Nov 2021	2756
DMS	16000	11 Oct 2021	3066
IM3	30000	16 Aug 2021	7559
PBR	18000	23 Aug 2021	4280
BRD	17000	12 Sep 2021	3756
PDG	15000	30 Aug 2021	2568
MGJ	14000	25 Oct 2021	2413
MAG	14000	01 Nov 2021	2638
PMG	18700	18 Oct 2021	2593
BUR	15600	27 Sep 2021	2542
TSI	18000	06 Sep 2021	3704
MKV	13000	08 Sep 2021	3054
PR1	11500	20 Sep 2021	2451
PSJ	18700	04 Oct 2021	3352

Sample Preparation

Sample extractions were performed according to Wang et al. [32] with slight modifications. In short, one-litre defrosted sewage sample was extracted using Oasis HLB cartridges (6 cc, 500 mg, Waters). The cartridges were conditioned by 6 mL of methanol (MeOH) followed by 6 mL of deionized water. Samples were percolated through the cartridges and vacuum-dried for about 15 minutes. The target compounds were eluted with 12 mL MeOH. The extracts were then concentrated to 1 mL under a moderate stream of nitrogen. For quality control, deionized water was treated identically to wastewater as a procedure blank in each batch of analysis.

Qualitative Analysis by LC-QTOF-MS

Suspect screening analysis was performed by using a liquid chromatography system coupled with a quadrupole time-of-flight mass spectrometer (Waters, VION Ion Mobility QTOF MS; LC-QTOF-MS) in both ESI (+) and ESI (-) modes. A C-18 column was used for reversed-phase liquid chromatographic separation. In both ESI mode, water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used as mobile phases A and B, respectively. The LC method was completed within 20 minutes, with the mobile phase A gradient set as follows: 99% at 0-minute and 0.5-minute, 65% at 16-minute, 0% at 18-minute, and 99% at 20-minute. Other system parameters included a column temperature of 40°C, a flow rate with 0.5 mL/min and an injection volume of 10µL. The mass spectrometer acquired data in the m/z range of 500-1000. Identified compounds were grouped according to their usage and confidence level of identification.

RESULTS AND DISCUSSION

Chromatogram Analysis

Chromatogram analysis was conducted focusing on chromatographic peaks with an intensity response at least ten times higher than the procedural blanks. Tentative candidates were selected based on a signal to noise (S/N) ratio >3, a mass accuracy tolerance of 10 ppm, and an isotope ratio difference (IRD) <10%. Anti-inflammatory, antibacterial, and analgesics were confirmed with MS library, while personal care products (PCPs), plasticizers and flame retardants were identified after the visual inspection of spectra.

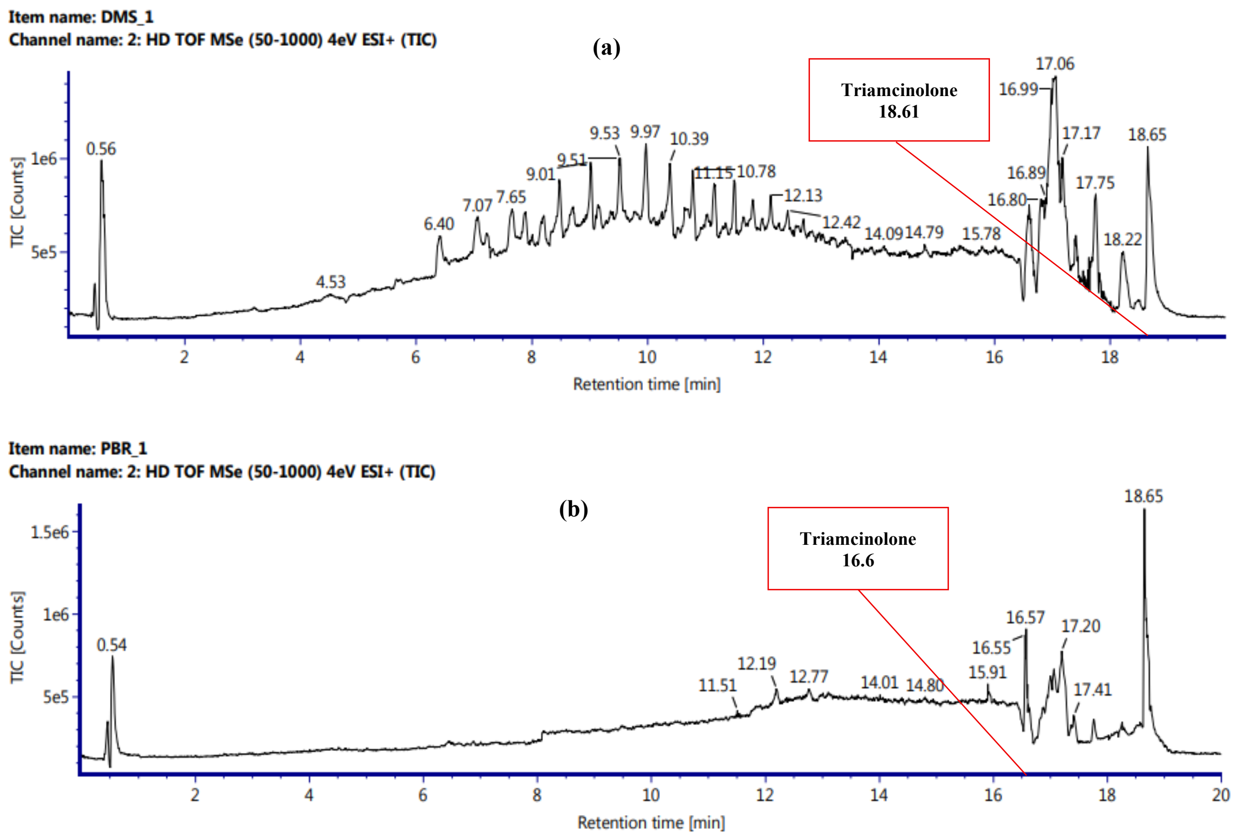

Figure 1. ESI positive mass spectrometry chromatogram of station (a) DMS and (b) PBR.

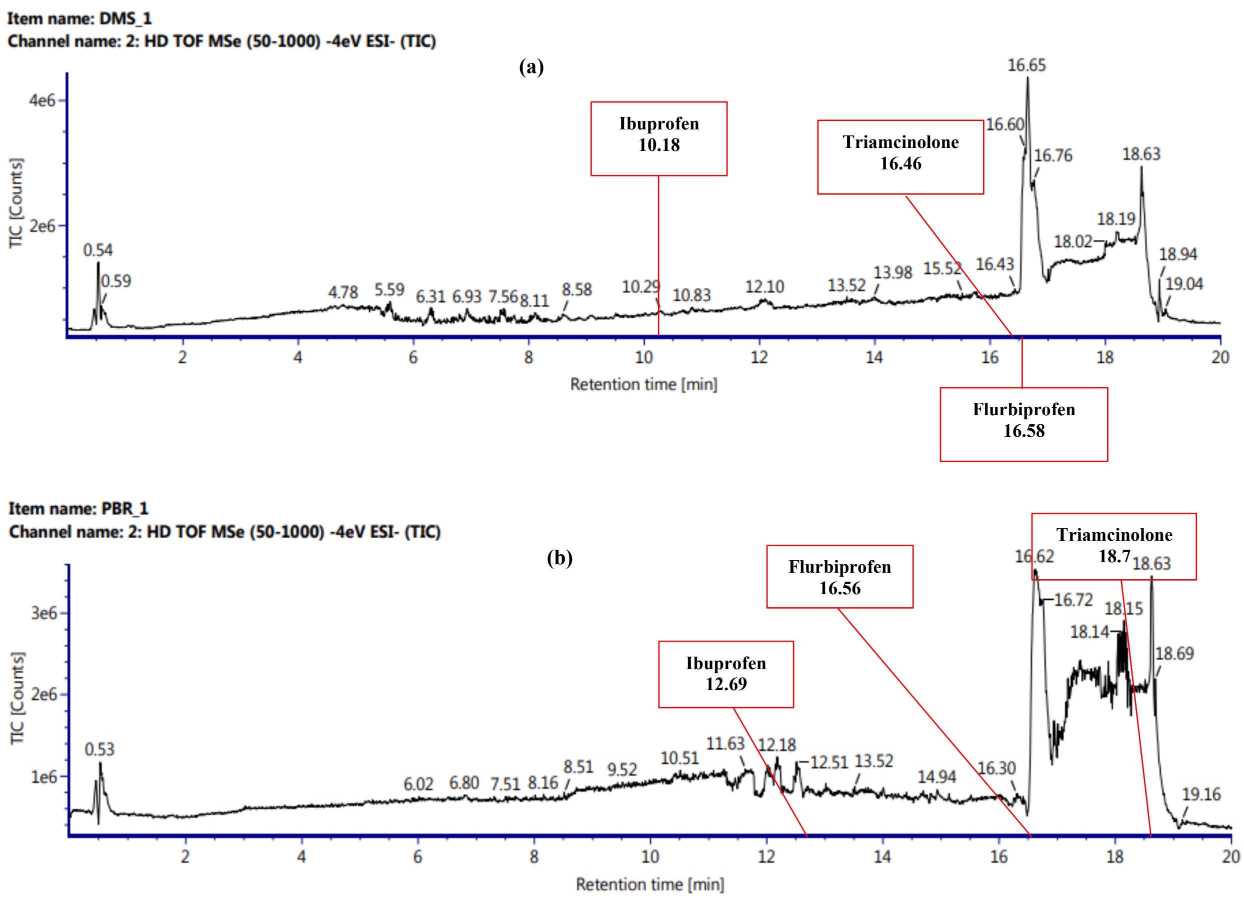

Figure 2. ESI negative mass spectrometry chromatogram of station (a) DMS and (b) PBR.

Based on the chromatogram obtained from ESI positive mode, triamcinolone was the only anti-inflammatory compound detected in all stations, with an observed m/z value of 395.186. On the other hand, ibuprofen (analgesic), flurbiprofen (analgesic), and triamcinolone (anti-inflammatory) were the most prominent compounds identified in the ESI negative mode chromatograms across all stations. Ibuprofen, flurbiprofen, and triamcinolone were detected with observed m/z values of 251.129, 289.089, and 393.171, respectively. **Figure 1** and **Figure 2** showed the ESI positive and ESI negative, mass spectra of station DMS and PBR, respectively, as representatives to demonstrate the retention times of the detected triamcinolone, ibuprofen, and flurbiprofen. Since ibuprofen is an effective pain reliever and sold over-the-counter medicine, its presence in raw wastewater was expected and readily identified [37].

Emerging Micropollutants in Raw Wastewater Samples

A total of 22 pharmaceuticals, 3 PCPs, 1 flame retardant, and 1 plasticizer were detected in raw wastewater samples using suspect screening in both ESI positive and negative modes. Each raw wastewater sample contained at least 8 different compounds. The WWTPs with the highest number of contaminants were DMS (17 compounds), MMJ (16 compounds) and MGJ (15 compounds), whereas station PBR had the least number of compounds (10 compounds). **Figure 3** shows the distribution of the identified compound across different chemical classes.

Pharmaceuticals were the most frequently detected EMPs, while fragrance and plasticizers showed the lowest detection frequencies, respectively, as demonstrated in **Table 2**. Pharmaceuticals dominated among EMPs in the wastewater samples due to the continuous introduction of newly formulated drugs into the market and the incomplete metabolism of many compounds in the human body. Consequently, pharmaceutical residues are frequently discharged into WWTPs through human excretion [38][39].

As shown in **Figure 4**, the detected pharmaceutical EMPs were most abundant in the following order: anti-inflammatory > analgesics > antibacterials, across all 14 WWTPs. In other words, anti-inflammatory compounds were the most frequently detected pharmaceuticals in raw wastewater samples from all stations. The global consumption of anti-inflammatory drugs exceeds thousands of tons annually, making them among the most commonly detected pharmaceuticals in the environment [40]. These compounds also exhibit high environmental persistence and resistance to biodegradation due to their high stability and reactivity [41].

As shown in **Table 2**, triamcinolone was the most frequently detected EMP among all 14 WWTPs in ESI positive mode. In ESI negative mode, flurbiprofen, ibuprofen, and triamcinolone were detected and identified in all 14 WWTPs. Ibuprofen and flurbiprofen are widely consumed analgesics with similar effects in relieving pain and fever [42][43]. These compounds commonly enter WWTPs through human urine and faeces following consumption [44]. The detection of ibuprofen in domestic wastewater with the concentrations exceeding hundreds of ng/L to µg/L had been reported by the researchers from Johor and Selangor in Malaysia [6][45]. Triamcinolone, often formulated in lotions and ointments for skin treatment, may enter WWTPs when washed off the skin [46]. Previous studies have also reported that triamcinolone is frequently detected in river water and WWTPs effluent [47][48][49]. Pharmaceuticals often been the main screening targets by researchers due to their ecotoxicological effects [50-51].

In contrast, phenylbutazone (anti-inflammatory) and galaxolidone (fragrance) were detected only once in ESI positive mode, at stations PR1 and MGJ, respectively. Galaxolidone was determined as the most abundant synthetic musk in the influent wastewater samples in Bangkok and Pattaya, Thailand [52]. Meanwhile, monomethyl phthalate (plasticizer) and endosulfan sulfate (insect repellent) were each detected only once in ESI negative mode, at stations MMJ and BRD, respectively. Phthalates are the endocrine-disrupting chemicals which can cause adverse effects, including reproductive failure, respiratory problems, allergic symptoms. Phthalates and their metabolites can enter raw wastewater by excretion and discharge directly into aquatic environment [53-54]. Phthalates such as monomethyl phthalate are frequently detected as urinary phthalate metabolites in Asia [55][56]. Additionally, tris (butoxyethyl) phosphate, a flame retardant, was detected in samples from both Station BRD and Station PDG.

Recently, suspect screening of emerging micropollutants in the influents from wastewater treatment plants has been investigated by researchers from different countries. **Table 3** below summarized the EMPs detected in influent wastewater from Ho Chi Minh, Beijing, and Denmark [26, 57-58]. Pharmaceutical compounds were the most prevalent category detected in the previous studies and this study. Since treated wastewater is an alternative source of clean water supply, thus the removal efficiency of WWTPs should be a focus for future studies to ensure the standard and quality of water [59-60].

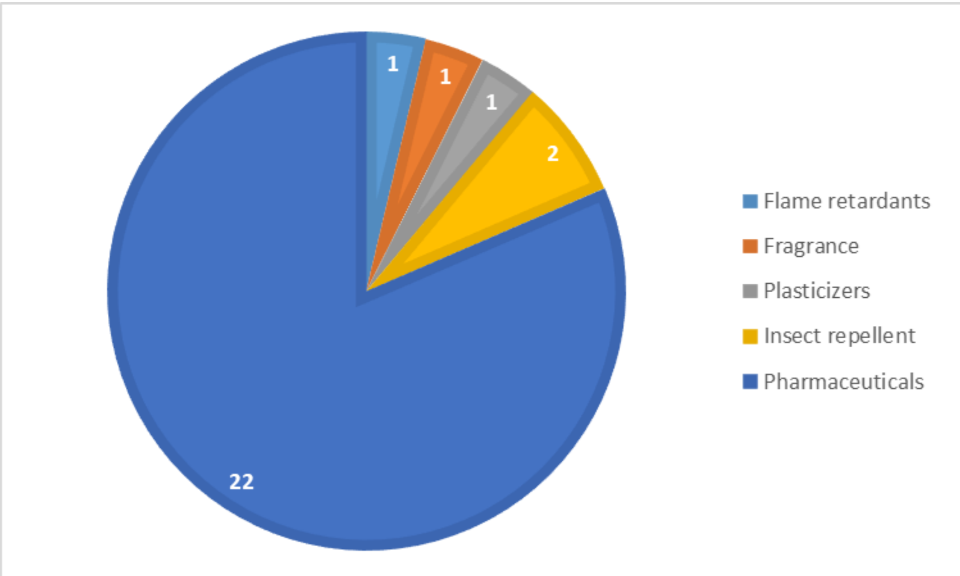

Figure 3. Pie chart showing an overview of the main groups of chemicals identified in raw wastewater, with the number of chemicals identified.

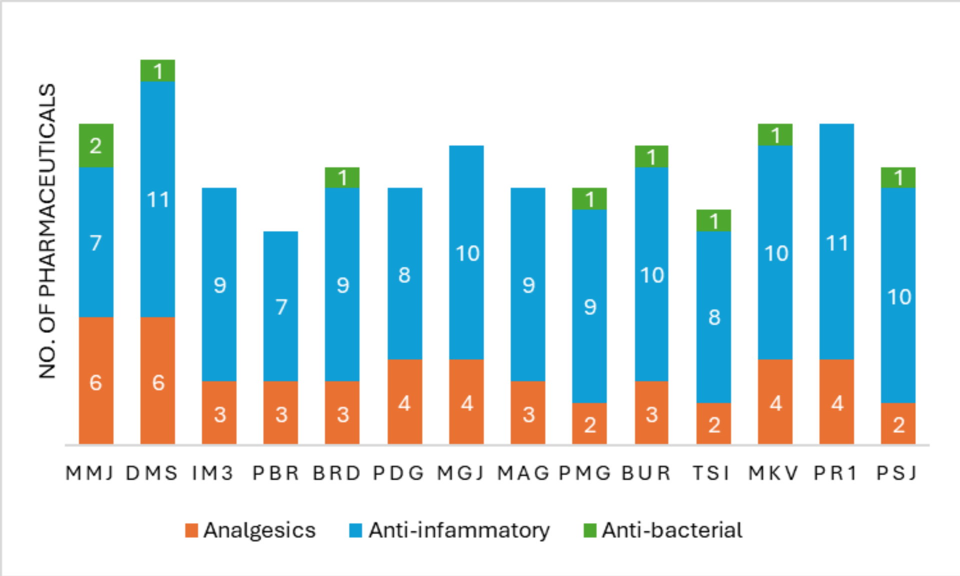

Figure 4. Quantity of three types of pharmaceuticals in raw wastewater from 14 WWTPs in both positive and negative modes.

Table 2. Detection frequency of emerging micropollutants among 14 WWTPs.

Group	Compound	Detection Frequency (%)
Pharmaceuticals (Analgesic)	Etodolac	28.6
	Flurbiprofen	100
	Ibuprofen	100
	Paracetamol	21.4
	Phenacetin	14.3
	Morphine	42.9
Pharmaceuticals (Anti-inflammatory)	Beclomethasone	71.4
	Betamethasone	57.1
	Betamethasone acetate	92.9
	Clobetasol propionate	71.4
	Cortisone	57.1
	Fluocinonide	57.1
	Hydrocortisone	57.1
	Hydrocortisone acetate	35.7
	Methylprednisolone aceponate	35.7
	Phenylbutazone	7.14
	Prednisone	71.4
	Prednisolone acetate	71.4
	Sulindac	28.6
	Triamcinolone	100
Pharmaceuticals (Antibacterial)	Sulfamethoxazole	14.3
	Trimethoprim	50.0
Flame retardants	Tris (butoxyethyl) phosphate	14.3
PCP (Fragrance)	Galaxolidone	7.14
Plasticizers	Monomethyl phthalate	7.14
PCP (Insect repellent)	Methoxyfenozide	21.4
	Endosulfan sulfate	7.14

Table 3. Comparison of the amount of EMPs detected in wastewater influent from different locations according to previous studies.

Location of WWTPs	EMP class	Number of compounds detected	Reference
Ho Chi Minh, Vietnam	PPCPs	11	[57]
	Illicit drugs	3	
	Artificial sweetener	1	
	Tobacco	3	
	Insect repellent	1	
Denmark	Pharmaceuticals	21	[26]
	Antibiotics	8	
	Food additives	4	
	Industrial chemicals	9	
	Pesticides	6	
	Others	13	
Beijing, China	PPCPs	16	[59]
	Plasticizers, flame retardants	7	
	Insecticide TPs	2	
	Pesticides	6	
	PAHs*	4	
	Steroids, hormones	10	
	Phenols	5	
	Food additives	4	
	Medical intermediate	7	
	Others	38	
Kuantan, Pahang, Malaysia	Pharmaceuticals	22	This study
	Insect repellent	2	
	Flame retardants	1	
	Plasticizers	1	
	Fragrance	1	

*PAHs: polycyclic aromatic hydrocarbons

CONCLUSION

In conclusion, 27 EMPs, including pharmaceuticals, PCPs, plasticizers, and flame retardants, were identified through suspect screening from 14 WWTPs in this study. Pharmaceuticals were the most frequently detected, while plasticizers were the least. Ibuprofen, flurbiprofen, and triamcinolone were present in all wastewater samples. The presence of EMPs in raw wastewater may contaminate other water sources, such as rivers, lakes, and marine environments, posing a threat to aquatic organisms when discharged from WWTPs. Therefore, future studies should focus on the quantitative analysis of both influents and effluents of WWTPs to better assess the quality of water discharged into natural water bodies. Implementing preventive measures and advancing wastewater treatment technologies should be a priority for researchers to overcome the ongoing challenges on water pollution.

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