Environ Sci Pollut Res
DOI 10.1007/s11356-015-4812-0

RESEARCH ARTICLE

Toxicological assessment of hospital wastewater in different treatment processes

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Received: 15 March 2015 / Accepted: 28 May 2015

Abstract This study surveyed the hospital wastewater characters focusing on antibiotic contamination in seven hospitals in Bangkok. It detected 19 antibiotics of which the high-frequent detection were quinolones such as ofloxacin + levofloxacin, norfloxacin, ciprofloxacin including sulfamethoxazole. Norfloxacin and ciprofloxacin appeared the highest concentrations of 12.11 and 9.60 μg/L, respectively. Most antibiotic concentrations in the wastewaters of the studied hospitals gave a good correlation (r^2=0.77–0.99) to the amount of usage. In this study, batch acute toxicity tests were performed to assess the toxicity of hospital wastewater on mixed liquor, freshwater algae (*Chlorella vulgaris* and *Scenedesmus quadricauda*), and microcrustacean (*Moina macrocopa*). The hospital wastewaters could inhibit the mixed liquor growth and gave similar toxic levels among test species: algae and microcrustacean (9.81–13.63 and 2.62–3.09 TU, respectively). The conventional activated sludge (CAS) and rotating biological contactor (RBC) could remove fluoroquinolones and tetracycline via biomass adsorption. After treatment, most of treatment could reduce the toxicity. Nevertheless, the effluent gave slight toxicity on some test species which might be caused from chlorination and a common toxicant (NH_3-N).

Keywords Antibiotics · Bioindicator · CAS · Ecotoxicology · Hospital wastewater · RBC

Introduction

Hospitals typically use numerous chemicals such as pharmaceuticals, radionuclides, solvents, and disinfectants in the process of diagnosis, equipment disinfection, and laboratory activities. After administration, antibiotics are incompletely metabolized; excretion fraction is commonly 10–90 % of the consumed amounts depending on their properties (Kummerer and Henninger 2003). The partial-metabolized antibiotics are excreted via patient excreta largely in the urine and partially in feces and are disposed into a hospital sewer system (Ternes and Joss 2006). Although discharged wastewater from hospitals is commonly treated by an on-site treatment system prior to being discharged to the central domestic sewer system, nevertheless, there have been reports of some residues of pharmaceutical products in domestic wastewater. The occurrences of antibiotics in hospital wastewater were in ranges of 0.01–35.5 μg/L (Schroder et al. 2012; Verlicchi et al. 2012; Santos et al. 2013) which are 1000 times greater than the concentrations detected in domestic wastewater and surface water (Kummerer 2009). This suggests the limitation of a hospital wastewater treatment system for elimination of pharmaceutical products. Several biological processes are commonly used for treatment of such wastewater of which the most popular one is a conventional activated sludge (CAS) process. Typically, CAS capability is limited in the removal of nutrients and trace xenobiotic including pathogens in wastewaters

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Responsible editor: Philippe Garrigues

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(Radjenovic et al. 2008). In case of hospital wastewater, a CAS-mixed liquor is adversely affected by a pulse loading of disinfectants existing in cocktail concentrations; subsequently, there is a loss of active biomass from the system (Carucci et al. 2006). Recently, pharmaceuticals still have not been controlled by the discharged wastewater quality standard even in many developed countries. Therefore, information of chemicals/pharmaceuticals from hospital wastewater including their treated properties is limited (Schroder et al. 2012). Likewise with other wastewater containing toxic chemicals, it is necessary to assess the risk to aquatic organisms of the hospital effluents. Although, many previous studies report acute and chronic toxicity values of a single antibiotic on a direct toxic study of aquatic organisms; nevertheless, the exposed concentrations are of higher-order magnitudes than the real concentrations existing in hospital wastewater (Andrieu et al. 2015). In a real situation, antibiotics are usually existent as a mixture in the hospital wastewater and in the treatment processes. At present, limited studies have been able to pinpoint the ecotoxicity of the hospital wastewater especially for the mixed pharmaceuticals including antibiotics. Hence, this study is focused on (1) surveying concentrations of antibiotics in hospital wastewater in relation to the usage of antibiotics; (2) investigation of the efficacy of different hospital wastewater treatment processes namely CAS and RBC (rotating biological contactor); and (3) evaluation of the acute toxicity of hospital wastewater and the treated ones using a mixed liquor of CAS, fresh algae (*Chlorella vulgaris* and *Scenedesmus quadricauda*) and microcrustacean (*Moina macrocopa*) as bioindicators.

Materials and methods

Determination of antibiotic concentrations in hospital wastewater

In total, the number of hospitals in Bangkok is 137 for medical treatment serving 7.98 million residences. This study investigated seven hospitals located in Bangkok for wastewater characteristics. They are both private and governmental and are classified as tertiary healthcare. The wastewater was collected from an outflow of a grit remover using a grab sampling method, which was performed during Dec. 2013–Mar. 2014. Analysis focused on 19 antibiotics listed as common antibiotics detected in hospital wastewaters (Schroder et al. 2012; Verlicchi et al. 2012; Santos et al. 2013). Sample extraction and instrumental analysis are performed as described by the EPA Method 1694 (2007). Briefly, the wastewater samples were extracted by Vertipak$^{\mathrm{TM}}$ HCP cartridges (6 mL, 200 mg) and were eluted by pure methanol. The target antibiotic compounds in the extracts were analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). This equipment is composed of an HPLC (Agilent 1100 series, Palo Alto, USA) equipped with an Atlantis T3 column (100×2.1 mm, 3 μm) under 40 °C analysis. The detector is a triple quadrupole mass spectrometer (TSQ quantum discovery max; Thermo Fisher Scientific, Waltham, MA, USA) equipped with electrospray ionization operating in both positive and negative modes. The mobile phases for a positive mode were the mixture of 0.1 % formic acid in pure acetonitrile and 0.1 % formic acid in ultrapure water. For a negative mode, a mixture of pure acetonitrile and 1 mM ammonium acetate was used.

Investigation of antibiotic usage and antibiotic concentration in wastewater

Firstly, occurrence of antibiotic concentration in hospital wastewater was surveyed in seven hospitals in Bangkok area. Afterwards, only three hospitals were selected for investigation of the performance of different treatment systems. Most hospitals use CAS (with and without chlorination) and only one use RBC; therefore, CAS processes of H1 and H2 hospitals were investigated for their treatment performances. For other treatment process, RBC in H3 hospital was additional selected to be studied comparatively. Nevertheless, only two of seven hospitals permitted our survey in order to correlate antibiotic usage and antibiotic concentration in the wastewater. Antibiotic usage records were from hospitals namely H1 and H2 (private). The monthly data of all medicine prescriptions issued to patients were collected from Jan. 2013 to Dec. 2013 for H1 and from Aug. 2013 to Jul. 2014 for H2. Only the data of antibiotics administered to in-patient department (IPD) was calculated for a total usage amount per year as Eq. (1):

$$\text{Antibiotic usage(kg/y)} = (\text{IPD prescription, unit/y})(\text{Antibiotics, mg/unit})\left(10^{-6}\right) \tag{1}$$

Performance of CAS and RBC for hospital wastewater treatment

From a survey of wastewater treatment plants in seven hospitals, six plants use CAS, only one use RBC. The CAS processes of H1 (without chlorination) and H2 (with chlorination) hospitals were selected to be investigated for their treatment performances. The RBC in H3 hospital was additionally selected to be studied comparatively. The information related to the wastewater characters of these three hospitals is shown in Table 1. Because it is reported that hospitals normally generate a maximum flow rate during 9 a.m. to 12 p.m. (Boillot et al. 2008), the wastewater samplings for this study were conducted over the same time frame (10 a.m.–12 p.m.) during Aug. 2014 to Oct. 2014 with a total of three sampling rounds.

Table 1 Information of three studied hospitals

Information	H1	H2	H3
Type	Private hospital	Private hospital	Governmental hospital
Sizing (beds)	150	550	242
Staff (persons)	700	2000	750
Patients per month (persons)			
IPD	600	1600	1500
OPD	12,000	50,000	35,000
Wastewater generation (m^3/d)	150	500	225
Wastewater treatment process	CAS	CAS	RBC
Disinfection process	None	Chlorination	Chlorination
Maximum treatment capacity (m^3/d)	240	900	2400

CAS conventional activated sludge, *RBC* rotating biological contactor

The sampling location of an influent was after a screening unit of a treatment process, and effluent was collected after sedimentation unit or chlorination unit (when available). All samples were preserved at 4 °C and analyzed as described in the standard methods for the examination of water and wastewater (APHA, 2012).

Bioassays of toxicity

Three toxicity bioassays on the mixed-liquor biomass, freshwater algae, and microcrustacean were carried out in order to evaluate toxicity of the raw hospital wastewaters, treated wastewaters of CAS, and RBC.

Effect on the mixed liquor

The activated sludge biomass or the mixed liquor is commonly employed to evaluate toxicity of wastewater because it can interpret an adverse effect of a pollutant on biomass activity in a biological treatment process. Because various staff practices in a hospital affect wastewater properties, therefore a total of six samples of the H1 hospital wastewater were collected in order to observe time dependence of wastewater character during a working period (6 a.m. to 9 p.m.). A specific growth rate of a test-mixed liquor (from domestic treatment plant) exposed to the hospital wastewater was determined via a batch growth experiment. This batch test was carried out in duplicate of 100 mL wastewater. The control was a set of 100 mL nutrient broth with an equivalent COD concentration to the test wastewater. Then, the mixed liquor was inoculated into the water samples to make up turbidity of liquor equal to the McFarland standard No. 0.5 using a spectrophotometer at 600 nm (Thermo, Evolution 60S). Then, all test flasks were incubated at room temperature with continuous shaking (150 rpm). Changes of the absorbance intensity of liquor were determined by interval time at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h.

Effect on freshwater algae

This study employed an algal growth inhibition assay as described in the OECD No. 201 guideline (2002) for the measurement of acute toxicity. It was performed on two single species of the freshwater algae, *C. vulgaris* (TISTR 8580) and *S. quadricauda* (TISTR8610), which were obtained from Thailand Institute Scientific and Technological Research (TISTR). The wastewater was made up to a serial dilution to obtain 0, 6.25, 12.5, 25, 50, and 100 (%*v/v*). Then, the test algal cells were inoculated into the flasks to obtain 10^4 cells/mL. Afterwards, the test flasks were incubated under 26 °C by continuous shaking at 120 rpm. The constant illumination intensity of 16:8 h light:dark regime was provided to the test algae. Growth inhibition was calculated after 72 h exposure using the direct cell count measurement.

Effect on microcrustacean

The mobility inhibition/mortality of water fleas as the OECD No. 202 guideline (2004) was employed to evaluate the acute effect of freshwater organisms. *M. macrocopa* was obtained from Pathumthani Inland Fisheries Research and Development Center, Thailand. The dilution of the wastewater was performed as described in the algal growth inhibition assay using ISO test water. Twenty milliliters of each dilution was transferred into a 100-mL beaker in duplicate. Then, ten young male *M. macrocopa* aged <24 h were delivered into the test under room temperature and a photoperiod of 16:8 h light:dark regime. The numbers of immobilized and mortality of the water fleas were recorded at 24 and 48 h exposure. The data with 95 % confident limits were used to determine a 50% effective concentration (EC_{50}) and 50 % lethal concentration (LC_{50}) in terms of a water dilution (%*v/v*) using a Probit method.

Results and Discussion

Occurrence of antibiotics in hospital wastewater in relation to the antibiotic usage

Table 2 shows the average and range concentrations of the 19 selected antibiotics in the seven hospital wastewaters. It was found that only 11 of 19 antibiotics were detected of which OFX+ LFX, NOR, CIP, and SMX were the most frequently detected ones (100 % found). Most detected antibiotics appeared in similar ranges of concentrations detected in many hospital wastewaters worldwide. Among the antibiotics, NOR and CIP showed the concentration ranges of 6.40–24.00 and 4.00–24.00 μg/L, respectively of which NOR was obviously high compared with the other studies. SMX, TC, and AMC were detected in ranges of 0.24–6.40, 0.72–2.40, and 0.24–2.40 μg/L, respectively. LCM, RXM, and TMP concentrations were <1.6 μg/L.

In order to find the relationship between the concentrations of antibiotics in wastewater and that of usage, the amount of antibiotic usage in H1 and H2 hospitals was surveyed (Table 2) and focused only on high quantity in use per year. It is clearly seen that H2 hospital had higher consumption of the total antibiotics than that of H1. This is because H2 hospital has a larger number of patients than that of H1. The total antibiotic usage in H2 hospital was six times more than in H1 hospital although H2 hospital treated three times the number of in-patients over that of H1 hospital. It was estimated that there are approximately 4.13 and 9.64 g of antibiotics per patient per year for H1 and H2 hospitals, respectively. The average antibiotic usage in the USA was 17.00 g per capita a year, whereas that in Germany was only 3.85 g per capita a year (Kummerer 2004). In addition, the results showed that β-lactams such as penicillin and cephalosporin were the highest prescription at 75.23 and 78.93 % of total antibiotics of H1 and H2 hospitals, respectively. These antibiotics were commonly consumed intravenous ones in an intensive care unit (ICU) (Souza et al. 2009). The ß-lactams including other subgroups make up the largest share of human-use antibiotics in most countries, approximately 50–70 % of total antibiotic use, followed by sulfonamides, macrolides, and fluoroquinolones (Kummerer 2009). Although they were the highest amounts in usage in H1 and H2 hospitals, they were occasionally non-detected (Table 2). They have high-average excreted factors

Table 2 Antibiotic concentrations and detection frequencies in hospital wastewaters

Class	Antibiotics	Abbreviation	Antibiotic usage (kg/y[a])		Antibiotic concentration, μg/L			
					This study			Previous studies[c]
			Average	Range	Frequency[b] (*n*=7)	Average	Range	
Beta-lactam	Amoxycillin	AMC	34.01	2.26–65.75	29	1.32	0.24–2.40	0.9
Chloramphenicol	Chloramphenicol	CAM	2.33	0.30–4.35	0	<det	<det	0.07
Lincosamides	Lincomycin	LCM	0.33	0–0.65	29	0.16	0.16–0.160	0.2–2.0
Macrolides	Erythromycin	ERY	0.2	0.03–0.37	86	0.2	0.032–0.64	0.013–0.8
	Roxithromycin	RXM	0.63	0.02–1.24	29	0.92	0.24–1.60	0.4–2.2
	Tylosin	TYL	0	0	0	<det	<det	-
Quinolones	Ofloxacin + levofloxacin	OFX + LFX	2.33	0.50–4.59	100	3.31	1.60–4.80	0.2–35.5
	Norfloxacin	NOR	0.76	0.33–1.18	100	12.11	6.40–24.00	0.2–1.6
	Ciprofloxacin	CIP	5.39	1.54–9.25	100	9.6	4.00–24.00	0.9–101.0
	Lomefloxacin	LFLX	0	0	0	<det	<det	-
	Nalidixic acid	NA	0	0	0	<det	<det	0.4
Sulfonamides	Sulfamethoxazole	SMX	0.92	0.28–1.57	100	2.89	0.24–6.40	0.3–25.3
	Sulfamerazine	SMZ	0	0	0	<det	<det	0.04–0.25
	Sulfathiazole	STH	0	0	0	<det	<det	<det
Tetracyclines	Chlortetracycline	CTC	0	0	0	<det	<det	<det
	Oxytetracycline	OTC	0	0	0	<det	<det	<det
	Tetracycline	TC	0.24	0–0.49	71	1.42	0.72–2.40	0.04
Other	Trimethoprim	TMP	0.18	0.06–0.31	86	0.49	0.08–1.60	0.3–7.6

[a] Antibiotic usage data from H1 and H2 hospitals

[b] Number found in seven hospitals (%)

[c] References: Schroder et al. (2012); Verlicchi et al. (2012); Santos et al. (2013)

det detection limit

from human body in ranges of 0.40–0.95 (Kummerer and Henninger, 2003); nevertheless, it is not clear whether a few lactams found in wastewater were because of a possible cleavage of a lactam ring, analytical techniques, or their chemical properties (Kummerer 2009). In addition, fluoroquinolones, macrolides, tetracyclines, and sulfonamides were the other classes often prescribed in the H1 and H2 hospitals. A lower amount of chloramphenicol and aminoglycosides was recorded in H2 hospital while chloramphenicol was absent in H1 hospital. Plotting data between each antibiotic usage and its concentration in wastewater of H1 and H2 hospitals showed a good positive correlation for some antibiotics (r^2=0.77–0.99, data not shown). This is because macrolide, fluoroquinolone, sulfonamide, and tetracycline classes were less biodegraded in aquatic environment. The residual of antibiotic metabolism depends on chemical property which varies largely in ranges of 10–90 % (Kummerer and Henninger 2003). In the estimation of antibiotic concentration in wastewater by calculation of the ratio of antibiotic residuals and quantity of discharged wastewater of H1/H2 hospitals, the calculated value was much higher than that of the detected concentrations in this study (~0.02 % of the used antibiotic, data not shown). The large portion of antibiotic disappearance may be because antibiotics could be easily adsorbed onto the solids (Dorival-Garcia et al. 2013) along a collection system and chemically transformed by reacting with other disinfectants/chemicals (Kummerer 2009). In conclusion, the concentrations of antibiotics in the hospital wastewater correlated well with the amount of usage. However, most of the antibiotic concentrations found in the wastewaters were much lower than the EC_{50} values of those antibiotics.

Table 3 Physicochemical and microbiological characterization of hospital wastewater and treated wastewater

Parameter	H1 (CAS1)			H2 (CAS2)			H3 (RBC)			Domestic wastewater[a]	Standard
	Influent	Effluent	Efficiency (%)	Influent	Effluent	Efficiency (%)	Influent	Effluent	Efficiency (%)		
pH	7.44 (0.50)	7.14 (0.36)	-	7.74 (0.22)	7.81 (0.15)	-	7.61 (0.14)	7.75 (0.08)	-	-	5–9[b]
EC	944 (272)	1506 (375)	-	1255 (350)	661 (293)	47	901 (170)	843 (138)	6	-	-
TS	621 (77)	884 (76)	-	566 (115)	257 (59)	55	410 (55)	331 (121)	19	390–1230	-
TSS	73 (23)	42 (31)	42	213 (206)	3 (2)	99	17 (5)	4 (3)	76	120–400	-
TDS	544 (48)	842 (45)	-	356 (137)	253 (57)	29	393 (58)	328 (123)	17	270–860	≤500[b]
COD	371 (78)	109 (5)	70	390 (110)	161 (87)	59	368 (108)	151 (61)	59	250–800	-
BOD	125 (25)	22 (8)	82	102 (29)	6 (4)	94	95 (6)	4 (2)	96	11–350	≤20[b]
TOC	53 (10)	13 (5)	75	70 (21)	6 (1)	91	63 (10)	5 (0.3)	92	80–260	-
TKN	34 (2)	12.3 (10.7)	64	44 (8)	22 (15)	50	46 (3)	28 (11)	39	-	≤35[b]
NH_3-N	27 (4)	8.8 (8.4)	67	35 (7)	18 (12)	49	42 (2)	24 (8)	43	12–45	-
NO_2^--N	ND	2.3 (1.4)	-	0.03 (0.04)	0.27 (0.34)	-	ND	0.70 (0.41)	-	0	-
NO_3^--N	0.33 (0.5)	5.98 (2)	-	0.26 (0.4)	7.34 (7.0)	-	0.72 (1.0)	6.44 (3.3)	-	0	-
Ortho-P	3.80 (0.6)	2.55 (0.6)	33	3.02 (0.6)	3.78 (1.3)	-	3.67 (0.1)	3.36 (0.2)	8	4–12	-
TC	7.23 (7.15)	4.98 (4.63)	31	6.90 (6.46)	0.11 (1.15)	98	6.41 (6.43)	2.77 (2.57)	57	6–10	≤3.7[c]
FC	6.49 (6.23)	4.65 (4.87)	28	6.46 (6.38)	1.3 (0.28)	80	5.64 (5.39)	2.18 (1.72)	61	3–8	≤3.0[c]
Cd	0.007	NA	-	ND	0.002	-	ND	0.002	-	-	≤0.05[c]
Cr	ND	NA	-	ND	ND	-	ND	ND	-	-	≤0.05[c]
Cu	0.02	NA	-	ND	ND	-	0.007	ND	100	-	≤0.10[c]
Fe	0.289	NA	-	ND	ND	-	0.122	ND	100	-	-
Pb	0.141	NA	-	0.13	0.084	35	0.078	0.046	41	-	≤0.05[c]
Mn	0.081	NA	-	0.036	0.047	-	0.093	0.049	47	-	≤1.00[c]
Ni	0.023	NA	-	ND	ND	-	ND	ND	-	-	≤0.10[c]
Zn	0.623	NA	-	0.081	0.031	62	0.155	0.068	56	-	≤1.00[c]

All the values are milligram per liter except pH (unitless)

Total coliforms (TC) and fecal coliforms (FC) are log MPN/100 mL

Average (SD) values

No. of samples=3 except heavy metals=1

ND not detected, *NA* not analyzed

[a] Metcalf and Eddy, Inc (2004)

[b] Building effluent standards; type A, Ministry of Natural Resource and Environment

[c] Surface water quality standards, Ministry of Natural Resource and Environment

Hospital wastewater properties and treatment efficiency of CAS and RBC

The quantity of wastewater generated from each hospital was 0.9–1.0 m^3 per bed per day which is a similar range to that of 0.2–1.2 m^3 per bed per day in hospitals of industrialized countries (Verlicchi et al. 2012). These hospital wastewaters included all wastewater discharged activities such as gray waters (kitchen, laundries, patient care units, and staff hygiene) and treatment activities (treatment units and laboratory activities). The wastewater properties are summarized in Table 3. Overall characteristics show that all concentrations of the parameters were close to the untreated domestic wastewater properties (Metcalf and Eddy, Inc 2004). Similar concentration ranges of general contaminants to that of the hospital wastewaters from previous studies were found (Boillot et al. 2008). Moderate organic concentrations in terms of COD and BOD_5 were 250–800 and 11–350 mg/L, respectively comparable to a common domestic wastewater. Nevertheless, relative low biodegradability by a BOD_5/COD ratio of 0.26–0.34 appeared to a typical ratio of 0.4–0.8 in domestic wastewater. It indicates a possibility of presence of persistent biodegradable compounds which might be retarding microbial activities in biodegradation in a treatment process. TKN and NH_3-N were in relative moderate concentration (27 mg NH_3-N/L) in H1 hospital, while relative high concentrations (35–42 mg NH_3-N/L) in H2 and H3 hospitals. NO_2-N was present in only H2 influent while a low NO_3-N (<0.72 mg/L) was detected in all wastewaters. A low level of heavy metals (0.00–0.62) was found as of that in other hospitals (Emmanuel et al. 2005; Boillot et al. 2008). For biological parameters, low concentrations of total coliforms (TC) and fecal coliforms (FC) were detected as 6–7 log MPN/100 mL and 5–6 log MPN/100 mL, respectively. Commonly, FC concentrations of <8 log MPN/100 mL indicate a presence of antimicrobial agents in the wastewaters (Metcalf and Eddy, Inc 2004; Emmanuel et al. 2005).

For treatment efficiency, removal of organic matters by CAS2 and RBC gave similar capability by giving 58 %–59 % COD removal and 94 %–96 % BOD removal. It suggests that the organic matter as COD form was partially biodegraded in both CAS2 and RBC. For CAS1, it was better COD removal (70 %) but lower BOD removal (82 %). This indicates that H1 hospital generated more simple biodegradable COD in the wastewater relatively to that of H2 and H3 hospitals. Thus, higher TDS concentrations in the effluent of CAS1 were attributed to more biodegradation of organic matter in a form of COD. For nutrient removal, better efficacy of CAS1 in elimination of nitrogenous components i.e., TKN and NH_3-N were found by giving 64 %–67 % removal whereas that of 49 %–50 % removal of CAS2 and 39 %–43 % removal of RBC. In relation to COD degradation, it suggests that the partially biodegradable COD in wastewater of H2/H3 hospitals were possible in nitrogenous organic form. For coliform bacteria, the CAS1 removed only 2–3 log orders of

Table 4 Concentration of selected antibiotic in the influent, sludge, and effluent of different treatment processes

Antibiotic[a]	Antibiotic concentration, μg/L or μg/kg (dry weight sludge)												MIC_{90}[b] (mg/L)
	H1 (CAS1)				H2 (CAS2)				H3 (RBC)				
	Influent	Sludge	Effluent	Efficiency	Influent	Sludge	Effluent	Efficiency (%)	Influent	Sludge	Effluent	Efficiency (%)	
AMC	4.91	NA	NA	-	10.32	549	0.09	99	36.64	595	0.06	>99	2–8
LIN	0.02	NA	NA	-	0.01	3.22	0.00	36	0.04	1.10	0.00	97	32–128
CLA	0.01	NA	NA	-	0.03	0.00	0.01	69	0.15	2.22	0.06	57	64
OFX + LFX	1.73	NA	NA	-	1.32	15,424	0.33	75	1.25	6306	0.90	28	0.125/0.06
NOR	0.67	NA	NA	-	0.50	13,841	0.16	69	0.62	6305	0.22	64	0.12
CIP	2.98	NA	NA	-	3.44	49,804	0.71	79	4.35	25,844	1.70	61	0.25
SMX	0.43	NA	NA	-	0.04	126	0.02	34	1.41	118	0.03	98	76
TC	0.31	NA	NA	-	0.78	11,835	0.15	81	0.45	7048	0.15	67	256
TMP	0.06	NA	NA	-	0.01	30	0.01	0	0.08	0.00	0.00	95	4

No. of samples=2

NA not analyzed, *MIC_{90}* minimum inhibitory concentration (inhibited growth of 90 %)

[a] Ten antibiotics commonly found in hospital wastewater

[b] References: Nguyen et al. (2005); Petinaki et al. (2008); Sawant et al. (2007)

total coliforms and fecal coliforms; subsequently 4.98 and 4.65 log MPN/100 mL remained in the effluent. In contrast to CAS2 and RBC, chlorination was applied to the effluent, thus higher efficiencies of coliforms elimination by 3–5 log orders in that of CAS2 and RBC were found. This was because chlorine dosing was very effective in bacterial disinfection while possibly increasing toxicity of the effluent on some aquatic organisms (Emmanuel et al. 2005).

Because treatment performance of CAS2 and RBC gave similar efficiencies, therefore selection of some antibiotics highly used in H2 and H3 hospital was investigated for treatment capability. As shown in Table 4, AMC appeared the highest concentration in both influents; however, it was removed effectively >99 % by CAS2/RBC. Because it adsorbed easily on mixed liquors, therefore it means that AMC was easily biodegraded in CAS2/RBC. In addition, CAS2 showed high elimination of fluoroquinolones (OLF + LFX, NOR, CIP) 70–80 % including TC (80 %) via adsorption onto sludge. They commonly exhibit high-sorption potential on biomass (Dorival-Garcia et al. 2013). Nevertheless, the RBC performed higher removal of AMC, LIN, SMX, and TMP (95–99 %) compared to that of CAS2. It is possible that this RBC in H3 hospital was exposed to sunlight which helped in antibiotic photolysis via solar radiation. Photodegradation effectively accelerates half-life of antibiotics to <1 h (Batchu et al. 2014). In conclusion, almost all antibiotics were successfully removed by CAS2 and RBC by adsorption process except for AMC where biological process was dominant. Although most antibiotics exist in a very low level relative to the MIC (microbial inhibition concentration), mixtures of them might pose potential toxicity on organisms. The toxicity study on the treated wastewaters was conducted as presented in next section.

Toxicity study of hospital wastewater

Effect on the mixed liquor

Toxicity effect of hospital wastewaters (H1) at various sampling times on growth of mixed liquor is shown in Fig. 1. It reveals that the biomass could grow only in a nutrient broth (control) giving a normal sigmoidal curve. Stable concentrations of biomass were observed in the test wastewaters indicating toxicity on microbial growth. The wastewater at 3 p.m. in particular sharply decreased the growth after 6 h of exposure. This implies that some high toxic compounds gave high-potential adverse effect to biomass in CAS. It suggests that the hospital wastewater had different properties depending on the working time/activities. Several chemical substances were released in hospital wastewater such as radionuclides (Fischer et al. 2009), pharmaceutical care product (Lin and Tsai 2009), antibiotics (Verlicchi et al. 2012; Santos et al. 2013), and disinfecting solutions containing glutaraldehyde, a volatile, irritant, and toxic chemical compound (Jolibois et al. 2002). Likewise, a failure of biological wastewater treatment process of high-strength domestic wastewater with some specific toxic compounds such as phenol, cyanide, and thiocyanide on the microbial community was reported (Papadimitriou et al. 2007).

Effect on freshwater algae

Two freshwater algae employed in the tests belong to the *Chlorophyceae* class. As shown in Table 5, the 72-h EC_{50} of hospital wastewater (H1, H2, H3) for *C. vulgaris* was in a range of 13.8–17.61 % (*v/v*) and that of *S. quadricauda* was in a range of 9.81–13.63 % (*v/v*). It indicates that *S. quadricauda* was more sensitive than *C. vulgaris* in those wastewaters. Our results agree with the studies of Boillot et al. (2008) and Emmanuel et al. (2004) that the 72-h EC_{50} of raw hospital wastewater on *P. subcapitata* were 1.8–11.9 % (*v/v*). The effluents of CAS2/RBC showed toxicities of 1.94–2.42 TU. This might be the effects of residual chlorine (Park 2014) that remain in the effluents of CAS2/RBC. In conclusion, all hospital wastewaters gave similar toxic levels to the test algae. After treatment, the CAS2/RBC gave slight toxicity that might be caused by chlorination. A disinfectant reagent (NaOCl) can react with residual organic matters forming organochlorine compounds which are possibly toxic for aquatic organisms (Emmanuel et al. 2004). The other compound

Fig. 1 Effect of hospital wastewaters on mixed liquor of AS at various sampling times

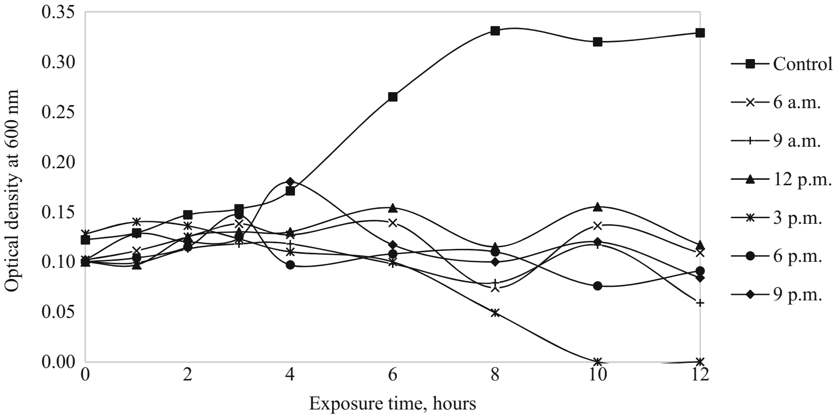

Table 5 Ecotoxicity of hospital wastewaters on freshwater algae and invertebrate

Hospital	Sample	Freshwater algae				Invertebrate		
		C. vulgaris		*S. quadricauda*		*M. micropoca*		
		72-h EC_{50} (%, *v/v*)[a]	TU	72-h EC_{50} (%, *v/v*)[a]	TU	Dose-response equation	48-h LC_{50} (%*v/v*)[a]	TU
H1 (CAS1)	Influent	13.83	7.23	9.81	10.20	$y=3.395x-0.127$	32.37	3.09
	Effluent	NA	NA	NA	NA	$y=3.1226x-0.4119$	54.09	1.85
H2 (CAS 2)	Influent	16.43	6.09	11.14	8.98	$y=3.4681x-0.4852$	38.16	2.62
	Effluent	41.33	2.42	45.80	2.18	$y=3.2555x-0.771$	59.25	1.69
H3 (RBC)	Influent	17.16	5.83	13.63	7.33	$y=3.2721x-0.1242$	36.82	2.72
	Effluent	51.60	1.94	87.10	1.15	$y=3.2389x-0.3828$	45.91	2.18

TU toxic unit ($TU=100/EC_{50}$), *ND* not detected, *NA* not analyzed, *y* probit value, *x* log concentration (%v/v)

[a] Volume of test water to volume of dilution water

which might be toxic to fresh algae is NH_3-N; however, the growth inhibition and toxicity levels to *Chlorella* sp. were 24,000 and 39,000 μM (408 and 663 mg/L), respectively, (Collos and Harrison 2014) which is much greater than the concentrations found in the studied wastewaters.

Effect of hospital wastewater on microcrustacean

The water flea, *M. macrocopa*, which was employed in this test, is capable of living in domestic wastewater (Nandini et al. 2004) consuming particulate organic matter. In this test, the 48-h LC_{50} of the wastewaters of H1, H2, and H3 gave toxicity levels to *M. macrocopa* by 32.37, 38.16, and 36.82 % (*v/v*) equivalent to 3.09 TU, 2.69 TU, and 2.72 TU, respectively (Table 5). These toxicity units ($TU=100/LC_{50}$) were slightly higher than 2 TU of a limit level that is proposed by some water agencies for industrial wastewater discharges (Emmanuel et al. 2005). It was reported that the toxicity of hospital wastewater on *D. magna* was about 52–71 TU (Emmanuel et al. 2005). This large difference of time unit between *M. macrocopa* in this study and *D. magna* might be because *D. magna* is more sensitive than *M. macrocopa* to toxic compounds (Ji et al. 2012). The 48-h LC_{50} of effluents of CAS1/2 and RBC showed a toxicity reduction of 54.09, 59.25, and 45.91 % equivalent to 1.85, 1.69, and 2.18 TU, respectively. In this case, the effect of chlorination was not found for the test water flea because there was no difference in toxicity between effluents of CAS1 (without chlorination) and CAS2 (with chlorination). The slight higher toxicity of RBC effluent might be caused from higher NH_3-N concentration (24 mg/L) compared to the CAS (9–18 mg/L). NH_3-N is known as a common toxicant on aquatic organisms (Theeppharaksapan et al. 2011). It can be concluded that the treatment of hospital wastewater could reduce the toxic effect on the test water flea. Chlorination did not give a negative effect on this organism.

Conclusion

General hospital characteristics were close to domestic wastewater properties but higher in antibiotic concentrations. The present levels of antibiotics in the hospital wastewater gave a good correlation to that of the usage. The AMC, OFX + LFX, NOR, CIP, and SMX were the most frequently detected ones. All hospital wastewaters gave similar toxic levels to mixed liquor/freshwater algae/microcrustacean. The CAS and RBC could remove fluoroquinolones and tetracycline via biomass adsorption. After treatment, most of the treatment could reduce the toxicity. Chlorination might cause a negative effect on the test fresh algae.

Acknowledgments This research was financially supported by the ASEAN University Network/Southeast Asia Engineering Education Development Network (AUN/SEED-Net) and Kasetsart University Research and Development (KURDI) grant no. S-K 4.56.

Compliance with ethical standards The authors of this manuscript would like to declare the following statements in compliance with ethical standards required by the journal:

This manuscript was developed based on our original research work as a single piece of work, and it has not been submitted to other journals for publication simultaneously.

The manuscript has not been published previously.

All co-authors of this manuscript have contributed to this work and agreed to the submission of this manuscript for publication in Environmental Science and Pollution Research Journal.

Conflict of interest The authors declare that they have no competing interests.

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