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Spatial analysis and source profiling of beta-agonists and sulfonamides in Langat River basin, Malaysia

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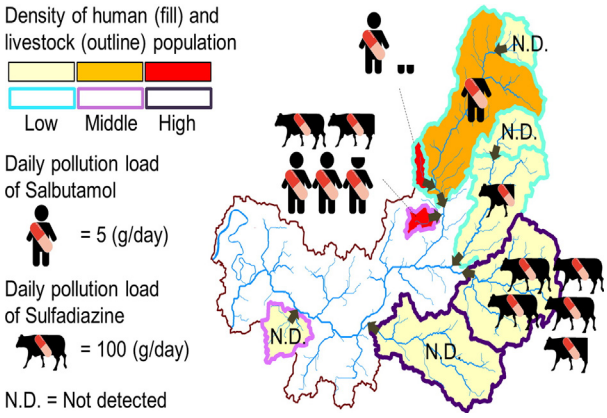
HIGHLIGHTS

- Beta-agonists and sulfonamides were quantified in the Langat River basin.
- Six compounds were widely detected in surface water especially from populated areas.
- Spatial analysis by GIS was used to estimate population density.
- Source profiling was based on estimated population density and monitoring results.
- Source profiling identified pollution sources of three compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Beta-agonists and sulfonamides are widely used for treating both humans and livestock for bronchial and cardiac problems, infectious disease and even as growth promoters. There are concerns about their potential environmental impacts, such as producing drug resistance in bacteria. This study focused on their spatial distribution in surface water and the identification of pollution sources in the Langat River basin, which is one of the most urbanized watersheds in Malaysia. Fourteen beta-agonists and 12 sulfonamides were quantitatively analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). A geographic information system (GIS) was used to visualize catchment areas of the sampling points, and source profiling was conducted to identify the pollution sources based on a correlation between a daily pollutant load of the detected contaminant and an estimated density of human or livestock population in the catchment areas. As a result, 6 compounds (salbutamol, sulfadiazine, sulfapyridine, sulfamethazine, sulfadimethoxine and sulfamethoxazole) were widely detected in mid catchment areas towards estuary. The source profiling indicated that the pollution sources of salbutamol and sulfamethoxazole were from sewage, while sulfadiazine was from effluents of cattle, goat and sheep farms. Thus, this combination method of quantitative and spatial analysis clarified the spatial distribution of these drugs and assisted for identifying the pollution sources.

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1. Introduction

Beta-agonists and sulfonamides are among the most commonly used veterinary drugs worldwide. Beta-agonists are used to treat cardiogenic shock, acute heart failure, bradyarrhythmias, asthma and chronic obstructive pulmonary disease (Yu et al., 2011), and also to feed them as a growth promoter in livestock feed for considerable muscle mass increase while decreasing fat accumulation (Mersmann, 1998). Similarly, sulfonamides are widely used in livestock productions for their therapeutic and prophylactic properties (Malintan & Mohd, 2006) as well as for growth promotion (Cheong et al., 2010). Environmental pollution of these drugs is concerned and beta-agonists may lead to adverse cardiovascular events (Salpeter et al., 2004), while sulfonamides may cause drug-resistant bacteria (Huovinen et al., 1995). Furthermore, beta-agonists and sulfonamides are used to treat diseases and infections for human, and they had been detected in waste streams from wastewater treatment plants, sewage treatment plants, hospitals and drug production facilities (Yu et al., 2011). Therefore, there is the potential for contamination of the water by such a wide range of treatments for both human and livestock. In Malaysia, the potential usage of banned beta-agonists in swine farms was identified (Ponniah et al., 2004), and salbutamol was detected in effluents of sewage treatment (Al-Odaini et al., 2010 and Al-Odaini et al., 2013). Likewise, sulfonamides were detected in effluents from swine farms (Malintan & Mohd, 2006), and the residues were detected in chicken meat products (Cheong et al., 2010). Since sulfonamides have high potentials to resist degradation and are highly mobile (Luo et al., 2011), they could be extensively transported from pollution sources flowing surface water.

The Langat River basin is located in the southern part of the capital region (*i.e.* Klang Valley) in Peninsular Malaysia, and has a major role in regional water supply. Urbanization in the Langat River basin drastically progressed due to the acceleration of economic growth since the 1980s. As a consequence, surface runoff had increased with a positive relationship with urban-related land uses (Noorazuan et al., 2003). Moreover, water quality in the Langat River basin has deteriorated due to wastewater from industry and households, municipal waste and agricultural runoff (Juahir et al., 2011). Drinking water treatment facilities have been frequently shut down due to a high concentration of ammonia in intake water over the last two decades (Hasan et al., 2010).

According to our preliminary survey, surface water was heavily contaminated by *Escherichia coli* (*E. coli*) around urban sites and *E. coli* were detected even in upstream regions. It is probably because household coverage by piped sewers is inadequate and septic tanks are poorly maintained. Furthermore, there is no specific regulation or federal law for the livestock effluent standards except for the regulations that were enforced through various state enactments and by-laws. Therefore, farmers usually treat their wastewater by sedimentation ponds or any biological waste treatment systems. In addition, some livestock species such as cattle, dairy cow and goat were sometimes released to graze outside. Both insufficiently treated livestock effluents and the excrement left in grazing fields could be transported into rivers. Thus, these various point sources and non-point sources would result in the severe contamination of *E. coli*.

Likewise, severe environmental pollution of beta-agonists and sulfonamides is concerned in the Langat River basin. There are populated cities and towns located in mid catchment areas and a large scale of oil palm plantation and livestock farms around the southern part of the mid catchment areas and entire downstream areas. The variety of anthropogenic influence could cause the contamination of these drugs and make the pollution complicated to identify the sources by environmental monitoring. Therefore, a spatial analysis and a source profiling are also necessary to visualize the occurrence and distribution as well as to identify the pollution sources.

Distribution of contaminants in river basin is basically followed by elevation and flow direction of surface water in a gravitational way, which means that occurrence of contaminants detected in a sampling point is subject to its catchment area. The pollutant load could become proportional to a number of pollution sources (*i.e.* point source) and/or an area of pollution sources (*i.e.* non-point source) in the catchment area. As long as there is no significant regional difference of coverage of sewage treatment system and hydrological impacts like abstraction of river water among catchment areas, the pollutant load in surface water would have a correlation with the number and/or the area of pollution sources. If that is the case, the pollutant loads of beta-agonists and sulfonamides could have a correlation with human and/or livestock population, considering their usage for treatments. Therefore, this study aimed to analyze these drugs throughout the Langat River basin for clarifying the contamination, and also to conduct the spatial analysis and source profiling for identifying the pollution source by using geographic information system (GIS).

2. Material and methods

2.1. Standards and reagents

Fourteen beta-agonist standards (cimaterol, terbutaline, salbutamol, zilpaterol, cimabuterol, ractopamine, clenbuterol, brombuterol, tulobuterol, mabuterol, hydroxymethyl clenbuterol, clenpenterol, isoxsuprine and mapenterol), 2 beta-agonist surrogates (ractopamine-d3 and clenbuterol-d9) and 12 sulfonamide standards (sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfadimethoxine, sulfaquinoxaline, sulfamethoxazole, sulfisoxazole and sulfachloropyridazine) were provided by Department of Veterinary Services in Ministry of Agriculture & Agro-Based Industry Malaysia. Methanol and acetonitrile were purchased from Fisher Scientific (USA).

2.2. Sampling sites and surface water collection

The locations of 14 sampling sites in the Langat River basin are shown in Fig. 1 and Table 1. The selection of the sampling sites was based on the main stream (*i.e.* the Langat River) and major tributaries. The sampling was conducted on the 22nd November, 2014. In each sampling site, surface water was collected by a stainless steel container and its one liter was filled into a polypropylene bottle. Meanwhile, pH and electrical conductivity (EC) were measured by LAQUAtwin (Horiba, Japan), and dissolved oxygen and water temperature were measured by Accumet AP84 (Fisher Scientific, Malaysia) on-site. Likewise, river width, water level and velocity in each sampling point were measured with a laser distance meter, a stopwatch, a water-level gauge and a plastic ball for estimating flow rate. The collected samples were put into a cooler box and transferred back to laboratory, and stored at 4 °C so as to prevent degradation of target compounds. The water samples were pretreated and analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) within 1 week after the sampling date.

2.3. Laboratory analysis

Chemical oxygen demand (CODMn), biochemical oxygen demand (BOD) and suspended solids (SS) were analyzed by standard methods. Ammonia nitrogen (NH_3-N) and phosphate-phosphorus (PO_4-P) were analyzed by a digital pack test apparatus (Digital Pack Test Multi, Kyoritsu Chemical-Check Lab. Corp., Japan). The number of *E. coli* was analyzed by Colilert method (IDEXX Laboratories Inc., USA).

2.4. Sample pretreatment

Sample pretreatments were separately taken for beta-agonists and sulfonamides in each water sample to fulfill acceptable recovery. In case of beta-agonists, 100 to 200 mL of the water samples were spiked with 10 μL of a surrogate standard, ractopamine-d3 (1 mg/L), and

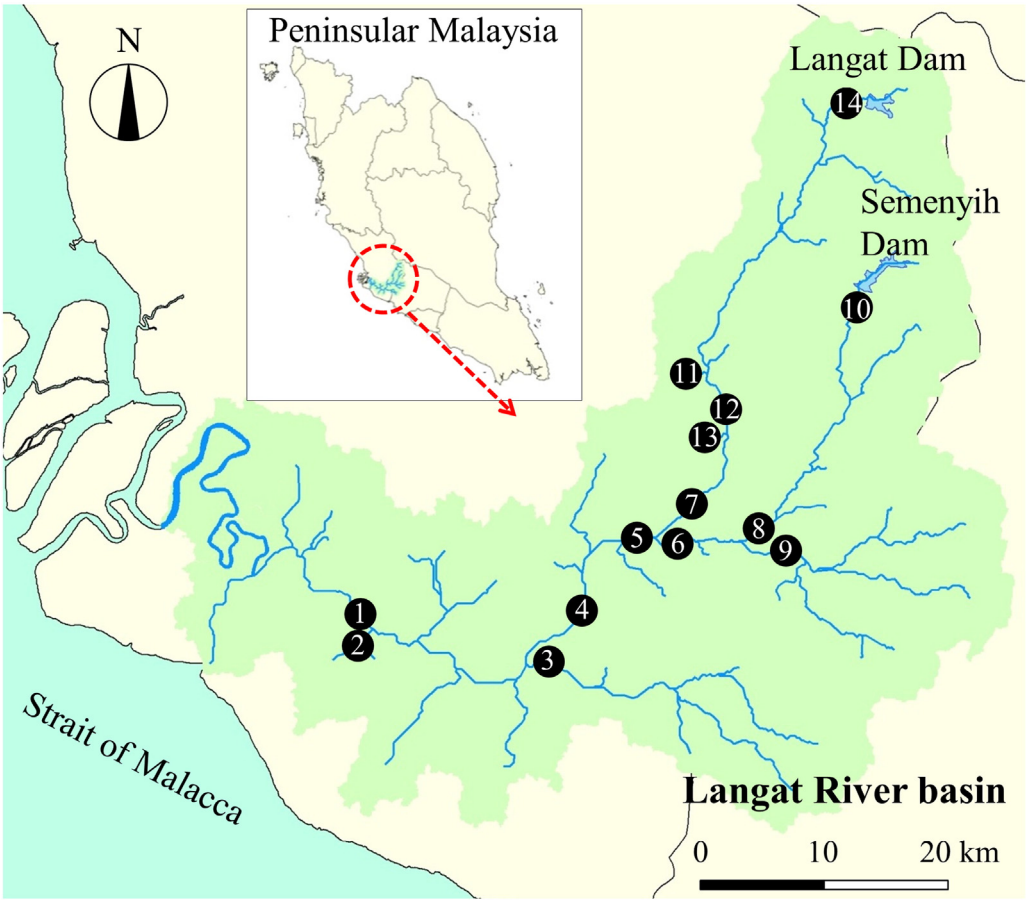

Fig. 1. Location of 14 sampling sites in the Langat River basin.

filtered through a GF/B glass microfiber filter (GE Healthcare UK Ltd., UK). The filter was put into a centrifugal tube and added with 5 mL of methanol. It was sonicated for 5 min and the eluate was mixed with the filtrate. The pH of the mixture was alkalized with 200 g/L of sodium hydroxide and adjusted at 10 to obtain a higher recovery in the following solid phase extraction. Solid phase extraction cartridge (Oasis HLB (6 cm^3/200mg), Water Corp, USA) was conditioned with 5 mL of methanol followed by 10 mL of pure water, and the alkalized solution was loaded into the cartridge at 5 mL/min. After the sample loading, the cartridge was dried up for 30 min and slowly eluted the retained beta-agonists with 5 mL of methanol twice. The eluate was gently evaporated with nitrogen gas and reconstituted with 480 μL of methanol added with 20 μL of an internal standard, clenbuterol-d9 (1 mg/L). The solution was filtered through a 0.2 μm regenerated cellulose syringe filter (Phenex, Phenomenex Inc., USA) and the filtrate was analyzed by LC–MS/MS.

In case of sulfonamides, no surrogate standard was spiked at the beginning of the sample pretreatment, and no pH adjustment by sodium hydroxide was taken before the solid phase extraction to obtain a higher recovery. At the reconstitution, 500 μL of methanol was added and no internal standard was added to be analyzed in an external standard method. Other pretreatment processes were the same as beta-agonists.

Table 1
Geographic information of 14 sampling sites in the Langat River basin.

Station	Longitude	Latitude	River/Tributary
S1	101′30″43 E	2′48″55 N	Langat River
S2	101′30″32 E	2′48″55 N	Tributary (from Banting)
S3	101′41″29 E	2′48″18 N	Labu River
S4	101′40″56 E	2′51″21 N	Langat River
S5	101′43″46 E	2′53″48 N	Langat River
S6	101′45″00 E	2′53″32 N	Semenyih River
S7	101′45″33 E	2′55″05 N	Langat River
S8	101′48″31 E	2′54″14 N	Semenyih River
S9	101′49″44 E	2′53″18 N	Tributary (from Beranang)
S10	101′52″25 E	3′03″23 N	Semenyih River (near Semenyih Dam)
S11	101′46″04 E	3′00″55 N	Tributary (from Balakong)
S12	101′47″08 E	2′59″35 N	Langat River
S13	101′46″23 E	2′58″03 N	Tributary (from Bandar Baru Bangi)
S14	101′53″01 E	3′12″56 N	Langat River (near Langat Dam)

2.5. LC–MS/MS analysis

LC–MS/MS (LCMS8030, Shimadzu, Japan) was used for the quantitative analysis. Mass spectrometric analyses were performed in multiple reaction monitoring (MRM) mode and the MS/MS parameters were optimized by flow injection analysis. A binary mobile phase was used with (A) 0.02% (v/v) formic acid and (B) methanol/acetonitrile (1:1), and Hypersil Gold C18 column (150 × 2.1 mm i.d.) with a particle size of 1.9 μm (Thermo Scientific Inc., USA) was attached to meet a good chromatographic isolation. The mobile phase gradient was as follows: 10% B (0 min), 10–95% B (0–6 min), 95% B (6–10 min) and 10% B (10–15 min) at a flow rate of 0.15 mL/min. The injection volume of the pretreated solution was 3 μL in beta-agonists and 5 μL in sulfonamides which were maximum volumes to avoid carry-over under the above settings. Table S1 summarizes the optimal MS/MS condition.

Linear calibration curves were used for all analytes with correlation coefficients over 0.995. The limit of detection (LOD) and limit of quantitation (LOQ) in each analyte were calculated as 3 and 10 times value of a standard deviation, respectively, which was calculated by a repeated

analysis of a certain concentration of standard solution of which signal-to-noise ratio was around 10. A recovery test of each analyte was conducted by spiking a known concentration of standard solutions into uncontaminated river water samples at the beginning of the sample pretreatment and analyzing them after the sample pretreatment. Table S1 shows the result of LOD, LOQ and recovery.

2.6. Spatial analysis and data compilation with GIS

Geographic information system (ArcGIS version 10.0, ESRI Inc., USA) was used for spatial analysis and source profiling of the analytes. Digital elevation data (Shuttle Radar Topography Mission (SRTM3)) were downloaded from United States Digital Service (https://dds.cr.usgs.gov/srtm/version2_1/SRTM3) to visualize a watershed boundary of the Langat River basin and catchment areas of each sampling point. These layers were merged with a sub-district boundary in Selangor State and a few district boundaries in Negeri Sembilan State. Moreover, statistical data of human population in each sub-district in 2010 were downloaded from Department of Statistics Malaysia (https://www.statistics.gov.my/), and census of poultry, cattle and dairy cow, swine, goat and sheep in each district of Selangor State and Negeri Sembilan State in 2012 were provided by Department of Veterinary Services (unpublished data). These data were added in the administrative map as attributes.

The populations of human and each livestock species were estimated in each catchment area based on how many percent of each sub-district area is overlapped with the catchment area. For instance, when a catchment area is overlapped with 60% of a sub-district area and 80% of another sub-district area, the same fraction of the human and livestock populations in each sub-district are combined to estimate total population of human and each livestock species in the catchment area. Furthermore, the estimated population of each category was divided by its land area to estimate density in the catchment area (*e.g.* density of human population: 100 person/km^2).

2.7. Source profiling

Nine catchment areas (S2, S3, S8, S9, S10, S11, S12, S13 and S14) were subject to a source profiling, whereas the rest of the 5 catchment areas (S1, S4, S5 S6 and S7) were excluded as they cover some of the 9 catchment areas which is not appropriate for the source profiling. A daily pollutant load (g/day) in each sampling point was calculated from the result of the quantitative analysis multiplied by the observed flow rate. The daily pollutant load was plotted against each estimated density of human and livestock populations in the 9 catchment areas, and their correlation coefficient was calculated. Those of which the correlation coefficient exceeded 0.40 were considered as possible pollutant sources. With the results of the correlation coefficient and the spatial analysis, the source profiling was conducted and the pollution sources of the detected contaminants were assessed.

3. Results and discussion

3.1. Water quality monitoring and assessment

Table 2 shows *in-situ* water quality monitoring results in 14 sampling sites. The pH was neutral in S4–S14 (6.28–7.26), whereas it was acid in S1, S2 and S3 (4.47–5.72) probably because of peatland around these points. The peatland exists at downstream areas of the Langat River basin and the low pH occurs because of acid sulfate soils (Ng et al., 1994 and Wöstena et al., 1997). The peatland had been extensively developed for oil palm plantation and it caused subsidence and oxidation of pyrite (Wöstena et al., 1997). Therefore, further acidification by the oxidized pyrite might arise. The S10 and S14 are located in the most upstream areas nearby Semenyih and Langat Dam, respectively, and the water quality was almost in pristine condition. In contrast, S8 and S9 are located in the mid catchment areas and the surface water was considerably turbid as the suspended solids were higher than 200 mg/L. The catchment areas of these points are located in rural areas and extensive land is used for oil palm plantation. Therefore, non-point source pollution due to surface runoff from the oil palm plantation might be a major reason of the suspended solids. Furthermore, S11, S12 and S13 showed high ammonia nitrogen (1.38–5.92 mg/L), biochemical oxygen demand (12.1–15.9 mg/L) and *E. coli* (4.11×10^4–2.05×10^5 MPN/100 mL). It was probably because of sewage from households and industrial effluents as these catchment areas are located in populated cities and towns. As a consequence, S6 and S7 received all these pollutant loads, and the pollutants were transported to downstream areas flowing S5, S4 and S1 towards estuary.

3.2. Quantitative results of beta-agonists and sulfonamides

A result of the quantitative analysis of beta-agonists and sulfonamides is shown in Table 3. Six compounds (*i.e.* salbutamol, sulfadiazine, sulfapyridine, sulfamethazine, sulfadimethoxine and sulfamethoxazole) were detected in most of the sampling points. In particular, sulfadimethoxine was considerably detected in S13 (3407 ng/L), and sulfadiazine was dominantly detected in S2, S9 and S13 (250.3–299.4 ng/L). In contrast, there was almost no contamination at S10 and S14 where the catchment areas had little anthropogenic influence. A stacked bar chart of the concentration and daily pollutant load of the detected contaminants were shown in Fig. 2. The daily pollutant load clearly showed that the pollution sources were mainly from the catchments of S9 (521.1 g/day) and S13 (2861.9 g/day), and the contaminants from the tributaries were confluent in the main stream such as S7 (1167.9 g/day) and S5 (533.4 g/day) and distributed towards estuary

Table 2
Quantitative results of water quality parameters and flow rate in sampling sites (n = 14).

Station	Water temp (°C)	DO (mg/L)	pH (−)	EC (mS/cm)	NH_3-N (mg/L)	PO_4-P (mg/L)	COD (mg/L)	BOD (mg/L)	SS (mg/L)	*E. coli* (MPN/100 mL)	Flow rate (m^3/s)
S1	28.3	3.48	4.47	0.24	1.80	<0.03	12.5	0.6	265	2.40E+02	180.5
S2	29.7	3.15	5.28	0.27	1.97	<0.03	7.5	2.3	54	1.42E+03	0.0
S3	28.0	4.89	5.72	0.14	0.45	<0.03	9.6	2.3	122	8.52E+03	19.7
S4	31.1	4.27	6.61	0.14	0.99	0.05	8.7	4.7	165	8.70E+03	60.7
S5	30.9	4.18	6.84	0.14	1.19	0.04	6.2	3.9	146	1.54E+04	74.5
S6	31.1	4.60	6.65	0.09	0.34	0.03	9.2	1.9	151	1.42E+04	24.4
S7	33.4	2.94	6.85	0.18	2.17	0.06	8.3	7.9	134	1.42E+04	28.1
S8	32.2	5.11	6.36	0.07	0.43	0.08	11.2	5.8	204	2.24E+04	18.9
S9	33.3	4.86	6.67	0.13	1.67	0.03	3.7	7.7	220	1.16E+04	18.7
S10	28.0	4.90	6.28	0.05	0.34	<0.03	10.8	2.4	3	3.64E+02	0.2
S11	31.4	2.79	7.07	0.36	5.92	0.22	14.1	15.9	48	2.05E+05	3.8
S12	31.1	4.09	7.14	0.18	1.38	0.05	10.4	15.0	156	8.32E+04	8.2
S13	30.3	4.78	7.03	0.27	4.42	0.11	9.6	12.1	20	4.11E+04	8.9
S14	27.4	7.37	7.26	0.04	<0.2	<0.03	2.1	1.6	1	1.00E+01	5.1

Table 3
Concentrations of beta-agonists and sulfonamides in surface water (n = 14).

Compound	Concentration (ng/L)													
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
Beta-agonists														
Cimaterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Terubutaline	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Salbutamol	LOD	4.3	LOD	4.7	LOD	LOD	LOQ	LOD	LOD	LOD	19.1	4.7	19.1	LOD
Zilpaterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Cimabuterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Ractopamine	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Clenbuterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Brombuterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Tulobuterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Mabuterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Hydroxymethyl clenbuterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Clenpenterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Isoxsuprine	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Mapenterol	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Sulfonamides														
Sulfadiazine	6.6	268.4	LOD	13.0	11.9	26.6	24.3	36.8	299.4	LOD	4.8	LOD	250.3	LOD
Sulfathiazole	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Sulfapyridine	LOQ	6.3	LOQ	3.5	4.2	LOQ	7.5	LOQ	3.9	LOD	12.9	7.2	12.9	LOD
Sulfamerazine	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Sulfamethazine	LOQ	4.6	LOQ	LOQ	4.7	5.1	4.3	9.7	LOQ	LOD	5.9	4.6	LOQ	LOD
Sulfamethizole	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Sulfamethoxypyridazine	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Sulfadimethoxine	LOD	LOQ	LOD	LOQ	46.3	LOQ	412.5	LOD	LOD	LOD	LOD	LOD	3407.4	LOD
Sulfaquinoxaline	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Sulfamethoxazole	8.0	35.0	10.1	21.4	15.8	11.1	28.7	LOQ	15.0	4.7	59.8	20.7	43.2	LOD
Sulfisoxazole	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Sulfachloropyridazine	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD

LOD: limit of detection; LOQ: limit of quantitation.

flowing along S4 (261.0 g/day) and S1 (298.8 g/day). The tendency of the pollution was similar to the result of water quality monitoring (Table 2) as the mid catchment areas were heavily polluted and the pollutants were transported to the downstream areas. In addition, the daily pollutant load at S2 became disappeared because a water gate nearby S2 which is connected to the Langat River was closed and the water was stagnant on the sampling time.

3.3. Spatial analysis

The human population in each sub-district and livestock population in each district were classified with five levels and clearly visualized the distribution in a map of Selangor State and a few districts in Negeri Sembilan State merged with a boundary of federal territory of Kuala Lumpur and the Langat River basin (Figs. S1 and S2). The human population is apparently centralized in and around Kuala Lumpur, and a portion of the populated area is overlapped with the Langat River basin. In contrast, livestock population is obviously larger in Kuala Langat and northern parts of Selangor State than the capital region. Fig. 3 shows catchment areas of 9 sampling points merged with the classified density of human population. It was apparently visualized that the density of human population was obviously large in catchment areas of S11 and S13, whereas catchment areas S10 and S14 were less human population. On the other hand, populations of cattle and goat were larger in catchment areas of S2, S3 and S9 than others (Fig. 4). This visual recognition was rightly reflected to the estimated densities of human and

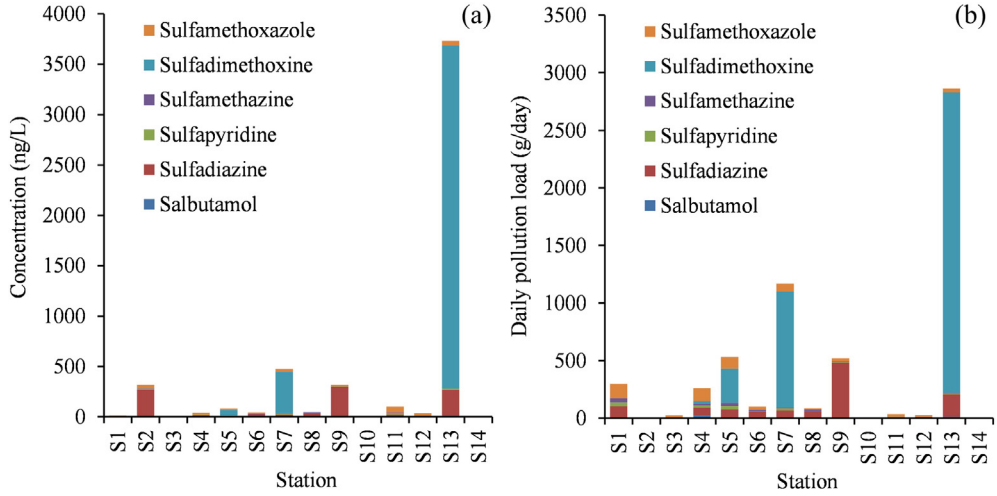

Fig. 2. Stacked bar graph of (a) concentration and (b) daily pollutant load of the detected beta-agonist (salbutamol) and sulfonamides (sulfadiazine, sulfapyridine, sulfamethazine, sulfadimethoxine and sulfamethoxazole) in surface water.

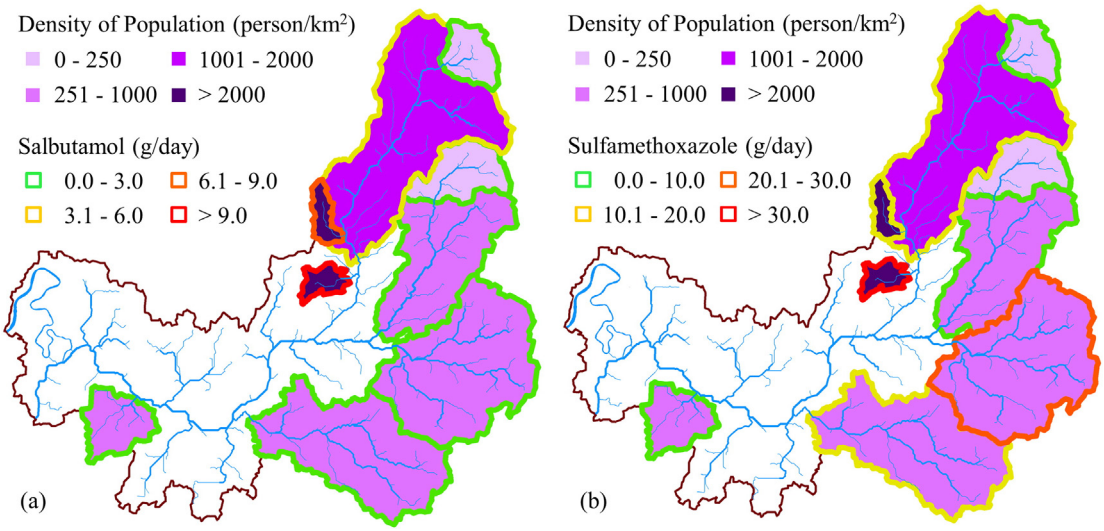

Fig. 3. Daily pollutant load of (a) salbutamol and (b) sulfamethoxazole at 9 sampling points visualized with estimated densities of human population in the respective catchment areas.

livestock populations among the catchment areas (Table S2). In addition, most of swine farms in Selangor State were localized in coastal areas of the Langat River basin and its estimated density was significantly high in S2.

3.4. Source profiling

Salbutamol and sulfamethoxazole were correlated with the estimated density of human population as correlation coefficients were 0.65 and 0.51, respectively. They were detected in S11, S12 and S13 of which catchment areas are located in populated cities and towns while few livestock farms exist. Salbutamol is used to relieve bronchoconstriction in asthma and to prevent pre-mature labor (Hashem et al., 2011), and more than 70% of unchanged salbutamol and its metabolite is excreted in urine (Morgan et al., 1986). Likewise, sulfamethoxazole is frequently used in combination with trimethoprim for the treatment and prophylaxis of *Pneumocystis carinii* pneumonia in patients with HIV (Mitra et al., 1996), and the half of the administered sulfamethoxazole is excreted as unchanged free drug (Shah et al., 1989). Therefore, they would be discharged *via* untreated sewage. According to Japan Sanitation Consortium, the coverage of sewerage diffusion in urban areas of Malaysia has been served about 70% (Japan Sanitation Consortium, 2011). It means that sewage from the unserved households would be directly discharged into rivers. Moreover, removal efficiencies of salbutamol and sulfamethoxazole by sewage treatment plants had been reported at 90–98% (Jones et al., 2007) and 26–88% (García-Galán et al., 2011), respectively. It indicates that certain amount of these drugs would be discharged from sewage treatment plants through the effluents.

In addition to the contamination from the populated areas, an equivalent amount of sulfamethoxazole was detected in S3 and S9 where the catchment areas are located in rural areas and many livestock farms exist (Fig. 4). The pollutant load of sulfamethoxazole had a weak correlation with the estimated density of cattle ($r = 0.27$), while there was little correlation with that of other livestock species ($r < 0.20$). Since sulfamethoxazole is also used to treat intestinal infection and infections of respiratory tract and urinary tract as an antimicrobial (Peng et al., 2006), it might be used for cattle and discharged *via* effluents from cattle farms. According to our field survey, cattle farms in Malaysia usually had a solid trap to collect feces, and farmers flushed the floor of their farms for clean-up every day. The effluent was retained at the solid trap and the supernatant was discharged into drains. Moreover, cattle were released

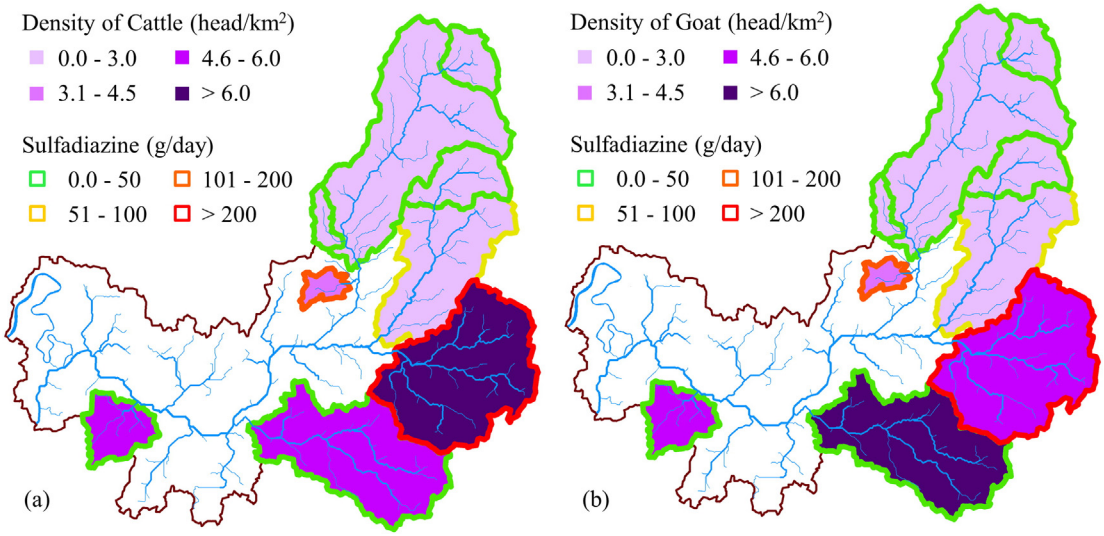

Fig. 4. Daily pollutant load of sulfadiazine at 9 sampling points visualized with estimated densities of (a) cattle and (b) goat in the respective catchment areas.

outside their farms to graze during daytime, and their excrement would be washed away into rivers by surface runoff. Therefore, point sources of effluents from cattle farms and non-point sources of their excrement might be the contamination source.

Sulfadiazine was highly detected in S9 and also detected in S6 and S8, and the daily pollutant load was correlated with the estimated density of cattle ($r = 0.51$), goat ($r = 0.53$) and sheep ($r = 0.67$). These areas are located in rural areas and many livestock farms exist (Fig. 4). Since sulfadiazine is used as a veterinary antibiotic to prevent and treat diarrhea and other infectious diseases (Heuer et al., 2008), effluents from these livestock farms could be contaminated and the untreated drug could be discharged into rivers. Furthermore, sulfadiazine is also used to treat burns and chronic wounds and prescribed to patients, and unchanged sulfadiazine is excreted in urine (Long, 1941). Therefore, the detection at S13 would be because of untreated sewage. According to our field survey, the breeding system of goats was similar to cattle and they were also released to graze outside during daytime. Therefore, non-point source of the excrement of goats could be one of the pollution sources. However, there was no correlation with the estimated density of poultry. According to our field survey, most of poultry farms in Malaysia were an enclosed system and poultry feces were collected and the dried feces were sold as an organic manure. Therefore, there was little effluent from the poultry farms. In addition, there was also little correlation with the estimated density of swine because swine farms are located in coastal areas of the Langat River basin and most of the 9 catchment areas did not cover the swine farm areas.

Sulfadimethoxine was considerably detected in S13 and distributed to S7 and S5, whereas it was not detected in S11. The catchment areas of S11 and S13 are located in populated areas, but this contrastive result led to a negligible correlation with the density of human population. In addition, sulfadimethoxine was not detected in S8 and S9 like sulfadiazine. Therefore, the considerable pollutant load at S13 would be neither due to sewage nor effluents from livestock farms. Since sulfadimethoxine is exclusively used as a veterinary medicine to treat infections especially for coccidian (Zhang et al., 2015), the pollution source might be specific facilities such as veterinary hospitals or manufacturers of this drug.

This source profiling did not work for sulfapyridine and sulfamethazine. They were not correlated with any estimated density of human and livestock populations. Sulfapyridine is used to control dermatitis herpetiformis (Duhring's disease) for human (Miao et al., 2004), and sulfamethazine is used for therapeutic purposes, treatment of infections and as a growth promoter for livestock (Lertpaitoonpan et al., 2009). Therefore, the pollution source could be similar to other detected sulfonamides. However, a clear tendency did not appear because their daily pollutant loads were not significantly different among the sampling points. The daily pollutant load of salbutamol and sulfamethoxazole at S11 was almost half of S13 (Fig. 3), although the estimated density of human population in the catchment of S11 was almost double (Table S2) and the number of *E. coli* at S11 was about three times higher than S13 (Table 2). Thus, the source profiling could be affected by many uncertain factors such as coverage of sewage treatment plants and removal efficiency, environmental fate from pollution sources, and gaps between the estimated densities and the exact numbers. Nevertheless, this combination method of the quantitative and spatial analysis is instrumental to visualize geographical pollution areas of veterinary drugs, and it could assist for identifying the pollution sources. The source profiling could be more advanced if more detailed and precise statistical data as well as local information such as land use, coverage of sewage treatment plants and location of possible pollution sources can be referred to.

4. Conclusions

This study detected 6 compounds of beta-agonists and sulfonamides in the surface water collected in the Langat River basin. It was suggested that the main pollution source of salbutamol and sulfamethoxazole was from sewage, while sulfadiazine was discharged from effluents of cattle, goat and sheep farms. The source profiling did not work for sulfadimethoxine, sulfapyridine and sulfamethazine, but the areas of the pollution sources were identified by the spatial analysis. These drugs were discharged from mid catchment areas and distributed towards estuary. It might lead to adverse effects to vulnerable aquatic species or produce drug resistance in bacteria. Currently, GAHP (Good Animal Husbandry Practice) which is a component of MyGAP (Malaysian Good Agricultural Practices) is employed as a guideline for prudent use of veterinary drugs and waste management. However, GAHP is only mandatory for exporting farms. Therefore, efficient enforcements are required to control both the sewage and effluents from livestock farms in the entire river basin.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.01.040.

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