Pertanika J. Sci. & Technol. 25 (S): 119 - 128 (2017)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Occurrence, Source Apportionment and Environmental Risk Assessment of Pharmaceuticals in Klang River, Malaysia

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ABSTRACT

This study examined the presence and sources of 10 pharmaceuticals in Klang River were studied. The most common pharmaceuticals were caffeine and acetaminophen, 0.57-20.62 ng/mL and "not detected"-1.45 ng/mL. Water samples were clustered based on pharmaceutical concentrations. Source apportionment analysis showed that treated wastewater discharged from treatment plants contributed 18.43% of pharmaceuticals in Klang River. An environmental risk assessment by means of the risk quotient (RQ) was done whereby the latter was more than one for salicylic acid and diclofenac in surface water posing threats to the aquatic environment. Salicylic acid showed high risk for acute toxicity, while diclofenac showed high risk for chronic toxicity. The results indicated a need for regular monitoring on pharmaceutical levels in Klang River and increasing the efficiency of wastewater treatment here.

Keywords: Environmental risk assessment, Klang River, Malaysia, occurrence, pharmaceuticals, source apportionment

INTRODUCTION

Many studies have examined the presence of pharmaceuticals in water samples (Al-Odaini et al., 2013; Lindberg et al., 2014; Silva et al., 2014). Findings showed high frequency of pharmaceuticals detected in water samples, mainly from point sources, such as influent wastewater, treated wastewater, and receiving water (Al-Odaini et al., 2013; Carmona et al., 2014; Oppenheimer et al., 2011; Schaider et al., 2014). The presence of pharmaceuticals in aquatic ecosystems is due to its high consumption. Pharmaceuticals enter the water compartment through municipal wastewater, hospitals waste and pharmaceutical industries effluent (Cardoso et al., 2014; Collado et al.,

ARTICLE INFO

Article history:
Received: 28 September 2016
Accepted: 03 February 2017

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ISSN: 0128-7680

2014; Golovko et al., 2014). The discharge of treated wastewater effluent into receiving water is responsible for the contamination.

Klang River basin is a densely populated and developed area in Malaysia and flows through major urban areas: Kuala Lumpur - Petaling Jaya - Shah Alam - Klang. The objectives of this study were to study the occurrence and distribution of pharmaceuticals in Klang River and its tributaries, to interpret the contribution of sources of pharmaceuticals loadings into Klang River through source apportionment analysis utilising principal component analysis (PCA) with multiple linear regression (MLR) method and to assess the potential environmental risk of surface water by evaluating the ratio between the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) for these surface waters.

METHOD

Chemicals and Materials

The selected pharmaceuticals include caffeine (CAF), acetaminophen (ACT), salicylic acid (SAL), diclofenac (DIC), ibuprofen (IBU), mefenamic acid (MEF), gemfibrozil (GEM), carbamazepine (CBZ), estrone (EST) and methanesulfonic acid (MSA) purchased from Sigma-Aldrich (purity assay in range of 98-101%). Acetonitrile (ACN) (HPLC grade) was purchased from Merck (Darmstadt, Germany). Ultrapure water was produced by Barnstead Nanopure (Thermo Scientific).

Sampling

The Klang River (Figure 1) originates from the Ulu Gombak Forest Reserve and flows through Kuala Lumpur and Selangor before finally entering the Straits of Malacca. Klang River and its 13 tributaries run through a densely populated area and is the receiving water body for discharges from sewage treatment, hospitals, industries and urban through its tributaries and sewerage system. Therefore, the river is subjected to pharmaceuticals from point and non-point source. A sampling of surface water was conducted from August 2014 to October 2014. Water samples were collected at 6 sites on the main stem of Klang River and 7 sites on 5 tributaries (Figure 1). One litre of each sample was collected in polypropylene bottles, acidified using

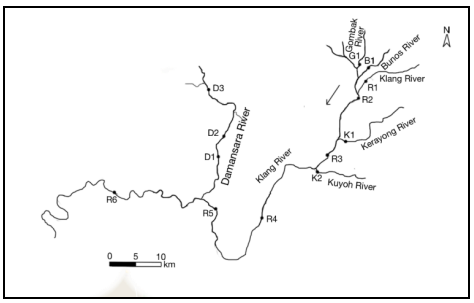

Figure 1. Sampling sites in the Klang River and its tributaries.
Note: The arrow indicates flow direction

hydrochloric acid (3 M) and vacuum-filtered using Whatman 45 µm GF/A filters (Whatman International Ltd Maidstone, England). The filtered wastewater samples were stored in the dark at 4ºC prior to online SPE-HPLC analysis.

Instrumentation

The online solid phase extraction high performance liquid chromatography (SPE-HPLC) was performed using Dionex Ultimate 3000 (Sunnyvale, CA, USA) system. The system consists of a large volume loop (10.2 mL) autosampler (WPS-3000SL Analyt.), dual gradient pump, left and right (Pump DGP-3600A), a thermostated column (TCC-3200 2x2P-6P) and a diode array detector (DAD) (PDA-3000 Photodiodearray). The system was equipped with a programmable 6 port/2 position switching valve for several modes (loading, clean-up, elution and separation). Data were processed by the Chromeleon Software v.6.8 (Dionex, USA). SPE clean-up was performed using Dionex IonPac AG14A RFIC (4.0×50 mm) (Thermo Scientific, USA), and analytical separation was performed with Acclaim Polar Advantage II (5 µm, 120 Å, 4.6×150 mm) (Thermo Scientific, USA). The temperature was set at 40°C. A gradient elution with a flow rate of 1 mL/min was used throughout the analysis. The DAD was set at wavelength of 220 nm (diclofenac, salicylic acid, mefenamic acid, estrone), 250 nm (acetaminophen) and 280 nm (caffeine, ibuprofen, carbamazepine, gemfibrozil). The gradient elution consisted of acetonitrile, 10 mM methanesulfonic acid (MSA) and ultrapure water.

Sample Extraction and Analysis

An online SPE-HPLC-DAD method was used to preconcentrate and separate the samples. The method had three steps. At equilibration mode, 10 mL of water sample was loaded onto sample loop using autosampler (fitted with a 100 µL syringe and 10 mL loop). Then, SPE column was positioned in loading mode using the switching valve. In the first step (loading), the left pump was used to load sample from sample loop onto SPE column at 1 mL/min and simultaneously the analytical column was equilibrated with the right pump. Co-retained sample matrix was then flushed out using a washing composition of 10 mM MSA and ACN (95:5). In the second step (elution), the switching valve was switched to elution position to couple the SPE column with the analytical column and analytes were transferred using gradient elution mobile phase composition. In the third step (separation), the switching valve was switched back into equilibration mode, disconnected the SPE column with the analytical column and analytes were separated in an analytical column using the right pump. All analytes were simultaneously analysed using DAD. Recoveries of 10 target pharmaceuticals were determined by spiking surface water samples at 5 ng/mL. The recoveries ranged between 86.6% and 108.6% and the reproducibility in relative standard deviation was between 0.8% and 10.2%. Limit of detections (LOD) were determined using regression method that ranged between 0.01 and 0.26 ng/mL.

Source Apportionment Method

Source apportionment analysis was conducted using Principal Component Analysis-Multi Linear Regression (PCA-MLR) method with XLSTAT 2014 (USA) software. The PCA was

used to analyse the most significant variables by excluding the less significant variables with minimum loss of original information (Kannel et al., 2007). Principal components (PCs) with Eigenvalue more than one were selected for factor analysis (FA). Then, PCA with varimax rotation was performed containing four pharmaceuticals detected in columns and sampling sites in rows to obtain varimax functions (VFs). The relationship between the principal component and the pharmaceutical is indicated by the factor loadings. Stepwise MLR was then performed on the significant factor scores to determine the source apportionment of each source based on total concentrations. The source apportionment from each factor was estimated using MLR utilising factor score values as independent variables and measured total pharmaceuticals as dependent variables. The basic equation of this model is:

$$Y = \beta 0 + \beta 1x1 + \beta 2x2 + \ldots + \beta p\text{-}1xp\text{-}1 + e \quad \text{Eq. (1)(Juahir et al, 2011)}$$

where Y is the response variable, x as explanatory variables x1, x2, xp-1, β as p parameters (regression coefficients) β0, β 1, β 2, ..., Bp-1 and e as the random error.

Ecotoxicological Risk Assessment

The risk of quotient (RQ) is a useful tool to characterise the potential ecological risk of contaminants in three trophic levels (fish, invertebrates and algae) of the aquatic environment (Gros et al., 2010). Based on EMEA guideline, RQ was calculated as the ratio between Measured Environmental Concentration (MEC) and Predicted No-Effect Concentration (PNEC) (Eq. (2)). The MEC is the maximum concentration of target compounds found in the effluent sample. The PNEC was estimated using the lowest values of acute EC_{50} or LC_{50} or the chronic No-Observed Effect Concentration (NOEC) (Kosma et al., 2014), divided by a default assessment factor (AF). For acute toxicity test results, PNEC was estimated using Eq. (3) and for chronic toxicity test results, the PNEC was estimated using Eq. (4) (Kosma et al., 2014).

$$RQ = \text{exposure/toxicity}$$

$$RQ = \text{highest concentration (MEC)/ PNEC,} \quad \text{Eq. (2)}$$

where,

$$PNECacute = EC_{50} \text{ or } LC_{50}/\ 1000 \quad \text{Eq. (3)}$$

$$PNECchronic = NOEC/AF \quad \text{Eq. (4)}$$

The ratio between the exposure concentration and predicted no effect concentration determines the potential environmental risk. A “high risk” is suspected when RQ≥1, “medium risk” is suspected when 1>RQ>0.1 and “low risk” is suspected when 0.1>RQ>0.01.

RESULTS AND DISCUSSION

Presence of Pharmaceuticals

The concentrations of selected pharmaceuticals in surface water samples are shown in Table 1. Surface water of Klang River showed presence of caffeine, acetaminophen, salicylic acid and diclofenac. Caffeine showed the highest mean concentration and ranged from 0.57-20.62 ng/mL. The variation in concentration could be due to the effect of dilution of compounds after discharge into surface water, and physical phenomena such as adsorption to sediments or suspended solids, biodegradation or photo degradation (Lopez-Serna, 2010) and concentrations of compounds in the surface water. A lower concentration of caffeine was reported in rivers in Korea (0.26 ng/mL) (Sim et al., 2010), and rivers in the United States (0.013-0.3 ng/mL) (Oppenheimer et al., 2011). Caffeine was reported as a promising marker for urban faecal contamination due to its persistence, solubility and high occurrences (Fenech et al., 2013).

Acetaminophen was detected in most of the sampling sites, while diclofenac and salicylic acid were detected in most of the tributaries of Klang River. The concentration of acetaminophen, diclofenac and salicylic acid in surface water ranged from 0.12-1.45 ng/mL, 0.05-3.21 ng/mL and 0.19-13.0 ng/mL, respectively. High concentration of these pharmaceuticals can be explained by high consumption of these pharmaceuticals in Malaysia and are available as over-the-counter medications. In addition, it was reported that acetaminophen is discharged as conjugates, and these conjugates might be broken down to its parent compound during wastewater treatment, resulting in the high amount of acetaminophen released into surface water (Al-Odaini, 2013). A comparable concentration for acetaminophen was reported in Malaysian rivers (maximum 0.35 ng/mL) (Al-Odaini, 2013) and Ebro River, Spain (n.d-0.71 ng/mL) (Lopez-Serna, 2012). The observed pharmaceuticals concentration range indicated that the studied water bodies were exposed to various sources including surface runoff from the urban zone, treated wastewater discharged from treatment plants and untreated wastewater.

A cluster analysis was performed to classify the river according to their pharmaceutical concentrations. As shown in Figure 2, sampling sites D2 and K1 were grouped in Cluster 1. High concentration of pharmaceuticals detected in these sampling sites may be due to the direct input of treated wastewater discharged from treatment plant that received wastewater from developed housing areas (Taman Tun Dr Ismail (TTDI) Jaya) and Kerayong River (K1). D1 and D3 from Damansara River (Cluster 2) showed moderate pharmaceutical concentration with discharges from Section 13, Shah Alam and Kampung Melayu Subang. Low concentrations of pharmaceuticals from six sites along Klang River were grouped in Cluster 3.

Table 1
Concentrations of pharmaceuticals in surface water samples (ng/mL)

River	Sampling site	Caffeine	Acetaminophen	Salicylic acid	Diclofenac
Klang	R6	1.46	0.46	n.d	n.d
Klang	R5	1.7	1.42	n.d	n.d
Klang	R4	2.65	1.39	n.d	n.d
Klang	R3	4.08	1.15	n.d	n.d
Klang	R2	0.62	0.12	n.d	0.04
Klang	R1	0.57	n.d	n.d	n.d
Damansara	D1	7.9	1.44	1.36	2.11
Damansara	D2	20.62	1.45	4.13	n.d
Damansara	D3	8.26	0.66	3.15	n.d
Kuyoh	K2	2.36	0.72	n.d	3.21
Kerayong	K1	4.92	1.11	13.0	1.87
Gombak	G1	0.74	n.d	n.d	0.05
Bunos	B1	2.59	1.40	0.19	0.23

*n.d = not detected

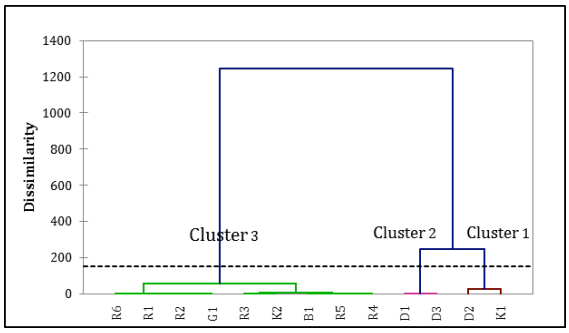

Figure 2. Dendogram showing cluster of sampling sites

Pharmaceutical Profile and Source Apportionment

In order to study the sources pharmaceutical in surface water samples, their presence in influent and effluent wastewater samples from six extended aeration wastewater treatment plants (WWTPs) located on Klang River were studied (Table 2). The WWTPs mainly treat wastewater from domestic sewage. Table 2 shows the frequency of pharmaceuticals detected in influent and effluent. Caffeine and acetaminophen were detected in all samples with the frequency of 87% to 100%. High removal rate of caffeine (96.2%), acetaminophen (97.2%) and salicylic acid (97.5%), while lower removal rate of diclofenac (38.7%) were observed. Similar results were also reported by Gros et al. (2010), Papageorgiou et al. (2016) and Dutta et al., (2014).

The PCA with Varimax rotation was applied using data set samples versus pharmaceuticals, to study the influence of pharmaceutical contamination in Klang River. The PCA results showed that 71.6% of the variance of the original data might be explained by the two main components. Table 3 shows factor loadings for the pharmaceuticals variables. The PC1 represents 40.23% of the data variability, and the highest loading variables in this PC were caffeine and acetaminophen (>0.75). The high removal rate of these two compounds indicated no significant contribution of these pharmaceuticals from treated wastewater into the river and hence, the sources of caffeine and acetaminophen were mainly from untreated wastewater. Diclofenac is the most influential variable in PC2, which explains 31.18% of the variance. The low removal rates of diclofenac resulted in high contribution of this compound from treated wastewater into the river. MLR analysis was performed on PCA scores to determine source apportionment of the compounds in all surface water samples. In this study, the factor scores from PCA for PC1 and PC2 as independent variables were regressed against total sum of four pharmaceuticals, as dependent variables. The resulting equation was:

$$\text{SumPharma} = 7.63+6.67*PC1+3.18*PC2 \quad \text{(Eq. 5)}$$

The standardised coefficients (β) of the model presented in Table 3 indicate the relative influence of the PCs. Pharmaceuticals from untreated wastewater greatly influenced the total pharmaceutical loading into receiving water (β = 0.8525), followed by treated wastewater discharged from treatment plant (β = 0.4063). The β values describe the relative relationship between pharmaceuticals and each VF. Thus, the source apportionment of pharmaceutical sources in Klang River came from untreated wastewater (81.57%) and treated wastewater discharged from treatment plant (18.43%), and these findings are consistent with a study conducted on Beijing River (Dai et al., 2015).

Table 2
Concentrations of pharmaceuticals in influent and effluent wastewater samples

Sample		Caffeine	Acetaminophen	Salicylic acid	Diclofenac
Influent	Frequency of detection (%)	100	100	50	83
	Range (ng/mL)	6.59-68.8	6.93-191.9	n.d-13.47	n.d-88.95
Effluent	Frequency of detection (%)	67	100	16	83
	Range (ng/mL)	n.d-1.81	0.14-0.76	n.d-0.48	n.d-0.35
	Removal rate (%)	96.2	97.2	97.5	38.7

Table 3
Factor loadings of the PCA analysis of pharmaceuticals in surface water samples

	PC1	PC2
Caffeine	0.8879	-0.0094
Acetaminophen	0.7857	0.1158
Salicylic acid	0.4575	0.6270
Diclofenac	-0.0451	0.9169
Eigenvalue	1.8088	1.0555
Variability (%)	40.4233	31.1839
Cumulative %	40.4233	71.6073
Standardise coefficient (β)	0.8525	0.4063

Note: Strong loading >0.75

Risk assessment

An assessment of the risk quotient (RQs) in the surface waters of the Klang River was done. Results for the RQ value were calculated from acute and chronic toxicity data for the three trophic levels (fish, invertebrates and algae) as shown in Figure 3 and Figure 4. Salicylic acid showed high risk for acute toxicity in fish, while diclofenac displayed high risk for chronic toxicity in fish (RQ>1). However, the levels of these pharmaceuticals were not toxic for invertebrates and algae. Similar findings were reported by Tewari et al. (2013). The findings indicated the significance of assessing and classifying the potential environmental impact of these pharmaceuticals (Gros et al., 2010; Kosma et al., 2014; Papageorgiou et al., 2016). As diclofenac showed high risk in chronic toxicity, this compound should be monitored in surface water.

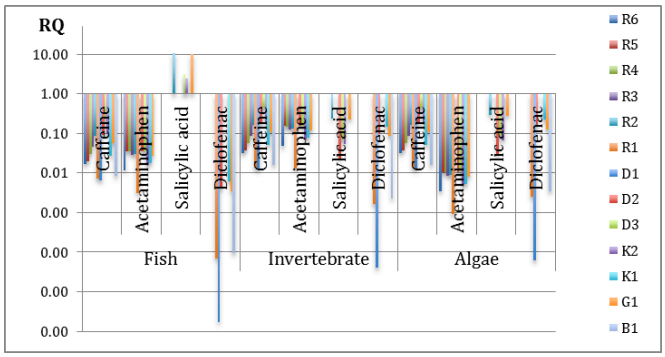

Figure 3. Risk quotients for pharmaceuticals in surface waters estimated for fish, invertebrates and algae for acute toxicity

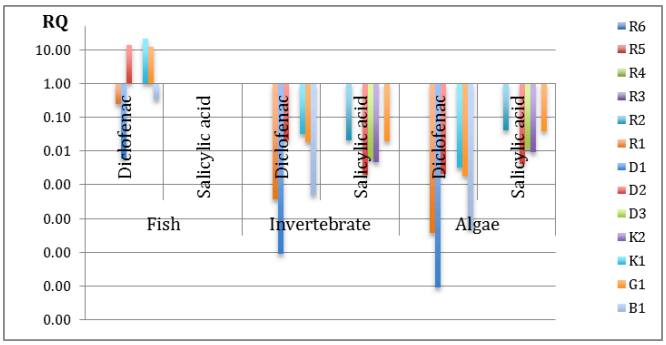

Figure 4. Risk quotients for pharmaceuticals in surface waters estimated for fish, invertebrates and algae for chronic toxicity

CONCLUSION

The occurrence and sources of 10 pharmaceuticals in the surface water of the Klang River were studied. The concentrations of pharmaceuticals varied in the range of "not detected" to 20.62 ng/mL. The significant contribution of pharmaceuticals from treated wastewater discharged from treatment plants (18.43%) indicated a need to increase efficiency of wastewater systems in eliminating pharmaceuticals. Results from environmental risk assessment showed salicylic acid and diclofenac exhibited RQ>1, which can threaten the aquatic ecosystem. Therefore, active monitoring is important to detect the presence of pharmaceuticals in order to protect the river quality.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support from Organisation for the Prohibition of Chemical Weapons (OCPW) (L/ICA/ICB/170997/11) and Research Management Centre (RMC) Universiti Teknologi MARA.

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