

## GIS-based analysis of the fate of waste-related pathogens *Cryptosporidium parvum*, *Giardia lamblia* and *Escherichia coli* in a tropical canal network

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### ABSTRACT

Urban canals play a major socio-economic role in many tropical countries and, particularly, Thailand. One of the overlooked functions that they perform is a significant attenuation of waste-related pathogens posing considerable health risk, as well as pollution attenuation in general. The study dealt with a comparison of three canals receiving: (i) municipal, (ii) mainly industrial and (iii) mainly agricultural wastewater, listed in order of progressively decreasing organic loading. The occurrence and fate of waterborne *Cryptosporidium parvum*, *Giardia lamblia* and *Escherichia coli* were monitored in the canals by both real-time PCR and conventionally for 12 months. The pathogens are etiological agents of an estimated 38% and 47% of diarrhea cases worldwide and in Thailand, respectively. The geographic information system (GIS) was used to evaluate and map point and, particularly, non-point pollution sources which allowed differentiating the canal sections in terms of predominant pathogen sources. The flowthrough canals, which can be viewed as waste stabilization ponds, were found to be efficiently removing the pathogens at the following generalized specific rates: 0.3 (*C. parvum*), 1.2 (*G. lamblia*), 1.8 (*E. coli*)  $\log_{10}/\text{km.d}$  in the dry season. The rates decreased in the rainy season for *E. coli* and *G. lamblia*, but increased for *C. parvum* which indicated different removal mechanisms. Data suggest that *E. coli* and *G. lamblia* were mainly removed through sedimentation and sunlight (UV) irradiation, while the likely mechanism for *C. parvum* was predation. Overall, the specific pathogen removal rates positively correlated with the canal organic loading rates in the rainy season. As an important result, an estimate of the municipal pollution mitigation by over 2,280 km canals in the Greater Bangkok suggests that concomitant to the pathogens at least 36–95 tons of BOD<sub>5</sub> is being removed daily, thereby saving the receiving Chao Phraya River and Bight of Bangkok, by far exceeding current, from major eutrophication problems.

**Key words** | canal network, *Cryptosporidium parvum*, *Escherichia coli*, geographic information systems, *Giardia lamblia*, removal mechanisms

### INTRODUCTION

Bangkok is one of the Asian cities with a very extensive canal network. With its 1,165 canals and 490 sub-canals representing, respectively, more than 2,280 km and  $3 \times 10^5$  km (5 m wide at the bottom, 10 m wide at the top

and 1.5 m deep), Bangkok can be easily compared to Amsterdam or Mexico. These canals and sub-canals carry approx.  $4.25 \times 10^7$  m<sup>3</sup> of water (Department of Drainage and Sewerage of Thailand, personal communication) which

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corresponds to  $4.2\text{m}^3/\text{person}$  at a given metropolitan population of  $10^7$ . Most of the canals were built a century ago for various purposes (transportation, flood management, irrigation and defense) but nowadays with the rapid urbanization of Bangkok they play a vital role of municipal waste and wastewater disposal facilities. A three-month health-risk assessment survey performed in August–October 2006 (data not shown) showed that the populations in the canal vicinity were in contact with canal water according to several scenarios through bathing and washing, swimming, fishing and subsistence agriculture. Investigating the occurrence of the selected pathogens, their distribution and removal in these canals is very important to both public health protection and notably for the assessment of wastewater treatment strategies in the developing countries.

Most countries in the tropics have large, undernourished and ill-housed populations with poor access to medical services. Few studies on pathogens in wastewater and surface water, particularly *C. parvum* and *G. lamblia*, have been undertaken in these countries representing more than 50% of the world's population. Cryptosporidiosis and giardiasis are among the most common parasitic infections worldwide with a yearly estimate of  $2.8 \times 10^8$  cases of infection by *Giardia* only (Lane & Llyod 2002). A recent study (Karanis *et al.* 2007) reviewed at least 325 water-associated outbreaks of parasitic protozoan disease. North American and European outbreaks accounted for 93% of all reports and nearly two-thirds of outbreaks occurred in North America. Over 30% of all outbreaks were documented in Europe, with the UK accounting for 24% of outbreaks worldwide. *Giardia duodenalis* (*G. lamblia*) and *C. parvum* accounted for the majority of outbreaks: 132 (40.6%) and 165 cases (50.8%), respectively.

Based on the World Health Organization (WHO) report, however, a majority of disease outbreaks (giardiasis) occur in Asia, Africa, and Latin America with some  $5 \times 10^5$  new cases reported each year (Lanata *et al.* 2002). A recent survey revealed that 37% of gastroenteritis in the USA was caused by *Cryptosporidium* and 14% by *Giardia*. The survey also showed that 62% of the treated water associated outbreaks were caused by *Cryptosporidium*. According to WHO (Lanata *et al.* 2002), pathogens *C. parvum*, *G. lamblia* and *E. coli* are estimated to be

the etiological agents of nearly 38% and 47% of the diarrhea cases worldwide and in Thailand (Ministry of Public Health-Thailand 2000). Universally, tropical areas accept water contaminant guidelines developed by temperate climate nations, despite the obvious differences in climatic conditions, which leads to an underestimation of actually higher risks of waterborne diseases in the tropical countries.

The first aim of this study was to carry out an extensive investigation of the occurrence of the pathogens *C. parvum*, *G. lamblia* and *E. coli* in municipal canals of the Greater Bangkok area (peri-urban Pathumthani province, Thailand), which furthered and expanded a previous study (Anceno *et al.* 2007b). The second aim was, with the aid of GIS, to evaluate the role of the canals as self-purification facilities achieving substantial removal of point and non-point waste organics in terms of BOD<sub>5</sub> concomitant with a high removal of the selected pathogens. The current study dealt with three different canals receiving: (i) municipal, (ii) mainly industrial and (iii) mainly agricultural wastewater, listed in order of progressively decreasing organic loading. The comparison would provide valuable insights into the behavior of the selected pathogens in the urban water environment and mechanisms of their removal which, in turn, would serve as a foundation for waterborne health risk mitigation.

## METHODOLOGY

### Land use map

Landsat<sup>TM</sup> (2003) multispectral imaging was used for map rendering. Unsupervised classification was done based on the aggregation of the land use classes depending on the spectral reflectance by using ERDAS IMAGINE<sup>®</sup> V8.7. For the purpose of this study, nine classes were finally retained. Specific land use along each canal was extracted considering a distance of 1 km from each canal sides—areas which could be significantly impacted by the canals. ArcView V3.3 (1992–2002) was used for all analyses (statistics of land use and evaluation of non-point pollution sources). Global positioning system (GPS) was used to record the latitude and longitude of the 14 sampling points. The nearest

neighbors interpolation method was used for the pathogens density maps. The resulting map with the sampling sites is presented in Figure 1.

### Evaluation of canal hydraulic regime and pollution sources

The mean flow of the water was monitored fortnightly from June 2006 to May 2007 by using a current meter (Model CM-2, Toho Dentan Co., Ltd), and hydraulic retention time (HRT) and Reynolds number ( $Re$ ) have been calculated. The latter ( $Re$ ) characterizes water flow in the canals and was estimated by using the mean water velocity and the hydraulic radius of the canals (data not shown). Main non-point sources of pollution considered included domestic pollution, agricultural run-off and urban run-off. To estimate the run-off, coefficient of run-off furnished by the Royal Irrigation Office of Bangkok was considered. As there was no such specific data to date, the coefficient of the Kwenoi River basin in Western Thailand was used as comparable to the study area in terms of land use, topography and geomorphology. Considering that only 50% of the run-off would get into the canals and that the run-off in the agricultural and urban area was the same, we sampled both run-offs for analysis and quantified the

loads from the annual run-off volumes according to Harremoës (1988). Using the run-off volumes and the land cover, pollution non-point sources (NPS) were evaluated while pollution point sources (PS) were investigated with the assistance of the Royal Irrigation Office of Pathumthani. Samples from both NPS and PS were analyzed as described below.

### Climatic data

Rainfall and solar radiation data were obtained from the meteorological facility of the AIT Water Engineering and Management program and also the Royal Irrigation Office of Pathumthani, Thailand.

### Water-sediment sampling and analyses

Samples were collected bimonthly from 14 sampling points (Figure 1) from June 2006 to May 2007. Water from canals was directly collected 20 cm from the surface at canal midpoints. Separate 1 l and 2 l samples were transported to the laboratory within 2 h in sterile, wide-mouth, screw-capped bottles. Sediment samples were obtained with the help of an Ekman dredge and placed in individual plastic bags. The samples were stored on ice during transport to the

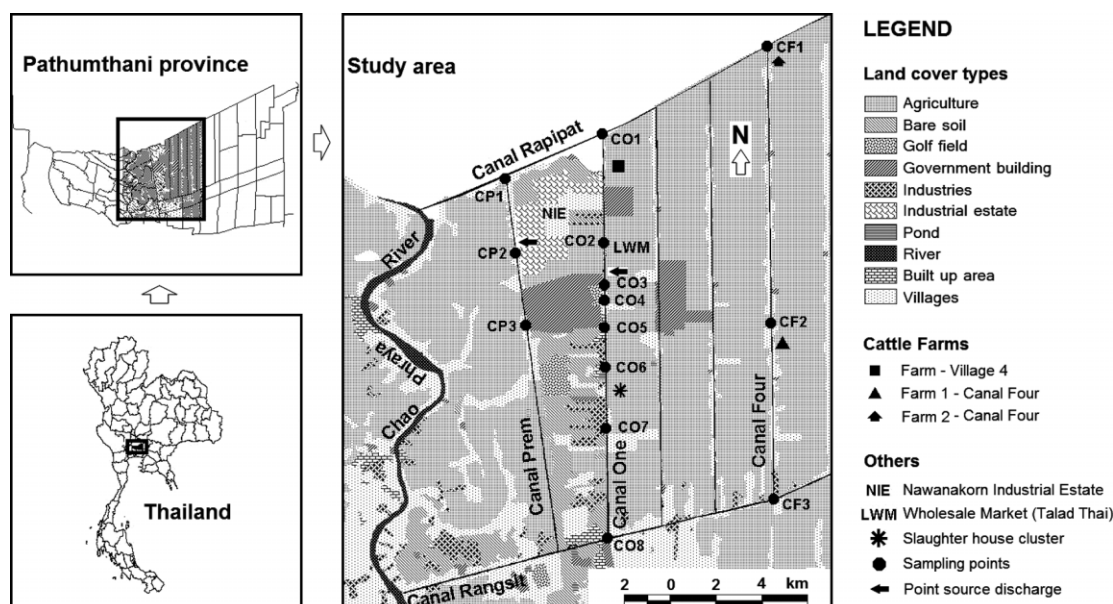


Figure 1 | Land use map of the study area and sampling points.

laboratory. In all cases, the elapsed time between sampling and physico-chemical parameter analyses (APHA 1998) did not exceed 6 h.

### Enumeration of fecal coliforms

Total coliforms, fecal coliforms and *E. coli* in wastewater samples were determined using the most probable number (MPN) method according to APHA (1998). Bacterial numbers in sediment were determined by the methods used by Karim *et al.* (2004). Results were reported as MPN per 100 ml of wastewater or 100 g of sediment.

### Quantification of the protozoan parasites

Preparation of water concentrates, DNA extraction, purification and real-time PCR amplification of *Cryptosporidium* oocyst wall protein (COWP 702, 151-bp) and *Giardia*  $\beta$ -giardin ( $\beta$ -giardin P241, 74-bp) coding gene fragments was carried out according to Anceno *et al.* (2007a). A 20- $\mu$ l reaction mixture contained  $1 \times$  iQ<sup>TM</sup> SYBR Green Supermix (Bio-Rad, USA), 0.5  $\mu$ M each of either COWP P702 or  $\beta$ -Giardin P241 primer pairs and DNA template. Thermocycling, carried out using an iCycler (BioRad, Hercules, CA, USA), consists of 10-min initial denaturation at 95°C and 45 cycles of 30-sec denaturation at 95°C, 1 min annealing at 60°C and 30 sec extension at 72°C.

Protozoan parasites in sediments were quantified by using the method described by Karim *et al.* (2004). A 20-ml aliquot of each sediment sample was processed for *Giardia* and *Cryptosporidium* (oo)cysts enumeration. The weight of the samples was recorded and dry matter determined as described (Pepper *et al.* 1995). Sediment samples were mixed with 30 ml of sterile deionized water. After homogenization, the samples were passed through a series of two sieves of gradually finer mesh (opening 2 mm, No. 10, Fisher Scientific Co., USA; 300  $\mu$ m, No. 50, Gilson Co., USA) to remove fibrous and coarse particulates. The samples were transferred to 750-ml plastic centrifuge bottles and concentrated by centrifugation at  $1,050 \times g$  for 10 min. The supernatant was aspirated off without disrupting the pellet. The (oo)cyst containing pellet was twice washed in deionized water. Elution solution was added to achieve a

final volume of 20 ml. Further downstream processing was performed as described previously (Anceno *et al.* 2007a).

### Removal rate evaluation

The evaluation of the removal rates was based on assumptions detailed herein. Canals were divided into different sections interposed by sampling points. Each section could be therefore considered as a pond. The upstream sampling point  $o$  represented the influent with concentration  $C_o$  and the subsequent downstream sampling points  $i$  with concentrations  $C_i$  were the effluent for the sampling section  $o \rightarrow i$ .  $Q_o$  and  $Q_i$  were, respectively, daily flow rate at points  $o$  and  $i$ .  $C_p$  and  $Q_p$  represented the concentration and flow rates of the discharged pollutant at point sources  $p$  between sampling points  $o$  and  $i$ . Several non-point sources of pollution were also taken into account.  $C_{dw}$  and  $Q_{dw}$  were, respectively, concentration and flow rate of pollutant from domestic wastewater.  $C_{ar}$  and  $Q_{ar}$  were, respectively, concentration and flow rate of pollutant from agricultural run-off.  $C_{ur}$  and  $Q_{ur}$  were, respectively, concentration and flow rate of pollutant from urban run-off.  $L_i$  was the length of the segment  $o \rightarrow i$  and  $L_t$  the total length of the canal section studied.  $R_i$  was the pollution load removed in the section  $o \rightarrow i$ . Assuming the NPS were equally distributed along the canals and the volumes were proportional to the length, the removal efficiency of any section  $o \rightarrow i$  could therefore be evaluated by the following Equation (1).

$$C_i \cdot Q_i = C_o \cdot Q_o + \left( \sum_{p=1}^{\infty} C_p \cdot Q_p \right) + \left[ \left( \frac{C_{dw} \cdot Q_{dw} + C_{ar} \cdot Q_{ar} + C_{ur} \cdot Q_{ur}}{L_t} \right) * L_i \right] - R_i \quad (1)$$

Concentrations  $C$  are in  $\text{kg}/\text{m}^3$  for  $\text{BOD}_5$ , (oo)cysts/ $\text{m}^3$  for *C. parvum* and *G. lamblia*, and  $\text{MPN}/\text{m}^3$  for *E. coli*. Flow rates  $Q$  are in  $\text{m}^3/\text{day}$ , distances  $L$ , in metres, and  $R$  either in kg of  $\text{BOD}_5$  or number of pathogens removed per day. Results were transformed into the removal rates as  $\log_{10}$  units/ $\text{km} \cdot \text{d}$  (Table 4).

### Data analysis

Data was compiled using MS Excel (Microsoft Corp., USA) and statistical analyses performed using the SPSS 10.0 for

Windows (SPSS Inc., USA). Two-tailed Pearson's product moment correlation ( $\alpha = 95\%$ ,  $99\%$ ) was used to determine correlations.

## RESULTS AND DISCUSSION

### Land use map

Coupling field investigations to the GIS technique provides a synoptic yet more accurate view of the dynamic interplay of several factors contributing to the overall biogeophysical (biogeochemical) state of a study area in question. The approach allows for data updates on a short- and long-term basis, improving our understanding of how natural and major socio-economic forces transform the environment and impact on public and environmental health. It also provides a better perspective for research and policy-making institutions alike in terms of suggesting practical strategies in dealing with economic progress. It is important to note from [Figure 1](#) that Canal One was mainly surrounded by the urbanized area, Canal Four was in the mainly agricultural area, while Canal Premprachakorn was receiving mainly industrial discharge.

### Hydraulic regime and pollution load of the canals

The characteristics and hydraulic regimes of the canals are shown in [Table 1](#). The hydraulic retention times (HRT) of Canal One and Canal Premprachakorn were twelve and five times higher during the dry season than the rainy season, respectively. A less notable seasonal HRT fluctuation was observed in Canal Four. The seasonal mean of the Reynolds number (data not shown) showed that for both rainy and dry seasons the water flow in all canals was

turbulent ( $Re > 2.3 \times 10^3$ ). Canal One was the most polluted in terms of organic load during both the rainy and dry seasons which can be explained by the diverse PS discharges into this canal. Being highly urbanized and surrounded by land area of various usages, the daily total pollution load was approx. 7,427 kg BOD<sub>5</sub> (98% from PS and 2% from NPS). In contrast to Canal Four, we did not find a significant seasonal fluctuation in pollution load in Canal One. The former, although less populated and situated in the agricultural area, was three times more polluted (BOD<sub>5</sub>) during the rainy season than the dry season. This was probably due to the organic run-off from the agricultural soils as the major pollution contributor in the rainy season (75%) and to a lesser extent in the dry season (5%), while canal Rapipat remains the major pollution source in the dry season. In contrast to these two canals, Canal Premprachakorn was 1.4 times more polluted during the dry season than the rainy season. It was evident that regardless of seasonality this canal received 99% of its pollution load from the Nawanakorn Industrial Estate and Canal Rapipat.

### Pathogens in water and sediment

As seen in [Table 2](#) and [Figure 2](#) (visualized concentration gradients of the pathogens along the canals), Canal One was the most contaminated canal. The sampling point CO3 which received discharges from the major Talad Thai wholesale market constituted the most polluted canal section. On the other hand, the observed attenuation of *C. parvum*, and *G. lamblia* and *E. coli* concentrations along the canals confirmed the pollution mitigation role of the canals. [Figure 3](#) shows the values of the physico-chemical parameters and concentrations of the pathogens in the

**Table 1** | Hydraulic regime and pollution load of the canals\*

Canals	Shape	HRT (d)		Mean flow (m <sup>3</sup> /d)		Pollution load (kg BOD <sub>5</sub> /m <sup>3</sup> .d)	
		RS	DS	RS	DS	RS	DS
CO	Trapezoidal ( $L = 21.5$ km)	1.5	18	761,572	171,051	0.015	0.014
CF	Trapezoidal ( $L = 19.6$ km)	29	30	23,440	13,548	0.009	0.003
CP	Trapezoidal ( $L = 24.8$ km)	1.5	8	662,327	286,051	0.007	0.010

\*Canal One (CO), Canal Four (CF), Canal Premprachakorn (CP); rainy season (RS), dry season (DS).

sampling points over a year of investigation. The monthly data (not shown) indicated a moderate seasonal fluctuation with slightly higher concentrations of pathogens found in the rainy season. It is interesting to note that during the study period the number of the fecal coliforms including *E. coli* in all the canals and the number of *G. lamblia* in Canal One and Canal Premprachakorn increased significantly from March to June with a peak in May. The rainy season in Thailand was in June–November but during our investigations the study area also received a significant amount of rainfall in March, triggering run-offs which caused the increase in the pathogen numbers in the canals (Melnick *et al.* 1977; Juarez-Figueroa *et al.* 2003). The positive correlation between the rainfall and the concentration of *G. lamblia* in Canal One and Canal Premprachakorn corroborated this finding. No similar trend was found for *C. parvum*.

## Correlations

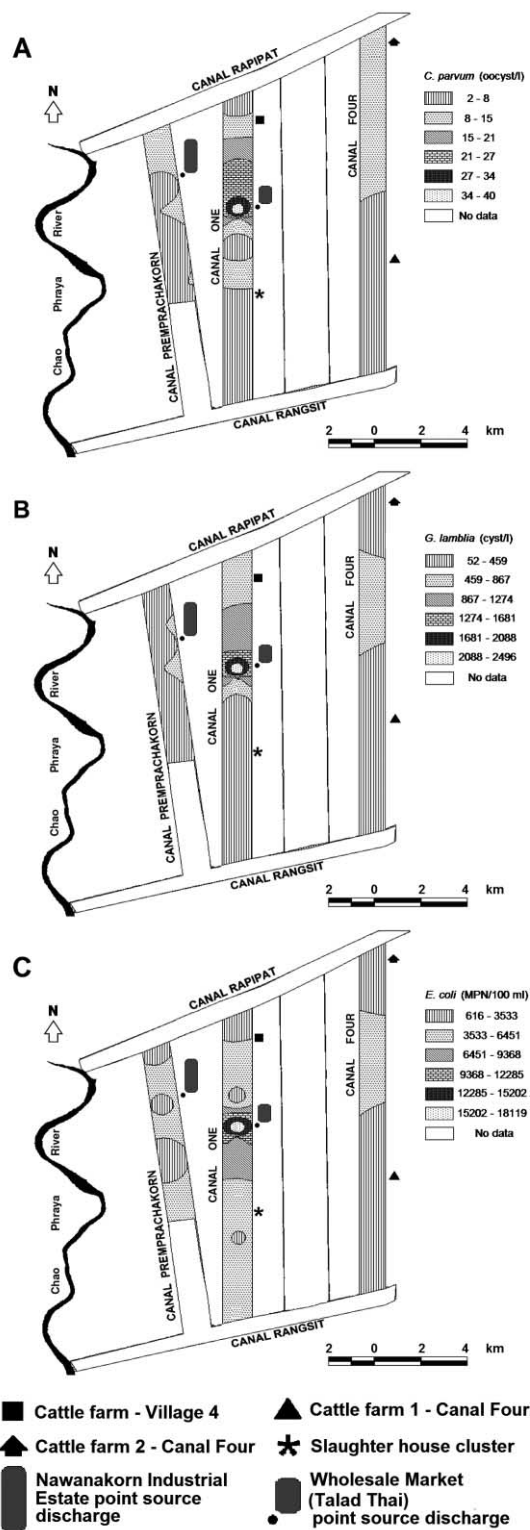
The interdependence of pollution load, DO, pH, solar radiation (seasonally) and temperature in the somewhat complex biogeochemical dynamics of pathogen removal, especially of fecal microorganisms, was well established in the literature (Curtis *et al.* 1992; Davies-Colley *et al.* 1999). In the present undertaking the following correlations were found from the two-tailed Pearson's product moment correlation analysis (Table 3). In 50% of Canal One sampling sites, a significantly negative correlation was found between *E. coli* concentration and the solar radiation ( $r = -0.67$ ;  $p < 0.05$ ). Similar correlation trends were also noted between *G. lamblia* concentration and solar radiation in 63% of Canal One sampling sites ( $r = -0.62$ ;  $p < 0.05$ ) and 33% of Canal Premprachakorn sampling sites ( $r = -0.72$ ;

$p < 0.05$ ), confirming the role of solar radiation in the removal of *E. coli* and *G. lamblia*. Rainfall positively correlated with the number of *Giardia* in 50% and 33% of the samples from the Canal One ( $r = 0.7$ ;  $p < 0.04$ ) and Canal Premprachakorn ( $r = 0.7$ ;  $p < 0.03$ ), respectively, confirming the role of rainfall in the pollution of the canals through run-offs. A significantly positive correlation was found between the fecal coliforms and *E. coli* in some sampling sites of the Canal One ( $r = 0.72$ ;  $p < 0.03$ ), Canal Four ( $r = 1.0$ ;  $p < 0.001$ ) and Canal Premprachakorn ( $r = 0.97$ ;  $p < 0.001$ ). Significantly positive correlations were also found between *E. coli* and total coliforms in 50% of Canal One sampling sites ( $r = 0.69$ ;  $p < 0.04$ ) and in 60% of Canal Four sampling sites ( $r = 0.89$ ;  $p < 0.001$ ). Another strong positive correlation was found between the total and fecal coliforms in some sampling sites of Canal One ( $r = 0.72$ ;  $p < 0.04$ ), Canal Four ( $r = 0.97$ ;  $p < 0.001$ ) and Canal Premprachakorn ( $r = 0.94$ ;  $p < 0.001$ ). The BOD<sub>5</sub> correlated positively with the total coliforms (minimum  $r = 0.6$ ; minimum  $p < 0.05$ ) and fecal coliforms (minimum  $r = 0.6$ ; minimum  $p < 0.05$ ) in at least 50% of all canal sampling sites. The BOD<sub>5</sub> also correlated with *E. coli* ( $r = 0.9$ ;  $p < 0.01$ ) in 75% of Canal One sampling sites. Positive correlations were found between the DO and chlorophyll *a* ( $r = 0.64$ ;  $p < 0.05$ ) in all the water samples from the Canal Premprachakorn, between the DO and temperature ( $r = 0.76$ ;  $p < 0.02$ ) in 50% of Canal One sampling sites, and between the DO and pH ( $r = 0.66$ ;  $p < 0.05$ ) in 50% of Canal One sampling sites. Positive correlation was also found between chlorophyll *a* and pH ( $r = 0.65$ ;  $p < 0.05$ ) in 50% of the samples from Canal One. The most interesting but least expected correlation was found between *E. coli* and the protozoan parasites. A significant positive correlation between *E. coli* and *G. lamblia* ( $r = 0.75$ ;  $p < 0.05$ ) and

**Table 2** | Log<sub>10</sub> concentrations of the pathogens in the canal water and sediment\*

Canals	<i>C. parvum</i>		<i>G. lamblia</i>		<i>E. coli</i>	
	Surface water (oocysts/l)	Dried sediment (oocysts/kg)	Surface water (cysts/l)	Dried sediment (cysts/kg)	Surface water (MPN/100 ml)	Dried sediment (MPN/100 g)
CO	1.2	0	3.4	2.7	4.1	5.1
CF	0.9	0	2.5	2.3	3.3	4.3
CP	1.3	0	1.5	3	3.4	4.3

\*Values are averages; see Table 1 for the canal name abbreviations.

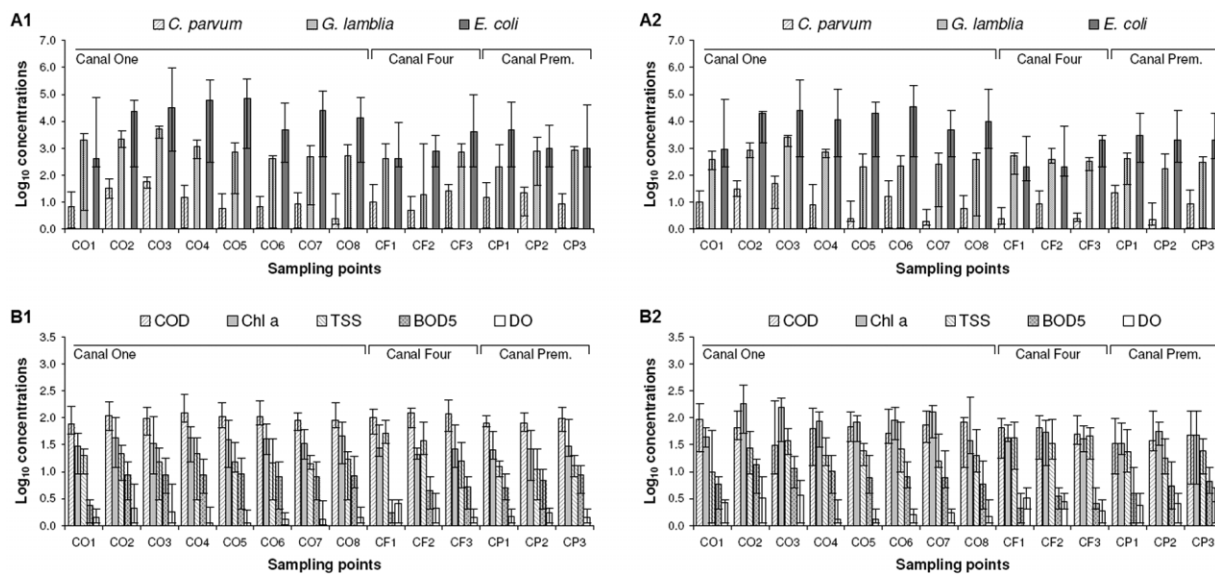


**Figure 2** | Canal network schematic showing the concentration gradients of *C. parvum* (A), *G. lamblia* (B) and *E. coli* (C) in water (width of canals is not to scale).

between *E. coli* and *C. parvum* ( $r = 0.73$ ;  $p < 0.03$ ) were found in 37.5% and 25% of Canal One sampling sites, respectively. Although this result was found only in a few sampling points of the canal, it appears to corroborate previous findings by Anceno *et al.* (2007b) for a network of flowthrough canals behaving as pond systems, though it contradicts many other studies which failed to establish a significant correlation between the indicator bacteria and the protozoan parasites in conventional ponds systems (Harwood *et al.* 2005).

**Removal rates and mechanisms**

Although it was previously assumed that the canals were merely diluting pollution from various sources, the comparison between the flow rate fluctuations and pathogen density (data not shown) along the canals revealed that the pathogen density was decreasing from upstream to downstream while the flow was decreasing in the same direction. This finding suggests that the dilution was not the only factor to be considered and it could be inferred as discussed below that a synergy of several mechanisms enhanced the overall pathogen removal in the canals. These mechanisms are assumed to include a large scale sedimentation (Grimason *et al.* 1993), as was confirmed by the presence of the pathogens in the sediment. Table 2 shows at least comparable or even elevated concentrations of *G. lamblia* cysts in sediments as compared to overlaying water, corroborating the importance of sedimentation in the pathogen removal. The strong positive link between *G. lamblia* removal and the solar radiation in the correlation studies also confirmed a major role of the latter in pathogen removal (Davies-Colley *et al.* 2000). It is an accepted view that sunlight facilitates pathogen inactivation via solar (UV) irradiation (Clancy *et al.* 2000; Davies-Colley *et al.* 2000) concomitant to a subsequent increase in water temperature (Jenkins *et al.* 1997). Neither *C. parvum* oocysts were found in the sediments, nor was correlation between solar irradiation and (oo)cyst concentrations observed. The considerably high apparent removal of *C. parvum* ( $1.4 \log_{10}/\text{km.d}$ ) achieved in Canal One is also partially contributed by predation. As the most polluted canal, it could harbor such diversity of microflora including pathogen predating higher organisms like other protozoans



**Figure 3** | Median log<sub>10</sub> concentrations of the pathogens (A1, A2 → rainy, dry season) and physico-chemical parameters (B1, B2 → rainy, dry season) in water columns. Line bars (+/–) represent 95% confidence interval. Parameters: chemical oxygen demand (COD, mg/l), five-day biological oxygen demand (BOD<sub>5</sub>, mg/l), total suspended solids (TSS, mg/l), chlorophyll *a* (Chl *a*, μg/l), dissolved oxygen (DO, mg/l), *C. parvum* (oocysts/l), *G. lamblia* (cysts/l), *E. coli* (MPN/100 ml); see Table 1 for the canal name abbreviations.

and/or invertebrates (Fayer *et al.* 2000; Simek *et al.* 2001; Stott *et al.* 2001). Overall, the removal of *G. lamblia* was greater than that of *C. parvum*, comparing well with literature. Unlike in the case of *C. parvum* and *E. coli*, a more efficient removal for BOD<sub>5</sub>, *E. coli* and *G. lamblia* was observed in the dry rather than in the rainy season in Canal One and Canal Premprachakorn although the pollution load was higher in the rainy season (Table 4). This could be explained by the longer HRT, lower flow rate and longer exposure to the sunlight during the dry season. The removal rate of organics in both rainy and dry season in terms of BOD<sub>5</sub> was better achieved in Canal One (0.3 log<sub>10</sub>/km.d) and Canal Premprachakorn (0.2 log<sub>10</sub>/km.d) than in the Canal Four (0.1 log<sub>10</sub>/km.d of BOD<sub>5</sub>) in the dry season, inferred to be caused by the higher pollution loads in the two previous canals. All the pathogens were better removed in Canal One followed by Canal Premprachakorn and to a lesser extent in Canal Four which was fairly surprising as the latter was the least polluted canal. Interestingly, a strong positive correlation (0.5 < *r* < 0.9) has been found between the loading rates in the canals and the removal of both pathogens and organics in the rainy season. This agrees with the findings for conventional ponds where the rate of treatment is higher when the wastewater is more polluted, while the converse is true when the wastewater is

stabilized leading to low pollutant concentrations (Shilton *et al.* 2000). During the dry season, such positive trend of correlation with a loading rate was found only for the removal of *Giardia* (*r* = 0.4).

### Canal treatment capacity

The Greater Bangkok area has a population of approx. 10 million and produces daily more than 3 × 10<sup>6</sup> m<sup>3</sup> of wastewater (Anonymous 2006). The city is only partially served by dedicated treatment facilities with an actual wastewater treatment capacity of approx. 2 × 10<sup>6</sup> m<sup>3</sup>/d. The remaining untreated wastewater (1.2 × 10<sup>6</sup> m<sup>3</sup>/d) is discharged into the canal network. Extrapolating the removal rate values based on the presented results over the overall Bangkok area, one can arrive at important general conclusions. The data gathered from Canal One situated in the most urbanized area was the most suitable for the purpose. From the removal rates of the different sections of the canal and the given length, a minimal removal rate of 16–42 kg of BOD<sub>5</sub>/km.d was estimated. The 2,280 km of canals in the Greater Bangkok area could therefore potentially remove at least 36–95 tons of BOD<sub>5</sub>/d before the water is discharged into the Chao Phraya River and subsequently into the Bight of Bangkok.



**Table 3** | Pearson's product moment correlations among pathogens and physico-chemical parameters

	T	DO	Chl a	TC	FC	Ec	GI
DO	CO(0.76;0.02;4/8)* CP(0.70;0.04;1/3)		CP(0.64;0.05;3/3)				
pH		CO(0.66;0.05;4/8)	CO(0.65;0.05;4/8)				
BOD <sub>5</sub>				CO(0.69;0.04;4/8) CF(0.60;0.05;2/3) CP(0.73;0.05;2/3)	CO(0.64;0.05;5/8) CF(0.60;0.05;2/3) CP(0.70;0.05;2/3)	CO(0.89;0.01;6/8)	
TC					CO(0.72;0.04;6/8) CF(0.97;0.001;3/3) CP(0.94;0.001;2/3)	CO(0.69;0.04;4/8) CF(1.0;0.001;1/3) CP(0.89;0.001;2/3)	
FC						CO(0.72;0.03;4/8) CF(1.0;0.001;1/3) CP(0.97;0.001;1/3)	
Cp						CO(0.73;0.03;2/8)	
Ec							CO(0.75;0.05;3/8)
Rad						CO(-0.67;0.05;4/8)	CO(-0.62;0.05;5/8) CP(-0.72;0.05;1/3)
Rf		CO(0.70;0.04;4/8) CP(0.72;0.03;1/3)					

\*Canal(*r*-values; *p*-values; applicable sampling sites/total sampling sites). In all cases, *N* = 12 and correlation coefficients with significance levels > 0.05 were omitted. Abbreviations: temperature (T), dissolved oxygen (DO), chlorophyll *a* (Chl *a*), total coliforms (TC), fecal coliforms (FC), *E. coli* (Ec), *G. lamblia* (GI), *C. parvum* (Cp), solar radiation (Rad), rainfall (Rf); see Table 1 for the canal name abbreviations.

**Table 4** | Geometric means for the generalized specific removal rates of organics and pathogens in the canals\*

Season	<i>C. parvum</i>			<i>G. lamblia</i>			<i>E. coli</i>			<i>BOD<sub>5</sub></i>		
	CO	CP	CF	CO	CP	CF	CO	CP	CF	CO	CP	CF
Rainy	1.4 (0.4–3.0)	0.3 (0.2–0.5)	0.1 (0.1–0.1)	1.9 (0.7–3.8)	1.3 (1.1–1.4)	0.4 (0.1–0.7)	2.5 (1.2–5.4)	2.1 (2.0–2.2)	0.9 (0.7–1.1)	0.2 (0.1–0.4)	0.1 (0.1–0.1)	0.03 (0.0–0.1)
Dry	0.7 (0.5–0.8)	0.4 (0.2–0.5)	0.1 (0.0–0.1)	2.1 (0.8–4.4)	1.3 (1.1–1.5)	0.7 (0.5–0.8)	2.2 (2.1–2.3)	2.2 (2.1–2.3)	0.9 (0.7–1.1)	0.3 (0.0–0.7)	0.2 (0.2–0.2)	0.1 (0.0–0.1)

\*Removal values including minimum-maximum in parentheses are reported as log<sub>10</sub> units/km.d; see Table 1 for the canal name abbreviations.

## CONCLUSIONS

The presented investigation covered several aspects (from the spatio-temporal number fluctuations of the selected water-related pathogens and relevant physico-chemical parameters to the GIS-based analysis of the removal of waste organics and selected pathogens) in an attempt to better understand the mechanisms underlying pathogen removal in the tropical canal network and its role as a major vehicle for surface water pollution mitigation. For the study period covered herein, the occurrence and removal rates of pathogens and waste organics in terms of BOD<sub>5</sub> were found to be both spatially and seasonally dependent, correlating well with the land use types and the identified pollution sources in the vicinity of the canal sections. One can safely assume that this network of canals is critically vital in terms of services rendered to treat tons of wastewater, the services representing a substantial economic value. However, despite the substantial removal performed by the canals, the residual densities of pathogens still remain a major public health threat. The data generated in this pioneering study will contribute to developing an integrated pathogen management approach and provide a baseline for the microbiological quality monitoring of the canal water. The results are also useful in the currently on-going quantitative microbial risk assessment of the major sections of population exposed to canal water according to a variety of scenarios. The data are of a major value for the modeling of the environments similar to the described, the environments which comprise a significant portion of global population.

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