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IDENTIFICATION OF PHARMACEUTICAL RESIDUES IN TREATED SEWAGE EFFLUENTS IN JOHOR, MALAYSIA

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Abstract: The introduction of pharmaceutical residues into aquatic environment has threatened the livelihood of aquatic organisms worldwide. The entrance of these residues into the environment originates from sewage effluents discharged from domestic wastewater treatment plants. Up to date, their presence in the sewage effluent is not monitored in Malaysia. Therefore, this study aims to identify the presence of pharmaceutical residues in the effluent domestic sewage treatment plants employed in Johor Bahru, Malaysia. Briefly, ten pharmaceutical compounds, including acetaminophen, sulfathiazole, sulfamethazine, sulfamethoxazole, clarithromycin, trimethoprim, lincomycin, carbamazepine, naproxen and ibuprofen, were selected based on their worldwide consumption. Sewage samples from five different types of sewage treatment system were collected. The samples were filtered prior to solid-phase extraction. Finally, the extracted samples were analysed with LC-MS/MS. The analyses showed that only sulfathiazole was not present in all effluent samples. Acetaminophen recorded the highest concentration of 9299 ng/L in an Imhoff Tank. Meanwhile, the lowest concentration of pharmaceutical residue detected was sulfamethazine, i.e. 0.843 ng/L, in a sequencing batch reactor. Overall, six out from ten pharmaceutical residues were found in all sewage samples denoting the inefficiency of current biological treatment systems in removing trace pharmaceutical compounds from sewage.

Keywords: *Pharmaceutical residues, sewage treatment plant, sewage effluent, occurrences*

1.0 Introduction

The exponential growth of new pharmaceutical drugs in the market has significantly reduced the mortality rate of non-communicable diseases among human race. Concurrent with the consumption of pharmaceutical drugs, they are continually released into the aquatic environment via domestic sewage treatment systems. As those compounds contain specific bioactive ingredients that can trigger a response at low doses, it has brought forth detrimental effects to the aquatic community. For instance, pharmaceutical compounds such as 17α-ethinyl estradiol and 17β-estradiol can disrupt endocrine system (Jarque *et al*., 2015, Saaristo *et al*., 2010).

Endocrine disruptive compounds (EDCs) may mimic, block or modulate hormone synthesis, with many acting as agonists of estrogenic receptors or antagonizing androgen receptors (Scippo *et al*., 2004). These will permanently modify the organization and future function of the male reproductive system and alter male behaviour. Some evidences provided in previous studies observed that EDCs can inhibit spermatogenesis, reduced sperms count and lowered egg productions in fishes (Milnes *et al*., 2006, Saaristo *et al*., 2010). In other cases, fishes exposed to EDCs in surface water were found to possess two reproductive organs (Jarque *et al*., 2015). The effects did not include the "cocktail effect" where different types of pharmaceutical compounds can interact to produce adverse combination effects.

Several reasons leading to their presence in the waterways include the incapability of the conventional sewage treatment systems in removing the micropollutants, improper channel of sewage into domestic drainage system and also some other anthropogenic activities (Bolong *et al*., 2009, Verlicchi *et al*., 2012). Nonetheless, domestic sewage treatment plants are one of the main gates for pharmaceutical residues entering the water bodies (Zhang *et al*., 2008). Generally, domestic sewage treatment plants worldwide employ conventional biological treatment systems owing to its excellent performance in removing high organic and nutrient content from sewage (Verlicchi *et al*., 2012). Therefore, the removal of pharmaceutical compounds is frequently overlooked. In Malaysia, the biological systems include extended aeration, sequencing batch bioreactor, oxidation ditch, oxidation pond and Imhoff Tank. According to previous studies, biological treatment processes have shown poor removal efficiencies for pharmaceutical compounds, except acetaminophen and ibuprofen, due to their resistance towards biodegradation (Göbel *et al*., 2007, Zhang *et al*., 2008, Sipma *et al*., 2010, Ryan *et al*., 2011).

Presently, the determination of pharmaceutical residues in sewage effluent in Malaysia is very little. Detection of pharmaceutical residues in sewage and surface water in the country only emerged in 2010 (Al-Odaini *et al*., 2010). However, the effluent and water samples analysed were randomly picked. Therefore, this study aimed to evaluate the

presence of pharmaceutical residues in sewage effluent for different types of biological treatment system utilized in Malaysia.

2.0 Methodology

2.1 Chemicals

All solvent used were of HPLC grade. Acetic acid and ammonium acetate were obtained from Wako (USA). Meanwhile, hexane, acetone, hydrochloric acid (HCl), formic acid, methanol and tetrasodium ethylenediaminetetraacetate (Na_4EDTA) were purchased from J.T. Baker (USA). Ultrapure water (18.2 MΩ.cm) for washing and standard preparation was produced by Milli-Q Direct Q-5 (UK).

2.2 Internal and Recovery Standards

Sulfathiazole-$^{13}C_6$, sulfamethoxazole-$^{13}C_6$, trimethoprim-d_9, sulfamethazine-$^{13}C_6$, sulfathiazole-$^{13}C_6$, trimethoprim-d_9, acetaminophen-d_3, acetylsalicylic acid-d_3, and ibuprofen-d_3 were employed as internal standards; while, isoproturon-d_5 and fenoprop were utilized as recovery standards in this study.

2.3 Sample Collection

Wastewater samples were collected from five different types of sewage treatment plants. Specific details of the selected sewage treatment plants were outlined in Table 1. Sewage samples before and after treatment were collected to observe the presence of targeted pharmaceutical residues. Samples were collected using opaque plastic bottle via grab sampling technique. The sample bottles were rinsed twice with sewage before collection. The sample bottles were kept in polystyrene box with ice to maintain its temperature below 4°C in order to minimize biochemical degradation before processing the samples.

2.4 Sample Preparation

Filtration apparatus were cleaned with acetone and hexane to prevent any organic contaminants. The filter paper was rinsed with distilled water followed by wrapping with aluminium foil before drying in the oven for 2 hours at 100^{o}C. The sample bottles were inverted several times to homogenize the content before filtration. 200 to 250 mL of the samples were filtered through 0.45 µm GF/F filter paper (Whatman, UK) to remove any suspended particles. Filtered samples were then stored in sample bottles and labelled accordingly.

Table1: Details of selected WWTPs for sampling

Type of WWTPs	*Location*	*Average Flow (m^3/day)*	*Population of Equivalent (PE)*	*River Discharged*
Extended Aeration (EA)	Taman Laguna	2343	2445	Melayu river
Oxidation Pond (OP)	Taman Perling	4,661	5295	Melayu river
Oxidation Ditch (OD)	Taman Tan Sri Yakob	11,682	14865	Melana river
Sequencing Batch Reactor (SBR)	Taman Impian Emas	18,531	24965	Skudai river
Imhoff Tank (IT)	Taman Ungku Tun Aminah	268	214	Skudai river

2.5 Solid-Phase Extraction (SPE)

The SPE were conducted with a 12-sample SPE vacuum manifold from SUPELCO (USA). SPE cartridge Oasis© HLB (6mL, 200 mg) purchased from Waters (USA) was used in this experiment. The SPE cartridges were conditioned sequentially with methanol and acidic water (pH 2) twice. Approximately 200 µL of 6N hydrochloric acid (HCl) was added to the filtered samples to adjust pH to acidic condition and 400 µL of Na_4EDTA were added to filter sample to inhibit the formation of precipitation. 100 µL of 0.5 ppm Internal Standard listed in section 2.2 were added into two blank and filtered samples. The sample bottles were connected to the HLB cartridge by using PTFE tube and labelled accordingly for the loading phase. The flowrate of SPE cartridge were set to 1 drop/sec. Upon the completion of the loading phase, SPE cartridges were dried for about 30 minutes. Once all the SPE cartridge were completely dried, 12 mL of methanol was used to elute each SPE cartridge. Finally, the eluents were evaporated under a gentle stream of nitrogen gas until the final volume achieved approximately 0.5 to 1 mL. The concentrated eluents were then transferred into 2 mL amber vials using micropipette. Recovery standards were spiked in all eluted samples.

2.6 Liquid Chromatography Tandem with Mass Spectrometry Analysis.

The extracts were analyzed for targeted pharmaceutical compounds using Agilent 1200 high performance liquid chromatography (Agilent Technologies, USA) combined with an Agilent 6460 triple quadruple mass spectrometer (Agilent Technologies, USA). The analytes were separated using a ZORBAX Eclipse XDB-C18 column (4.6 x 150 mm i.d., 3.5µm particle size, Agilent Technologies, USA), at 35ºC. The mass spectrometer was

used in both positive and negative electrospray ionization mode. Targeted analytes were identified and quantified using multiple reaction monitoring (MRM) mode. A flowrate of 0.3 mL/min and an injection volume 10µL were used for all analyses. The mobile phases comprised of two mobile phases, where acetonitrile/methanol (50:50, v/v %) as mobile phase A, while ultrapure water consisting of 0.3% ammonium formate and 0.1% formic acid made mobile phase B. The column was initially equilibrated at 5% mobile phase A. The percentage of mobile phase A was increased in gradient until it reached 95% over a period of 28 minutes. For the mass spectrometry condition, both positive and negative ion mode were run at 250ºC gas temperature, 10L/min gas flowrate, 4000V capillary voltage and 40 psi nebulizer pressure.

3.0 Results and Discussion

3.1 Frequency of Detected Pharmaceutical Residues in Treated Wastewater Effluent

Figure 1 shows the frequency of detection of 10 targeted pharmaceutical residues in the sewage effluent. From 16 effluents collected, sulfathiazole was the only selected pharmaceutical that was not detected in any of the effluent samples. Six pharmaceutical compounds were detected in all samples collected, namely, acetaminophen, lincomycin, trimethoprim, sulfamethoxazole, clarithromycin and naproxen. The results revealed that these six pharmaceutical compounds are among the most frequently consumed pharmaceutical by resident living nearby these sewage treatment plants. Meanwhile, sulfamethazine was traced in 15 samples. This compound was not traced at the sample of effluent stage of EA. While, carbamazepine was traced at all sample except samples from Imhoff Tank. Ibuprofen was the least compound traced in this study with 68.75% percentage of detection (11 samples), suggesting that this pharmaceutical might have high rate of biodegradability during the treatment processes.

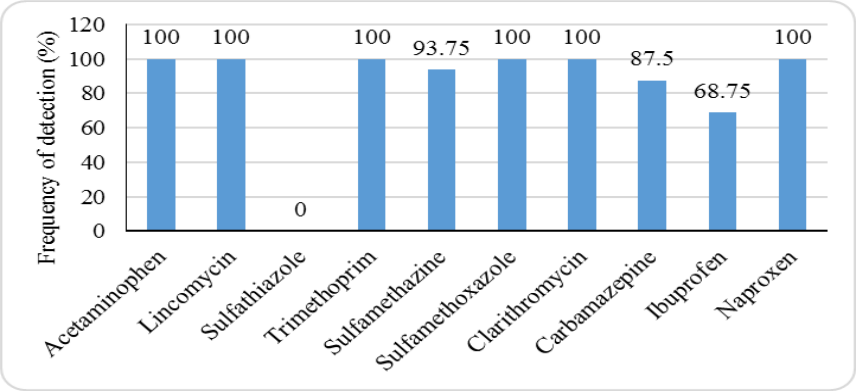

Figure 1: The frequency of detection (%) of targeted pharmaceutical in the samples of wastewater treatment plant (n=16)

3.2 Concentration of Pharmaceutical Residues in Treated Wastewater Effluent

Table 2 shows the concentration of the ten selected pharmaceutical residues detected in the treated wastewater effluent. The following discussion will focus specifically on the group of the pharmaceutical compounds.

Table 2: The concentration of selected pharmaceutical residues in treated wastewater effluents (ng/L).

WWTPs type	Stages	Acetaminophen	Lincomycin	Sulfathiazole	Trimethoprim	Sulfamethazine	Sulfamethoxazole	Clarithromycin	Carbamazepine	Ibuprofen	Naproxen
SBR	Influent	12433	3.95	ND	188	12.8	2239	5.28	18.6	108	898
	Effluent	309	3.49	ND	48.5	0.843	19.9	225	125	ND	29.2
EA	Influent	17120	28.8	ND	3.58	12.4	11.6	64.1	9.24	640	1082
	Effluent	8.33	7.41	ND	3.20	ND	4.50	475	12.8	95	831
IT	Influent	17712	18.0	ND	5.98	18.0	26.3	4.91	ND	103	124
	Effluent	9299	47.0	ND	2.52	10.4	9.55	7.79	ND	88	376
OD	Influent	28641	7.45	ND	37.3	7.92	78.9	91.1	11.6	769	127
	Effluent	5.05	69.1	ND	38.7	9.35	140	101	39.5	ND	143
OP	Influent	40165	35.7	ND	313	13.7	1532	41.1	17.0	569	3746
	Effluent	11.0	11.6	ND	17.0	1.87	28.6	22.5	5.49	203	232

ND: Non-detectable <Method detection limit (MDL)

3.2.1 Analgesic

The highest concentration pharmaceutical recorded at the influent of oxidation pond, i.e. 40,165 ng/L. While the lowest concentration of acetaminophen in the influent sample was recorded in extended aeration with a concentration of 17,120ng/L. The highest concentration of acetaminophen at the effluent sample was in Imhoff Tank (9299 ng/L) and the lowest concentration was from oxidation ditch (5.05ng/L). From five wastewater treatment systems selected, four of them showed good efficiency in removing acetaminophen with average removal efficiency up to 90% except for Imhoff Tank, which recorded less than 50% of removal suggesting its inefficiency in removing acetaminophen. Acetaminophen was expected to be easily determined and the

concentration would be quite noticeable owing to its popular usage as an active ingredient in many over the counter drugs to relieve pain or to reduce fever.

3.2.2 Antibiotics

The concentration for lincomycin, trimethoprim, sulfamethazine, sulfamethoxazole, clarithromycin and carbamazepine were 69.1 ng/L, 313.0 ng/L, 18.0 ng/L, 2239.0 ng/L, 475.0 ng/L and 125.0 ng/L, respectively. While the lowest concentration of these compounds, lincomycin, trimethoprim, sulfamethazine, sulfamethoxazole and clarithromycin were 3.49 ng/L, 1.86 ng/L, 0.843 ng/L, 4.50 ng/L and 4.91 ng/L, respectively. It is surprising that the concentration of lincomycin and clarithromycin increased at the treated effluent. This explains that lincomycin and clarithromycin were bioaccumulated during the wastewater treatment process rather than degraded.

3.2.3 Anticonvulsant

The highest concentration of carbamazepine was recorded at the sample of effluent of SBR, 124ng/L which higher than the concentration recorded at the influent stage, 18.6ng/L. From five sewage treatment plants, three of them, sequencing batch reactor, extended aeration and oxidation ditch show an increasing concentration of carbamazepine from the influent until the effluent stage. In addition, carbamazepine achieved poor percentage removal inImhoff Tank and Oxidation pond. Similar results were also reported by previous studies (Zhang *et al*., 2008)(Vieno *et al*., 2007, Joss *et al*., 2005) . This might be due to its persistent properties towards water-soluble nature.

3.2.4 Nonsteroidal Anti-Inflammatory Drug (NSAID)

The highest concentration of ibuprofen detected at the influent was 769 ng/L, at the oxidation ditch, while the lowest concentration was 88 ng/L at the effluent of Imhoff tank. The percentage removal of ibuprofen can achieve up to 100% removal. Naproxen recorded the highest concentration at the influent of oxidation dtich, 3746 ng/L, while the lowest naproxen concentration was recorded 29.2ng/L at the effluent of sequencing batch reactor. The concentration of naproxen is likely to accumulate in two sewage treatment plants (Imhoff Tank and oxidation ditch) which express inefficiency of those treatment systems in removing pharmaceutical residues. In contrast, the concentration of naproxen reduces in three other treatment plants including sequencing batch reactor, extended aeration and oxidation pond. The occurrence pattern of naproxen in the all treatment plants varied greatly proving the different behaviour of this compound during the treatment processes.

4.0 Conclusion

This study has proven that sewage treatment plant in Malaysia are incompetent in removing pharmaceutical residues. From all 10 targeted pharmaceuticals, 9 of them were detected in all the five sewage treatment plants. Acetaminophen, lincomycin, trimethoprim, sulfamethazine, sulfamethoxazole, and clarithromycin were consistently present in all effluent samples. Acetaminophen was the highest concentration among other pharmaceutical compounds studied, 40165 ng/L, while the lowest concentration pharmaceutical compound recorded is sulfamethazine, 0.843 ng/L. The removal efficiencies of pharmaceutical compounds from the selected sewage treatment plants varied greatly varied. Nonetheless, there still residue of the pharmaceutical compounds discharged from the treatment plants that can potentially harm the aquatic organisms in the long run.

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