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Faecal indicator bacteria and organic carbon in the Red River, Viet Nam: measurements and modelling

Huong Thi Mai Nguyen

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& l'Académie des Sciences et de la Technologie
du Viet Nam (Co-tutelle)

Ecole Doctorale Sciences de la Nature et de l'Homme :

Évolution et écologie

Institut d'écologie et des sciences de l'environnement de Paris

Equipe Communautés microbiennes dans les écosystèmes continentaux

**Faecal indicator bacteria and organic carbon in the
Red River, Viet Nam: measurements and modelling**

Par Huong Thi Mai NGUYEN

Thèse de doctorat de Sciences de l'Environnement

Dirigée par Emma Rochelle-Newall and Quynh Thi Phuong Le

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Summary

In many developing countries, poor water quality poses a major threat to human health and the lack of access to clean drinking water and adequate sanitation continue to be a major brake on social and economical development. Urbanization and untreated domestic and industrial wastewater are significant sources of organic carbon (OC) and faecal bacteria in aquatic ecosystems. This is particularly problematic in developing countries where efficient wastewater treatment is lacking and where human populations are rapidly increasing, becoming more urban and increasingly industrialized. Waterborne pathogens and OC from wastewater are particularly susceptible to shifts in water flow and quality and the predicted increases in rainfall and floods due to climate change will only exacerbate the problems of contamination. It is therefore imperative that we have an understanding of the distribution and the factors that control the distribution and dispersion of water borne pathogens. The Red River is the second largest river in Viet Nam and constitutes the main water source for a large percentage of the population of North Viet Nam. This thesis presents the results from observations and modeling of both faecal indicator bacteria (FIB) and dissolved and particulate organic carbon (DOC and POC) in the Red River basin, North Viet Nam. The objective of this work was to obtain information on the numbers of two FIB (*Escherichia coli* and total coliforms (TC)) and OC in the Red River and then to model these variables in order to investigate scenarios of the system on the 2050 horizon when the population in the area is estimated to have doubled.

For *E.coli* and TC, the results from 10 stations along the Red River showed that TC numbers reached as high as 39,100 colony forming units (CFU) 100 ml⁻¹, values that are considerably higher than the clean water limits set by QCVN 02:2009/BYT of the Viet Nameese Government (50 CFU 100 ml⁻¹ for informal domestic water quality). Significant seasonal differences were found for FIB with numbers being higher during the wet season. *E.coli* and TC

die-off rates ranged from 0.01 d^{-1} to 1.33 d^{-1} and were significantly higher for free bacteria than for total (free + particle attached) bacteria, suggesting that particle attachment provided a certain level of protection to *E.coli* and TC in this highly turbid river system. This data, along with other data collected from a range of sources on TC numbers was then modeled using the Seneque/Riverstrahler model in order to investigate the dynamics and seasonal distribution of *E.coli* and TC in the Red River (Northern Viet Nam) and its upstream tributaries. Indeed, although many studies have been published on the use of models to assess water quality through fecal contamination levels, the vast majority of this work has been conducted in developed countries and similar studies from developing countries in tropical regions are lacking. The results of the model show that, in general, the overall correlations between the simulated and observed values of TC follow a 1: 1 relationship. They also show that TC numbers are affected by both land use in terms of human and livestock populations and by hydrology (river discharge). The importance of diffuse sources of TC over point sources in this system was demonstrated, especially in the upstream part of the Red River. The scenario, based on the predicted changes in future demographics and land use in the Red River basin for the 2050 horizon, showed only a limited increase of TC numbers compared with the present situation at all station. This was particularly the case in Ha Noi even though the population is expected to double by 2050. DOC and POC concentrations were also measured and modeled along the Red River. The model results reflected the importance of land use, discharge and the dominance of non-point sources over point sources in this network. Indeed, as for *E.coli* and TC, the concentrations observed reflected the large amounts of industrial effluent, agricultural runoff, and domestic sewage that are discharged into the surface water of this river system. The model also allowed determining the net ecosystem metabolism in terms of OC respiration over the whole delta. It was found that the OC inputs to the Red River and the resulting heterotrophic respiration of this OC resulted in a system that was a strong CO_2 source. Recognizing and

understanding the link between human activities, natural process and microbial functioning and their ultimate impacts on human health are prerequisites for reducing the risks to the exposed populations. This work in tropical systems has been based on the application of a model developed on temperate environment after checking its applicability or appropriateness of the biogeochemical mechanisms for tropical environments. This thesis provides some new information on *E.coli* and TC and on OC in the Red River, Viet Nam as well as providing a base for discussion with decision makers on the future management of wastewater in the Red River basin.

Keywords: Red River, Faecal Indicator Bacteria, Organic Matter, Seneque/Riverstrahler model, human impacts

Tóm tắt

Ở các nước đang phát triển, ô nhiễm nước đặt ra mối đe dọa lớn đối với sức khỏe con người và thiếu nước sạch và vệ sinh môi trường vẫn tiếp tục là vấn đề chính cho sự phát triển. Đô thị hóa và nước thải sinh hoạt và công nghiệp không được xử lý là nguồn cung cấp đáng kể carbon hữu cơ (OC) và vi khuẩn vào các hệ sinh thái thủy sinh. Điều này đang là vấn đề đặc biệt quan trọng ở các nước đang phát triển, nơi hiệu quả xử lý nước thải còn yếu kém và nơi dân số đang gia tăng nhanh chóng, với tốc độ đô thị hoá và công nghiệp hóa tăng cao. Ô nhiễm vi sinh và OC từ nước thải ảnh hưởng tới dòng chảy và chất lượng nước, đồng thời với gia tăng lượng mưa và lũ lụt do biến đổi khí hậu sẽ làm các vấn đề ô nhiễm trầm trọng thêm. Như vậy, bắt buộc chúng ta nên có sự hiểu biết về phân bố và các yếu tố ảnh hưởng tới sự phân bố và phát tán của các tác nhân gây bệnh trong nguồn nước. Sông Hồng là con sông lớn thứ hai tại Việt Nam và là nguồn cung cấp nước chính cho bộ phận lớn dân cư miền Bắc Việt Nam. Luận án này trình bày các kết quả thu được từ những quan trắc thực tế và kết quả mô phỏng từ mô hình về các chỉ tiêu vi khuẩn chỉ thị phân (FIB) và cacbon hữu cơ dạng hòa tan và dạng hạt (DOC và POC) trong lưu vực sông Hồng, miền Bắc Việt Nam. Mục đích của nghiên cứu này là để thu được những thông tin về FIB và OC trên hệ thống sông Hồng và sau đó nghiên cứu ứng dụng mô hình mô phỏng các thông số này theo các kịch bản năm 2050 khi dân số ở khu vực này được ước tính tăng gấp đôi.

Về FIB, kết quả quan trắc tại 10 trạm dọc theo sông Hồng cho thấy giá trị FIB đạt tới $39.100 \text{ MPN } 100 \text{ ml}^{-1}$, cao hơn rất nhiều lần so với giới hạn cho phép về chất lượng nước sinh hoạt ($50 \text{ MPN } 100 \text{ ml}^{-1}$ cho nguồn cung cấp nước sinh hoạt theo QCVN02: 2009/BYT của Chính phủ Việt Nam). Có sự khác biệt đáng kể theo mùa đối với FIB, trong đó các giá trị cao hơn đáng kể đã được quan sát thấy trong mùa mưa. Tốc độ chết của FIB dao động từ $0,01 \text{ ngày}^{-1}$ đến $1,33 \text{ ngày}^{-1}$, trong đó tốc độ chết của FIB tự do cao hơn đáng kể so với FIB tổng số (tự do

+ gắn kết), điều này cho thấy dạng vi khuẩn gắn kết cung cấp một mức độ bảo vệ nhất định cho FIB trong hệ thống song có độ đục lớn. Kết quả này cùng với các số liệu khác thu thập từ nhiều nguồn khác nhau về FIB sau đó được mô hình hóa nhờ sử dụng mô hình Seneque / Riverstrahler để điều tra về động học và phân bố theo mùa của FIB ở sông Hồng (Bắc Việt Nam) và các nhánh chính thượng nguồn. Mặc dù nhiều nghiên cứu đã được công bố về việc sử dụng các mô hình để đánh giá chất lượng nước thông qua mức độ ô nhiễm phân, nhưng phần lớn các nghiên cứu này đã được tiến hành ở các nước phát triển và thiếu các nghiên cứu tương tự từ các nước đang phát triển ở các vùng nhiệt đới. Các kết quả của mô hình chỉ ra rằng, nhìn chung, các mối tương quan tổng thể giữa các giá trị mô phỏng và giá trị quan trắc của FIB theo mối quan hệ tỉ lệ 1: 1. Kết quả của mô hình cũng chỉ ra rằng giá trị FIB trong nước sông đang chịu ảnh hưởng bởi cả hai yếu tố là tình hình sử dụng đất, liên quan tới dân số và số lượng gia súc –gia cầm được chăn nuôi trong lưu vực, cũng như yếu tố thủy văn của hệ thống sông (lưu lượng nước sông). Như vậy, mức độ quan trọng của nguồn thải phát tán so với nguồn thải điểm cung cấp FIB trong hệ thống sông Hồng đã được chứng minh. Kết quả mô phỏng kịch bản, dựa trên sự thay đổi trong tương lai về dân số và sử dụng đất trong lưu vực sông Hồng năm 2050, cho thấy giá trị FIB tăng rất ít so với kết quả mô phỏng cho thời điểm hiện tại ở tất cả các trạm, điều này là đặc biệt đối với trường hợp tại trạm Hà Nội, khi mà dân số dự kiến sẽ tăng gấp đôi vào năm 2050.

Hàm lượng DOC và POC cũng được đo đạc và mô phỏng cho các vị trí dọc theo sông Hồng. Các kết quả mô hình phản ánh mức độ quan trọng của tình hình sử dụng đất, lưu lượng nước và nguồn thải phát tán hơn so với nguồn thải điểm trong mạng lưới sông Hồng. Cũng như đối với FIB, hàm lượng OC cũng phản ánh ảnh hưởng của nước thải công nghiệp, nông nghiệp và nước thải sinh hoạt được thải trực tiếp vào nguồn nước mặt của hệ thống sông này. Mô hình này cũng cho phép xác định các quá trình chuyển hóa của mạng lưới sinh thái về mặt trao đổi OC trong toàn bộ vùng đồng bằng. Nguồn cung cấp đầu vào của OC cho sông Hồng và kết quả của hô hấp dị dưỡng của các OC này đã tạo ra một nguồn CO₂ lớn trong hệ thống sông.

Nhận biết và hiểu được mối liên hệ giữa các hoạt động của con người, quá trình tự nhiên, hoạt động của vi sinh vật và các tác động cuối cùng của chúng đến sức khỏe con người là điều kiện tiên quyết cho việc giảm rủi ro cho các người dân tiếp xúc với nguồn nước ô nhiễm. Những nghiên cứu như vậy cho vùng nhiệt đới này đã được tiến hành dựa trên việc áp dụng mô hình được xây dựng và phát triển cho vùng ôn đới sau khi kiểm tra khả năng áp dụng hoặc phù hợp của nó theo các cơ chế sinh địa hóa cho môi trường nhiệt đới. Luận án này cung cấp một số thông tin mới về FIB và OC ở sông Hồng, Việt Nam cũng như cung cấp một cơ sở khoa học cho các nhà hoạch định chính sách về quản lý nước thải trong hệ thống sông Hồng trong tương lai.

Từ khóa: Red River Delta, Vi khuẩn chỉ thị phân, Chất hữu cơ, mô hình Seneque/Riverstrahler, tác động của con người.

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1 General Introduction

1.1. Human activities, microbial pathogens and organic carbon

Rivers are the major source of fresh water for industry, agriculture, domestic and leisure use. However, the conversion of natural landscapes to agriculture and increasing urbanization and industrialization has led to drastic changes in water quality in many of the World's rivers (Vorosmarty *et al.*, 2010). This problem is particularly pressing in developing countries where the rapid, recent industrialization and urbanization has led to dramatic decreases in water quality (Kumar *et al.*, 2014). Moreover, the consequences of human activities on water quality are all the more critical in these regions of the world where wastewater treatment facilities are often overloaded or inexistent and many people are exposed to illness and death through the use of unclean water (UNICEF/WHO, 2012).

Increasing urbanization, industrialization, agriculture and plantation forestry have been all linked to reduced water quality and ecological degradation in the Red River, Viet Nam (Trinh *et al.*, 2007; Le *et al.*, 2010; Luu *et al.*, 2012). Moreover, increases in rainfall and floods due to climate change are expected to further exacerbate these problems by increasing the transport of land-produced contaminants from land into the river. This, combined with the rapid shifts in land use that this tropical region is experiencing and the increasing urbanization and demand for clean water and sanitation mean that it is essential to understand the sources and controlling factors of contaminants in this and other tropical aquatic systems. One such way of obtaining an understanding of these factors is to use biogeochemical and hydrological modeling coupled with *in situ* and laboratory based measurements.

1.1.1 Aims and scientific questions of the thesis

The polluted water of Red River poses a threat to the health and livelihoods of local people

along the river in North Viet Nam. This thesis aims to provide information on some of the links between faecal indicator bacteria (FIB), organic carbon (OC), land use and hydrology in the Red River and Delta (North Viet Nam) using both experimental and modeling techniques. The work carried out aimed to identify the mechanisms that determine the transfer of FIB and OM within the hydrographic network, from the upper basin down to the sea, taking into account the influence of human activities and of climate change in its watershed. Therefore the first objective of the thesis was to investigate the seasonal variability of two FIB – *Escherichia coli* and Total Coliforms, and of DOC and POC concentrations in the Red River and its delta by identifying the environmental factors controlling the abundance of these microbes, determining their die-off rates as well providing information on the carbon dynamics in this river system. The second goal was to construct a dataset on TC, DOC, POC concentrations in domestic, industrial and agricultural sources in the Red River drainage network. This data, along with that collected during the survey work, were then used for implementing the existing SENEQUE/Riverstrahler model on the Red River to calculate TC and OC dynamics in the drainage network. The model was then used to estimate the influence of the point and non-point sources and environmental conditions on the retention or elimination of TC, organic matter and suspended solids in the Red River drainage system and to examine scenarios of what might occur in 2050.

1.1.2 Structure of the thesis

This PhD thesis contains 5 chapters of which 2 are data chapters written in form of scientific articles. This, the **first chapter** provides a short, general introduction to the thesis. **Chapter 2** gives a detailed description of the study area with background information on the Red River and its Delta and on the physical constraint data required for the modeling approach, such as the geomorphology, geology and lithology and also hydro-meteorology (temperature, rainfall

and hydrology). A detailed description of the Seneque/Riverstrahler model is also provided. The first section of **Chapter 3** presents an introduction to FIB and the specificities of developing countries. The second section then presents the results of the work on the seasonal variability of *E.coli* and Total Coliform numbers and die-off rates in the Red River basin, North Viet Nam. This section is published in the journal “Scientific Reports”. The third section of this chapter presents and discusses the results on modeling of FIB with the Seneque/Riverstrahler model. This chapter is in submission “Environmental Monitoring and Assessment” (submitted the 12th January 2016). The following chapter (**Chapter 4**) starts with a short introduction to OC in aquatic environments; the second section then presents the work on OC degradation and the modelling of OC and CO₂ fluxes in the Red River system. This article is in preparation for submission to the journal “Biogeochemistry” in the summer of 2016. **Chapter 5** is the final chapter of this thesis and provides some general conclusions and perspectives on this work on FIB and organic carbon in the Red River system. A complete list of references and three appendices are also given in **Chapters 6** and **7**, respectively.

2 Study site and Methods

2.1 Study site



Figure 2.1: Map of Viet Nam. The major cities and islands are noted. From the Maps of the World website.

2.1.1 Water resources in Viet Nam

Viet Nam is located in South East Asia. It is bordered to the North by China, to the west by Laos and Cambodia and to the east by the Eastern Sea (Fig. 2.1). The country has one of the highest population densities in the region ($273 \text{ people km}^{-2}$). It ranks 3rd in South East Asia, just after the Philippines with $307 \text{ people km}^{-2}$ and Singapore with $7,486 \text{ people km}^{-2}$. Moreover, Viet Nam's population is continuing to grow rapidly and is estimated to reach 126 million by 2040. Given population growth, it can be anticipated that the environment in Viet Nam will be subject to increasingly intense pressures and that conservation of the environment and the services it provides will be increasingly difficult.

Viet Nam has a dense network of rivers, 2,360 rivers of more than 10 km long with several

much longer ones such as the Red and Mekong Rivers. This network includes many trans-national rivers that have their source in other countries (Table 2.1). Indeed, around two thirds of Viet Nam's water resources originate from outside the country, making Viet Nam dependant on water resource decisions made in upstream countries. Surface water in Viet Nam comes from a total catchment area of 1,167,000 km² and the surface water potential is estimated at 835 billion m³ per year with the largest proportion in the Mekong delta region in the south of the country, followed by the Red River (Sông Hồng in Viet Nameese) delta region in the North (Fig. 2.2).

In Viet Nam extensive data on surface water quality is lacking. However, the information available reveals rising biological and chemical pollution levels in downstream sections of the major rivers (Berg *et al.*, 2007; Trinh *et al.*, 2007; Le *et al.*, 2010; Luu *et al.*, 2012; Navarro *et al.*, 2012; Ziegler *et al.*, 2013; Ozaki *et al.*, 2014). The upstream water quality of most rivers remains good, while downstream pollution mainly from urban areas (human and urban waste) and industries affects the water quality (Berg *et al.*, 2007; Navarro *et al.*, 2012; Ozaki *et al.*, 2014). The rapid economic and demographic growth that Viet Nam is experiencing is increasing the demand for clean water as well as increasing the amount of wastewater that needs to be treated. Indeed, in the context of global change and economic development, it is obvious that any socio-economic development is closely linked to the need for water of an adequate quality.



Figure 2.2: The Red River delta region in the North Vietnam.

2.1.2 Red River Basin

The Red River is a transboundary river basin that flows through Viet Nam, China and Laos. The total basin area is around 156,000 km² of which around 55 % are in Viet Nam (Table 2.1). A small part is located in Laos (1100km², or 0.65%) and with the remaining 44% located in China. The basin is delimited between latitudes 20°23 'to 25°30' North and longitudes 100°00 to 107°10' East. To the north the basin borders with the Yangtze River basin, to the East the Thai Binh basin, to the west with the Mekong River basin and the Ma River, and to the south with the Gulf of Tonkin. The length of the Red River in Viet Nam is about 328 km making it the second largest river (after the Mekong River) in the country.

Table 2.1: Water resources in the major rivers of Viet Nam (Truc, 1995).

River Basin	Catchment area		Total volume		
	Total area in Viet Nam (km ²)	Percentage in Viet Nam	Total (bill.m ³)	Total generated in Viet Nam (bill.m ³)	Percentage in Viet Nam
Me Kong	795,000	8	508	55	11
Red River – Thai Binh	156,000	55	137	80.3	59
Dong Nai	44,100	85	36.6	32.6	89
Ma – Chu	28,400	62	20.2	16.5	82
Ca	27,200	65	27.5	24.5	89
Ba	13,900	100	13.8	13.8	100
Ky Cung – Bang Giang	11,220	94	8.9	7.3	82
Thu Bon	10,350	100	17.9	17.9	100

2.1.2.1 Topography

The topography of Red River basin slopes from northwest to southeast. Mountainous terrain to the East and North dominates the upper catchment area and tends to decrease in a northwest – southeast direction with an average altitude of 1,090 m. The mountainous region on the border between Viet Nam and Laos has many mountains above 1,800m such as Pu Si Lung (3,076m), Pu Den Dinh (1,886m), Pu San Sao (1,877m). This range also separates the watersheds of the Red River and Mekong River systems. Within the Red River basin, Hoang Lien Son mountains divide the Da and Thao Rivers, two of the tributaries of the Red River. It is in this range that the Fansipan peak (3,143m), the highest mountain in Viet Nam, is found. The Tay Con Linh mountain that peaks at 2,419m divides the Thao and the Lo Rivers, the second and third tributaries of the Red River.

The average altitude of the river basin is high. The slopes vary between 6 and 8.5 degrees but can be quite steep such as in the Ngoi Thia (23 degrees) or Suoi Sap (25 degrees) streams and the Thao, Da and Lo River basins have an average altitude of 547m, 965m, and 884m, respectively (Le, K.L., 2009). The Lo River has the highest slope (1.8 mkm^{-1}), then the Da River with 1.5 mkm^{-1} , with the Thao River having the lowest slope (1.2 mkm^{-1}).

2.1.2.2 Climate

The Red River Basin is influenced by the Asian tropical monsoon. The North East monsoon occurs from November to April bringing cooler, dryer weather. The South West monsoon occurs from May to October and weather during this period is warmer and much more humid. Wind direction also generally depends on the orientation of the valley. It can vary from mainly from the west or northwest during the summer in the Da river basin to south-southeast in the Lo River

Basin. The average wind speed about $1-1.5\text{ms}^{-1}$ but these values can reach 40ms^{-1} during storm and cyclone events.

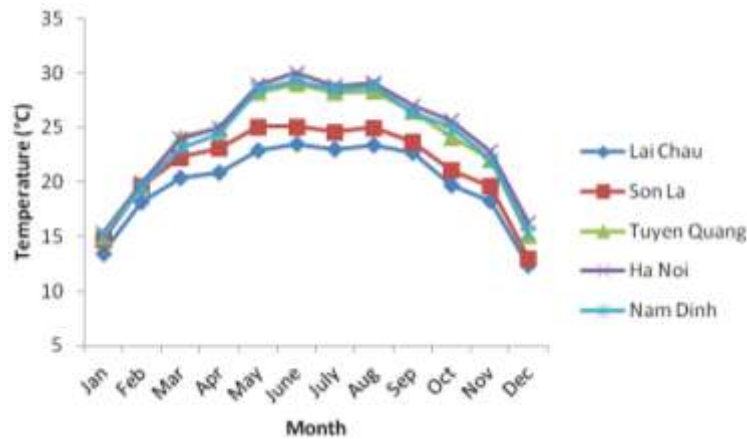


Figure 2.3a: Monthly air temperature in 2013 for a selection of cities in the Red River basin.

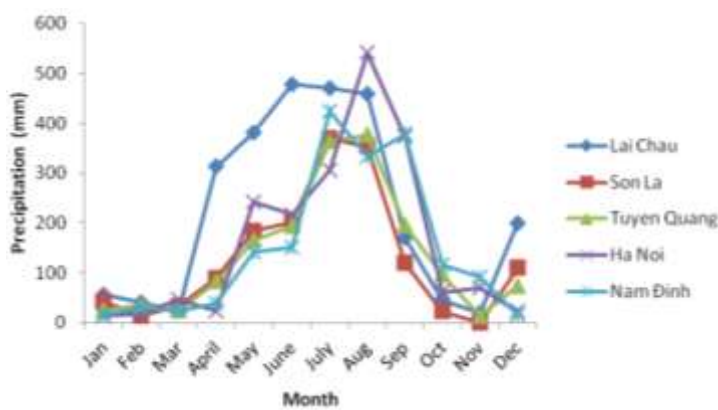


Figure 2.3b: Monthly precipitation for the same stations in 2013.

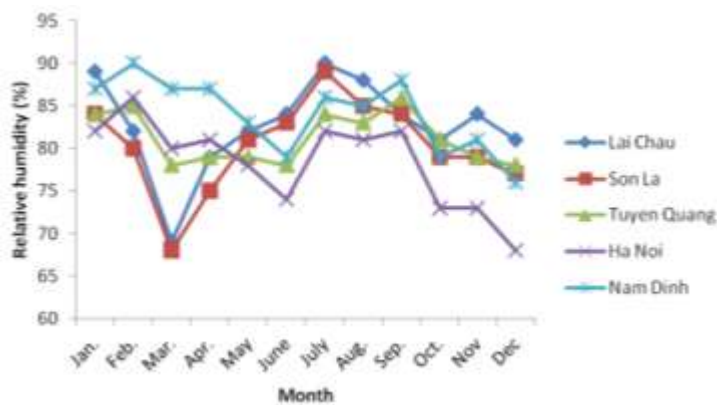


Figure 2.3c: Monthly relative humidity (%) for the same stations in 2013.

Temperature tends to increase gradually from upstream to downstream (Figure 2.3a). Highest temperatures usually occur in May with values of up to $37 - 41^{\circ}\text{C}$ observed in Son La. Lowest temperatures usually occur from October to January throughout the basin. Minimums of -0.2°C have been observed in Son La and occasionally snow can fall in the city of Sa Pa in the mountainous province of Lao Cai.

The annual radiation in the Red River Basin varies between $100 - 200 \text{ Kcal cm}^{-2} \text{ yr}^{-1}$ (average 60 to $80 \text{ Kcal cm}^{-2} \text{ yr}^{-1}$). It is lowest in January and February when total radiation is $5 - 8 \text{ Kcal cm}^{-2} \text{ month}^{-1}$ and highest in July. In the summer, the radiation balance is relatively uniform across the basin. In winter, the difference is higher with the radiation levels varying with altitude. This means that annual Hanoi (5m AMSL) has $72.5 \text{ Kcal cm}^{-2} \text{ yr}^{-1}$ but in Sapa (1570m AMSL) the radiation balance was only $44.7 \text{ Kcal cm}^{-2} \text{ yr}^{-1}$.

The monsoonal climate means that two distinct seasons are found. The rainy season usually lasts 5 months from June to October. Overall, rainfall is high but unequally distributed and varies between $1,200 - 2,000 \text{ mm}$, with an average of $1,800 \text{ mm yr}^{-1}$ (Le, 2009). The distribution of rainfall in the basin depends heavily on the topography (Fig. 2.2b). For example, Bac Quang, located in middle of the Lo river basin, has rainfall of up to $5,499 \text{ mm yr}^{-1}$. However, the cities located behind the mountains such as Yen Chau, Son La, Nghia Lo have much lower rainfall ($1,200\text{mm}$ to $1,600\text{mm yr}^{-1}$). In the plains, average annual rainfall varies from $1,400\text{mm}$ to $2,000\text{mm}$.

The average relative humidity in the basin is high and values from $80\% - 90\%$ are common (Fig. 2.3c). The first maximum occurs in February - March due to drizzly weather in late winter. The second maximum occurs around July - August when temperatures and rainfall are highest. The driest periods occur in May - June and around October - November period corresponding to the beginning and the end of the rainy season.

The Red River basin average annual evaporation varied between 730 - 980 mm yr⁻¹ in Thai Nguyen, 560 – 1,050 mm yr⁻¹ in the Midlands, and 700 - 990 mm yr⁻¹ in the Plains. Total average evaporation, determined over the period 1958 – 2006, at Son La is 932,8mm and 803,4mm at Thac Ba (Tran, 2007; Vu, 2009).

2.1.2.3 Hydrology

Red River system has three major tributaries: Da, Thao and Lo rivers. All three of these rivers originate from Yunnan (China) and then flow into Viet Nam. The Thao River (named the Nguyen River in China) originates in the Dali Lake at an altitude of 2,000 m at Wei Son in Yunnan Province, China. It then flows in a Northwest to Southeast direction and enters Viet Nam in the Bat Xat district, Lao Cai province. It then receives water from the Da River at Trung Ha and Lo River at Viet Tri before flowing into the Red River delta. The Thao River is considered mainstream of the river and the part of the river from Viet Tri to Ba Lat is known as the Red River.

The Red River delta has a network of interlacing canals and arroyos. It has several distributaries including the Duong and Luoc Rivers that flow into the Thai Binh River and the Tra Ly, Dao and Ninh Co Rivers. The Red River flows in the Gulf of Tonkin at Ba Lat, as well as through the Tra Ly, Lach Giang and Day Rivers.

The Da River, known as the Ly Tien River in China, originates in the high mountains of Yunnan province and flows in a Northwest to Southeasterly direction before entering Viet Nam at Ka Long Commune in Muong Te district, Lai Chau province. It then flows through Dien Bien, Son La and Hoa Binh provinces before joining with the Thao River at Trung Ha. The Da River is 1,010 km long, has a catchment area of 52,900km² of which 570 km and 26,800km² are in Viet Nam.

The Lo River also has its source in the high mountains of over 2,000 maltitude in the

Southwest Yunnan province, China. In China, the Lo River is known as the Ban Long River. It flows in a Northwest to Southeasterly direction before entering Viet Nam in Vi Xuyen District, Ha Giang province before flowing through Tuyen Quang, Phu Tho, Vinh Phuc provinces and emptying in the Thao River at Viet Tri. The mainstream of the Lo River is 470 km long and the total basin area is 39,000 km², of which 275 km and 22,600 km² are in Viet Nam.

The average total flow of the Red River system is about 127 km³, which of 48.7 km³ (38.3%) enters from China and Laos, 55.1 km³ (43.4%) from the Da River, 25.6 km³ (20.2%) from the Thao River and 33.3 km³ (26.2%) from the Lo River (Le *et al.*, 2007). The maximum and minimum flows for some selected gauging stations are given in Table 2.2. Due to distributaries in the delta, discharge at the Hanoi station is 40% lower than that at Son Tay, which is located immediately downstream from the confluence of the three main sub-basins (Le *et al.*, 2010).

River discharge also varies seasonally as a consequence of seasonal differences in rainfall. The annual flood season in the middle and upper rivers often begins in May – June and ends in September - October. Downstream flooding occurs from June to October. Some rivers in the highlands of Son La, Moc Chau (Da River Basin) the flood season extends from July to October. Flood flows represent around 70-80% of the annual flow and flow in June to August or July to September are consistently high, accounting for about 50-65% of the annual flow. July and August are generally the months with the highest average monthly flow, and these months alone account for 15-30% of annual flow. During the dry season flow accounts for 20-30% of annual flow. The lowest discharge occurs between January and April when flow accounts for less than 10% of the annual flow.

Table 2.2: Water level and flow of some main rivers at 2013 (GSO, 2013).

River – Station	Water-level (cm)		Flow (m ³ s ⁻¹)	
	Max	Min	Max	Min
Da river - Lai Chau	21,729	17,743	4,690	89
Da river - Hoa Binh	1,735	941	3,070	69
Thao river - Yen Bai	3,212	2,454	5,340	98
Thao river - Phu Tho	1,759	1,270	-	-
Lo river - Tuyen Quang	2,259	1,518	-	-
Red river - Son Tay	1,056	259	13,100	640
Red river - Ha Noi	722	34	6,960	145

2.1.2.4 Demography

Administratively, the Red River basin covers 25 provinces with a population of 32 million people (estimates for 2013) including the capital city of Hanoi and large port city of Hai Phong. The Red River basin has the largest population density in Viet Nam (Table 2.3). The delta provinces are most densely populated with the major cities of Hanoi (2,087 people km⁻²); BacNinh (1,354 people km⁻²); Hai Phong (1,260 people km⁻²) and Hung Yen (1,244 people km⁻²; data 2013)(GSO, 2013). In contrast, the mountainous provinces are less densely populated e.g. YenBai 112 people km⁻²; Hoa Binh 175 people km⁻². At present, about half of the inhabitants in the Red River basin live in rural areas with the rest living in cities, towns and townships. However, the process of urbanization is accelerating as is the rural exodus and the population density in the urban areas is expected to rapidly increase.

Table 2.3: Population in the Red River basin

	Area (km²)	Population (x10³ people)	Density (Person km⁻²)
Total in country	33,0972.4	89,708.9	271
Red River Delta	21,059.3	20,439.4	971
Ha Noi	3,324.3	6,936.9	2,087
Vinh Phuc	1,238.6	1,029.4	831
Bac Ninh	822.7	1,114	1,354
Quang Ninh	6,102.4	1,185.2	194
Hai Duong	1,656	1,747.5	1,055
Hai Phong	1,527.4	1,925.2	1,260
Hung Yen	926	1,151.6	1,244
Thai Binh	1,570.5	1,788.4	1,139
Ha Nam	860.5	794.3	923
Nam Dinh	1,652.8	1,839.9	1,113
Ninh Binh	1,378.1	927	673
Yen Bai	6,886.3	771.6	112
Hoa Binh	4,608.7	808.2	175

Generally, the educational and health conditions in the Red River basin are low, especially in the mountainous provinces such as Lao Cai, Yen Bai, Bac Kan, Bac Giang. Health related infrastructure is lacking and access to adequate sanitation is limited in these provinces. The delta provinces such as Vinh Phuc, Bac Ninh, Ha Nam, Ninh Binh have much higher economic growth and the health and education conditions are considerably better. This is particularly true in the Ha Noi metropolitan area which is the cultural center of the country. The midland plains of the Red River, where the capital city of Hanoi is located, also house the scientific, political and administrative services of the country. However, sanitary facilities such as wastewater treatment and garbage collection and treatment are still very low even in urban areas (e.g. Ha Noi) (Fig 2.4).

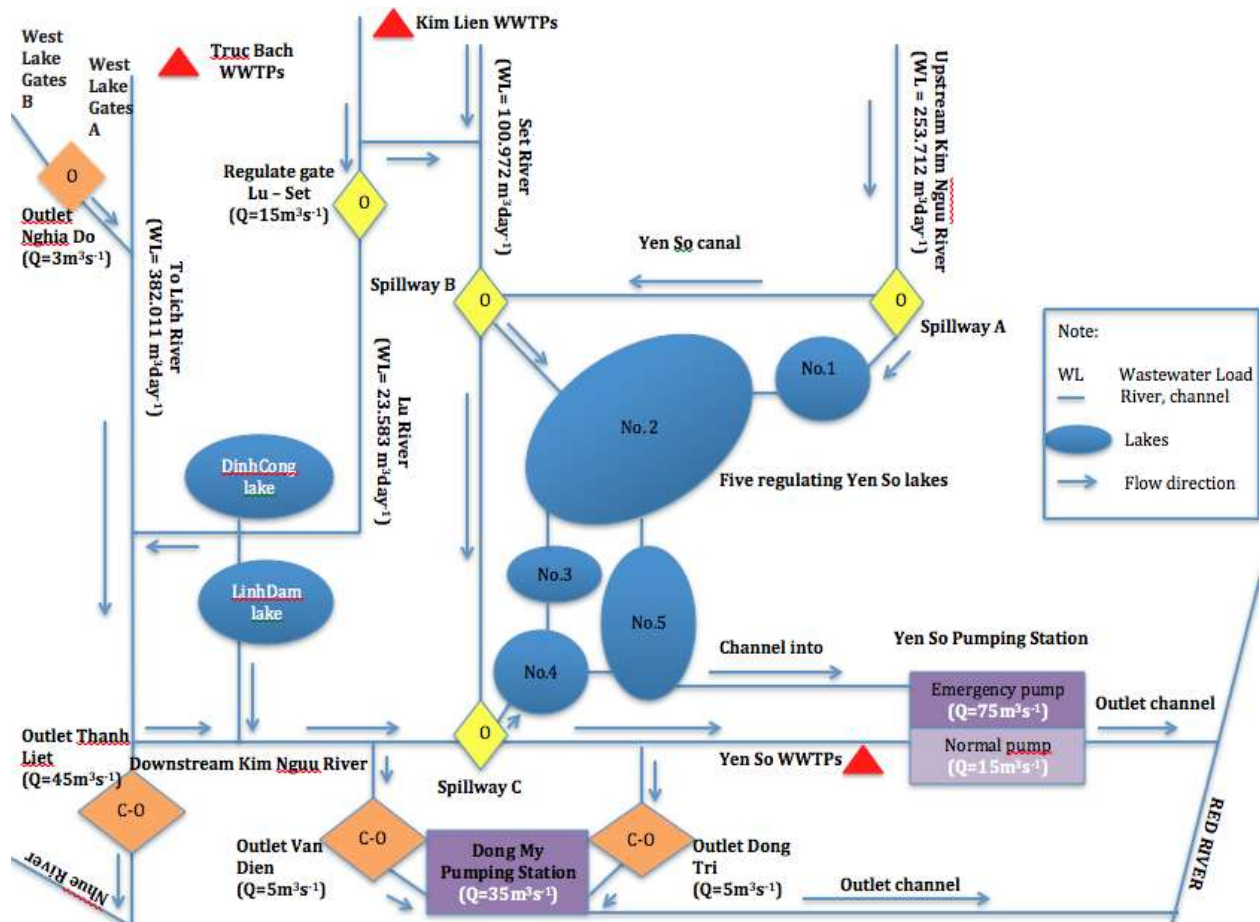


Figure 2.4: Scheme of wastewater routing in Hanoi city

In the center of Hanoi City, the drainage system is a combined system without separation of runoff, domestic and industrial wastewater. In 2013, total wastewater discharged in this city averaged $794,466 \text{ m}^3$ per day (Huan *et al.*, 2014). The wastewater treatment plant at the Yen So Park receives the wastewater flows from the Kim Nguu and Set Rivers and from Yen So Park. This plant is designed to treat a maximum of $200,000 \text{ m}^3$ wastewater per day, including $125,000 \text{ m}^3 \text{ day}^{-1}$ from the Kim Nguu river, $65,000 \text{ m}^3 \text{ day}^{-1}$ from the Set river, and an additional of $10,000 \text{ m}^3 \text{ day}^{-1}$ from sewer systems in the city (Figure 2.4). These rivers are heavily polluted by wastewater discharged directly from Hanoi City.

2.1.2.5 Economy and land use

The Red River Delta is one of eight economic regions formed within the Worlds' major river basins. It is characterized by rapid population growth, urbanization and industrialization, intensive agriculture all of which have negatively affected water quality. Indeed, the Red River Delta has been identified as one of the regions that will be most severely affected by climate change and human activities in the future (Chaudhry and Greet, 2008; UNCCD, 2008). The Red River Delta, along with the Mekong River Delta is a key economic and agricultural region in Viet Nam.

The economy of the region is based on industry, services, agriculture, forestry and fisheries. With 22.8% of the national population in 2011, this region contributed 676.9 billion Viet Nam Dong (25 billion USD); accounting for 28.4% of the GDP.

The most significant industrial zones are located in Hanoi, Vinh Phuc, Bac Ninh, Hung Yen, Hai Duong and Hai Phong provinces and the main sea ports are found Hai Phong and Quang Ninh. Industry is mainly metallurgy, chemicals, construction materials along with food processing and consumer goods production. Agricultural production is strongly based on irrigated and non irrigated crops and aquaculture. About 760,000ha are used for crop cultivation (mainly rice production) and for planted forests and about 120,000 ha are used for aquaculture. The production of hydroelectricity is important in the larger reservoirs (Hoa Binh, Thac Ba, Son La, Tuyen Quang).

Table 2.4: Structure of economic sector for Viet Nam and the Red River delta provinces. Values are given as a percentage of the gross domestic production (GDP, USD).

	Relative percentage of each activity (%)					
	2005	2007	2008	2009	2010	2011
Viet Nam						
Agriculture, forestry and fishery	21.0	20.3	22.2	20.9	20.6	22.0
Industry and Construction	41.5	42.0	40.4	40.8	41.6	40.8
Services	37.5	37.7	37.4	38.3	37.8	37.2
Red River Delta						
Agriculture, forestry and fishery	16.2	14.0	13.9	13.0	12.2	12.0
Industry and Construction	39.4	42.2	43.2	44.0	45.0	45.4
Services	44.4	43.8	42.9	43.0	42.8	42.6

Tourism is also an important economic activity in several of the provinces (Hanoi, Tam Coc, Bich Dong, Trang An – Ninh Binh, Ha Long – Quang Ninh). However, the economic structure of the sector is shifting as a function of the increase in the proportion of industry and construction at the cost of agriculture, forestry and fisheries.

Around 15% of the countries rice production occurs in the Red River Delta (Rutten *et al.*, 2014). However, recently large areas of rice paddies have disappeared as a consequence of the construction of housing and factories. Indeed, land use patterns in Viet Nam are expected to change dramatically in the future as a consequence of several global and local processes that interact at various scales and domains (Rutten *et al.*, 2014).

Table 2.5: Land use in the basin in 2013 (x10³ ha)

Thous. ha	Total area	Agricultural production	Forestry	Specially used	Homestead
Total in country	33,097.2	10,210.8	15,405.8	1884.4	695.3
Red River Delta	2,105.9	770.8	519.1	315.6	141.1
Ha Noi	332.4	149.7	24.4	70.0	37.0
Vinh Phuc	123.9	49.7	32.4	18.9	8.7
Bac Ninh	82.3	42.2	0.6	17.9	10.1
Quang Ninh	610.2	50.3	390.3	42.8	10.1
Hai Duong	165.6	84.6	10.9	30.6	15.6
Hai Phong	152.7	49.5	20.2	27.3	13.8
Hung Yen	92.6	53.2	-	17.7	10.0
Thai Binh	157	93.4	1.4	28.5	13.0
Ha Nam	86.1	43.4	6.3	16.0	5.7
Nam Dinh	165.3	93.4	4.2	25.5	10.9
Ninh Binh	137.8	61.4	28.4	20.4	6.2
Yen Bai	688.6	107.6	473.7	15.7	4.9
Hoa Binh	460.9	65	288.6	25.2	19.5

Along with climate change, other key drivers affecting land use in Viet Nam are technological change, population growth and international trade. Economic development and structural change will lead to considerable changes in land use with expansion of planted forests and urbanization at the expense of rice paddies, mangroves and other non-production forests, and shrub lands. This is directly related to the specific development trajectory of Hanoi and the surrounding industrial areas. Between 1999 and 2009, the Red River Delta has witnessed a very high pace of industrial activity that led to an expansion of urban land throughout the region. The new industrial areas are predominantly located in suburban areas at a distance of about 70-140 km from Hanoi.

2.2 Methods

2.2.1 Sampling strategy and laboratory analysis

2.2.1.1 Experimental work

From January 2013 to December 2014 monthly field surveys took place to collect water samples for a series of water quality measurements along the Red River. Ten stations (from Yen Bai- upstream of the Red River- to Ba Lat - downstream of Red River) were chosen. The main purpose of these surveys was to determine both seasonal (dry and rainy season) and year-to-year variations of water quality on the Red River. The ten selected stations were:

- Yen Bai that is representative of water quality upstream of the Red River after receiving water from China.

- Hoa Binh, characterizing the water quality of the Da River before receiving water from the Red River.

- Vu Quang, characterizing the water quality of the Lo River before receiving water from the Red River.

- Son Tay, located just after the confluence of the Da and Lo Rivers to the Red River, represents water quality of the Red River after receiving water from the Da and Lo River.

- Hanoi station, representative for water quality at mid river.

- Gian Khau, characterizing the water quality of the Day River after receiving water from the Red River.

- Quyet Chien, representative for water quality of the Tra Ly River before discharging of the Red River.

- Nam Dinh, representative for water quality of the Dao River after receiving water from the Red River.

- Truc Phuong, representative for water quality of the Ninh Co River after receiving water from the Red River.

- Ba Lat, illustrating the water quality of the Red River downstream before discharging to the Sea.

2.2.1.2 *In-situ* measurements

During the monthly sampling campaigns, surface samples were collected in a can of 1.5 liter (30 cm below the surface of the river) and no preservative was added (Fig. 2.5). The water samples were kept at 4°C to 10°C in an icebox before treatment, during transportation to the laboratory.



Figure 2.5: Sampling river water on the Red River.

Other physical-chemical parameters were measured directly in river water as: temperature (°C), pH, conductivity ($\mu\text{S cm}^{-1}$), salinity (‰), turbidity (NTU), TDS (total dissolved solids –

mg l^{-1}), TSS (total suspended solids – mg l^{-1}) and dissolved oxygen (DO, $\text{mg O}_2 \text{ l}^{-1}$) were measured using WQC-22A (TOA, Japan) and Hach (USA) probes.

2.2.1.3 Laboratory treatment

Upon return to the laboratory, all samples were treated immediately to avoid any changes during storage. The filtration was realized with a Gelman Science filter (Pall) equipped with a manual pump. Samples were filtered through:

- Whatman GF/F paper-filter (glass micro-fiber filters $0.7\mu\text{m}$) previously burned at 500°C (4 hours) for dissolved nutrient analyses as nitrogen (nitrite, nitrate and ammonia), phosphorus (phosphate), and for DOC. For the determination of SS and particulate organic carbon POC on the filter, GF/F filter-papers were pre-weighted.

After treatment, all samples were contained in disposable sterile polyethylene flasks except for DOC that was stored in brown glass bottles. The samples were stored frozen. Samples were prepared in duplicate for analysis in Laboratory of Environment (Institute of Natural Products Chemistry – INPC, Hanoi).

2.2.1.4 Laboratory analysis

Suspended solid (SS) values were determined as the weight of material retained on the Whatman GF/F filters per volume unit after drying the filter for 2 h at 120°C . POC was determined on the same burned pre-weighted filters, as was used for the TSS determination. DOC concentration was measured by a Shimadzu TOC-VE analyzer. Nutrients (N, P, Si) were spectrophotometrically determined on a Drell 2800 (HACH, USA) in the laboratory according to APHA (2012) methods.

FIB numbers (free and attached) were measured by a direct count method using Petrifilm E. coli/Coliform Count (EC) plate which contain Violet Red Bile (VRB) nutrients (APHA, 2001).

2.2.2 Seneque/Riverstrahler model

In order to integrate the measurements obtained on the spatial and temporal distribution of both FIB and organic carbon concentration into a general vision of the dynamics of these components within the entire river system, we have made use of an existing modelling tool, already adapted to the Red River basin (Le *et al.*, 2010; Le *et al.*, 2015), the Seneque/Riverstrahler Model. We here present the principle of this model, as well as the functioning of the software used to pilot the model.

2.2.3 Principles of the Riverstrahler model

The SENEQUE/Riverstrahler model (Billen *et al.*, 1994; Billen and Garnier, 1999; Garnier *et al.*, 1999) is a biogeochemical model (RIVE) of in-stream processes imbedded within a GIS interface (SENEQUE), providing a generic model of the biogeochemical functioning of whole river systems (from 10 to > 100000 km²), designed to calculate the seasonal and spatial variations of water quality (Ruelland *et al.*, 2007; Thieu *et al.*, 2009).

The basic version of RIVE involves 29 variables describing the physicochemical and ecological state of the system (Fig 2.6).

These include nutrient, oxygen, suspended matter, dissolved and particulate nonliving organic carbon concentrations, and algal, bacterial and zooplankton biomasses. Most of the processes that are important in the transformation, elimination and/or immobilization of nutrients during their transfer within the network of rivers and streams are explicitly calculated, including algal primary production, aerobic and anaerobic organic matter degradation by planktonic as well as benthic bacteria with coupled oxidant consumption and nutrient remineralisation, nitrification and denitrification, and phosphate reversible adsorption onto suspended matter and subsequent

sedimentation.

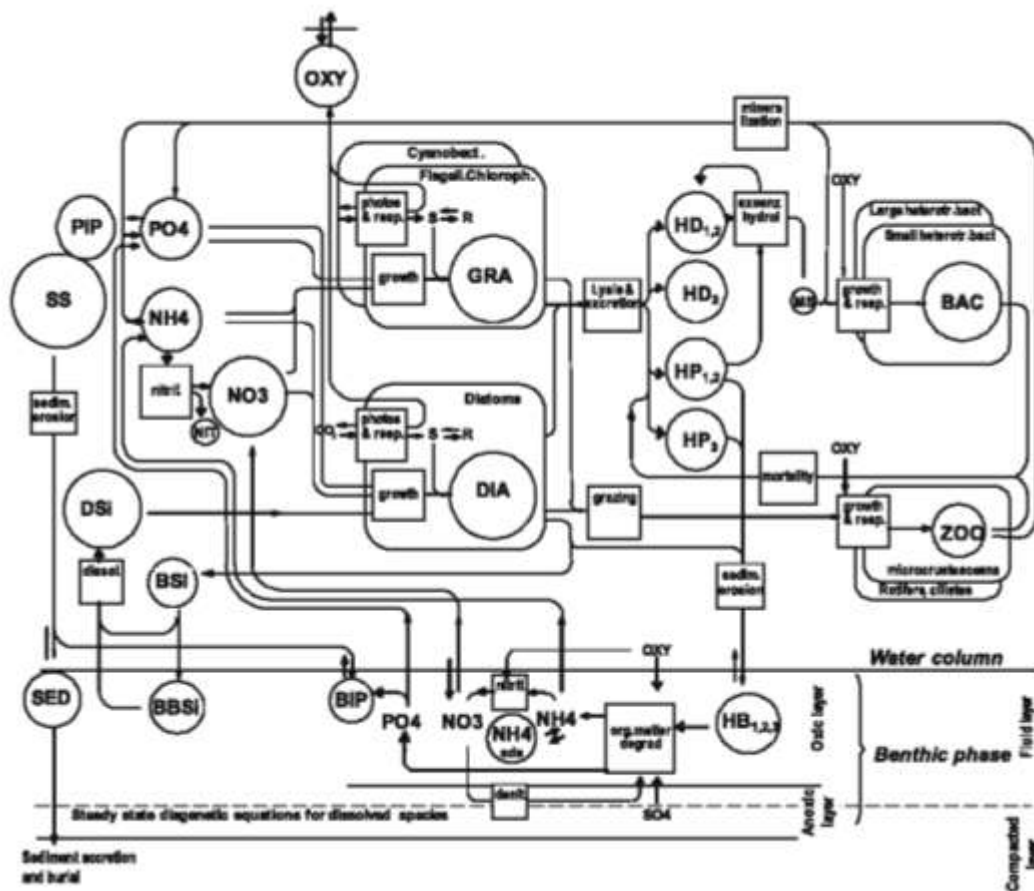


Figure 2.6: A schematic representation of the RIVE model of biogeochemical processes in aquatic systems. State variables include: DIA, diatoms; GRA, green algae; ZOO, zooplankton; BAC, heterotrophic bacteria; HD1,2,3, rapidly, slowly and non hydrolysable dissolved organic matter; HP1,2,3, rapidly, slowly and non hydrolysable particulate organic matter; OXY, oxygen; NH₄, ammonium; NH₄ads, ammonium adsorbed onto the sediment; NO₃, nitrate; NIT, nitrifying bacteria; PO₄, ortho-phosphate; PIP, particulate inorganic phosphorus; BIP, benthic inorganic phosphorus; Dsi, dissolved silica; BSi, biogenic detritic silica; BBSi, benthic biogenic silica; SS, suspended sediments; SED, deposited sediments. (From Thouvenot-Korppoo *et al.*, 2009).

The basic assumption behind the model is that basic biological and physico-chemical

processes involved in the functioning of the river system are the same from headwaters to downstream sectors, while the hydrological and morphological constraints controlling their expression differ largely along an upstream–downstream gradient as do the constraints exerted by the inputs of terrigenous material.

The model describes the drainage network of the river system as a combination of basins, represented as a regular scheme of confluence of tributaries of increasing stream order, each characterized by mean morphologic properties, connected to branches, represented with a higher spatial resolution. The framework of the drainage network is built as a combination of three kinds of objects (Fig. 2. 7):

- Upstream basins are idealized their complex drainage network structure as a regular scheme of confluence of tributaries of increasing stream-order, its having the same mean morphological characteristics.

- Branches are represented as major rivers, with a spatial resolution of 1 km of the detailed and realistic geographical.

- Reservoirs are represented as mixed reactors, connected either to branches at a defined position or to all stream-order rivers in a basin.

The hydrological constraints, i.e. the flow of water within each object, are deduced from measured discharge data at a number of key stations in the river network and expressed in terms of surface runoff and base flow for each elementary sub-basins, based on the recursive filter of Eckardt (2005). The climatic constraints are set by the seasonal variations of temperature (by streamorder) and light intensity. The other constraints consist of point sources (the discharge of wastewater from human settlements) and diffuse sources (characterized by the concentration of each variable associated with the superficial and base flow components of the discharge issued from each and use classes of the watershed). The calculations of water quality in the drainage

network are made at a ten days resolution.

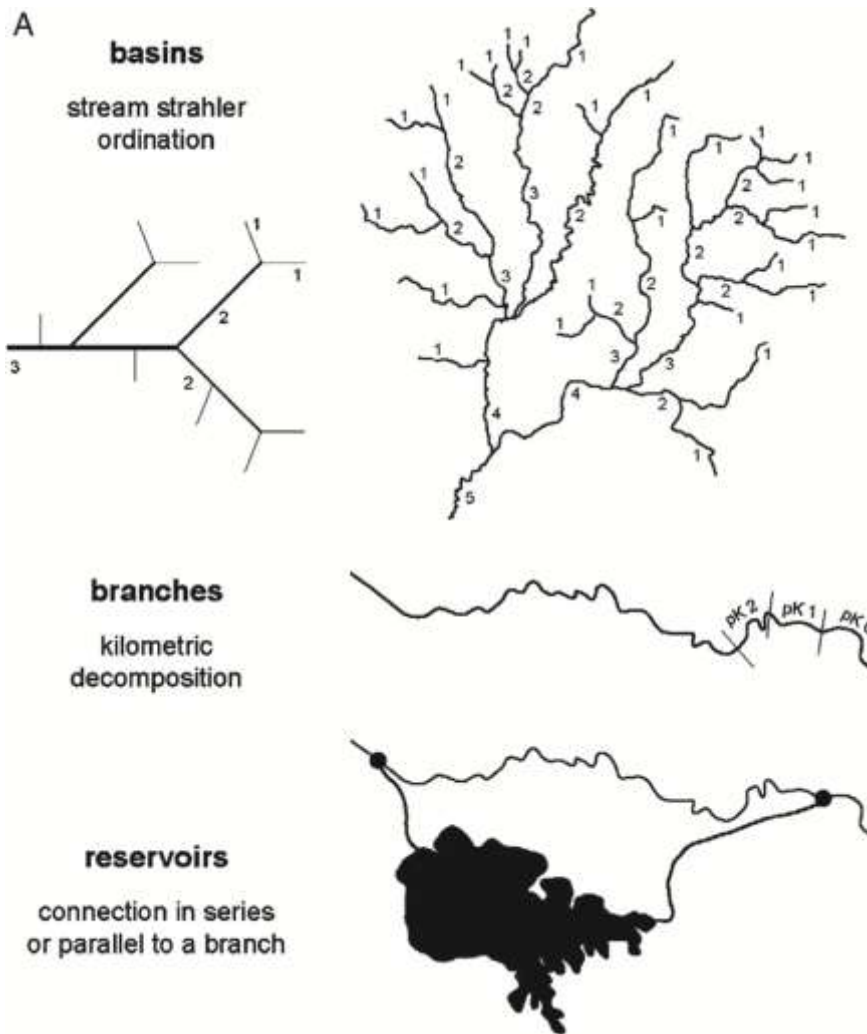


Figure 2.7: The three kinds of objects taken into account in the representation of the drainage network by the Riverstrahler model: basins, branches and reservoirs (Ruelland *et al.*, 2007).

The principles and data requirements of the Riverstrahler model are summarized in Fig 2.8.

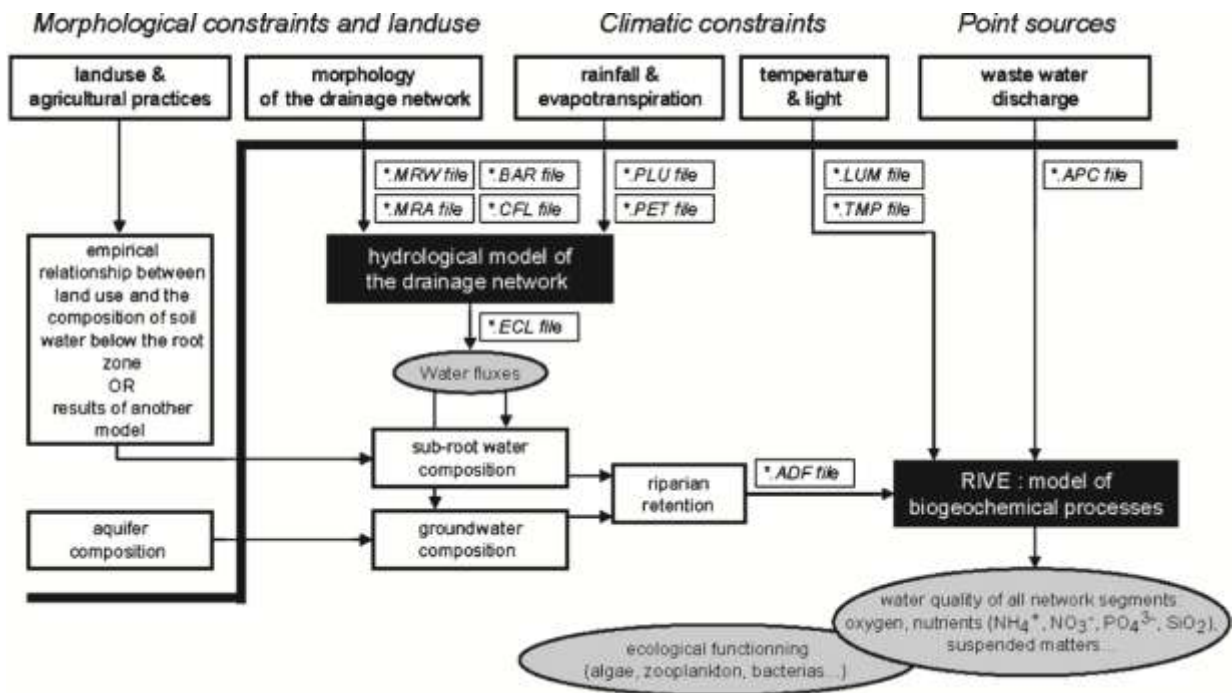


Figure 2.8: Principles of the calculation of water quality by the Riverstrahler model (Ruelland *et al.*, 2007).

2.2.3.1 The SENEQUE interface

The GIS interface SENEQUE, allows the user to run the Riverstrahler model for a definite project. It first allows the explicit delimitation the part of the basin concerned, and the degree of resolution required in its representation in terms of objects (branches and basins) combination. It then allows the testing of defined scenarios through the fixing of anthropogenic and hydrological constraints. The GIS files used are under Arc/Info or ArcView format however all other files are simple text files, conferring a large versatility in the dialog with other programs or applications. The results of the calculations are provided as seasonal variations of discharge or concentration of any variable at one station (either at the outlet of the tributary of a specified stream-order for a

basin, or at a specified kilometric position for a river branch) or as the longitudinal variations of discharge or concentration along a river branch at a specified time. A cartographic representation of the variable (with an adjustable color code) over all basins and branches of the project at a given time period can also be produced. Furthermore, the possibility exists to automatically compare the calculation results with measured data when these are stored in the database and the results from several scenarios of the same project can also be compared simultaneously (Fig 2.9).

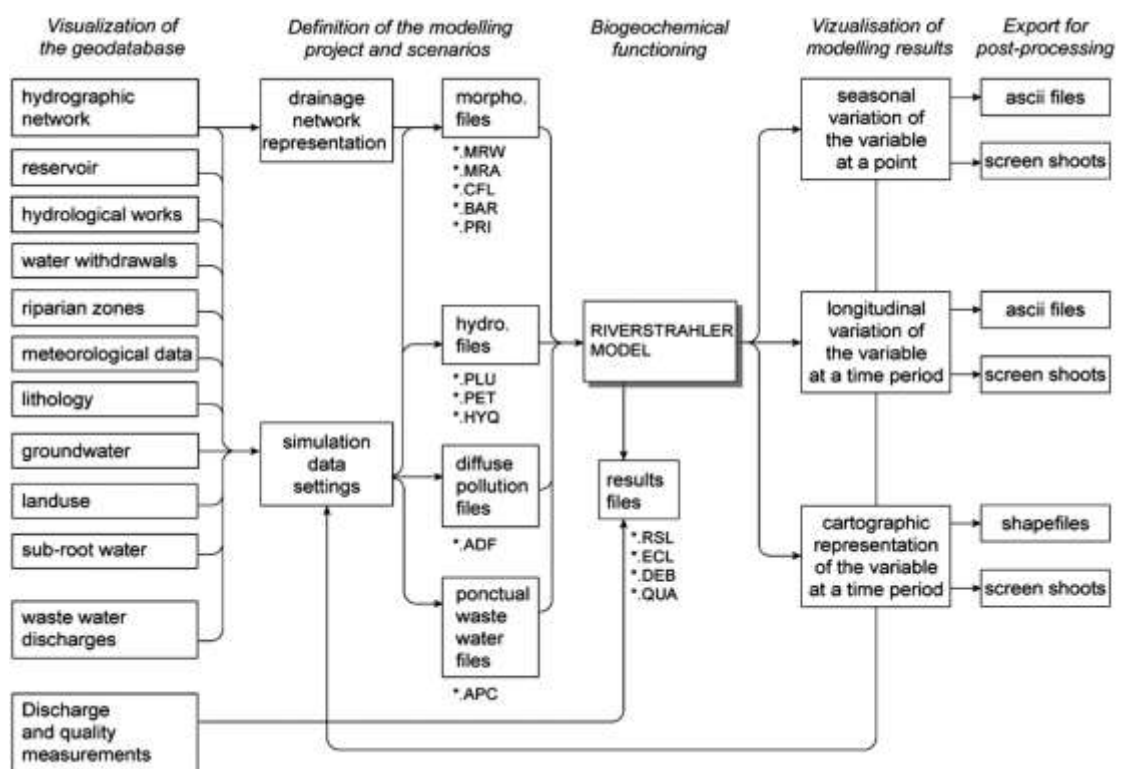


Figure. 2.9: Schematic representation of the functionalities of the GIS interface of the SENEQUE software (Ruelland *et al.*, 2007)

2.2.3.2 Spatial structure of the Red River as used in this thesis

For the purpose of modelling FIB and organic carbon in the lower tributaries of the Red River, where we have concentrated our measurements, the spatial resolution adopted for running the Riverstrahler model is rather coarse, with a focus on the course of the 3 main branches of the

river system (Da, Lo, Thao/Hong), and most of the remaining drainage network represented as idealized basins (Fig 2.10). The two major dams located on the Da and in the Lo basins are taken into account by considering the morphology (increased width and minimum depth) of the corresponding stretches of river. In this representation, our sampling stations correspond to the following kilometric point (pK) of the different branches as indicated in Table 2.6.

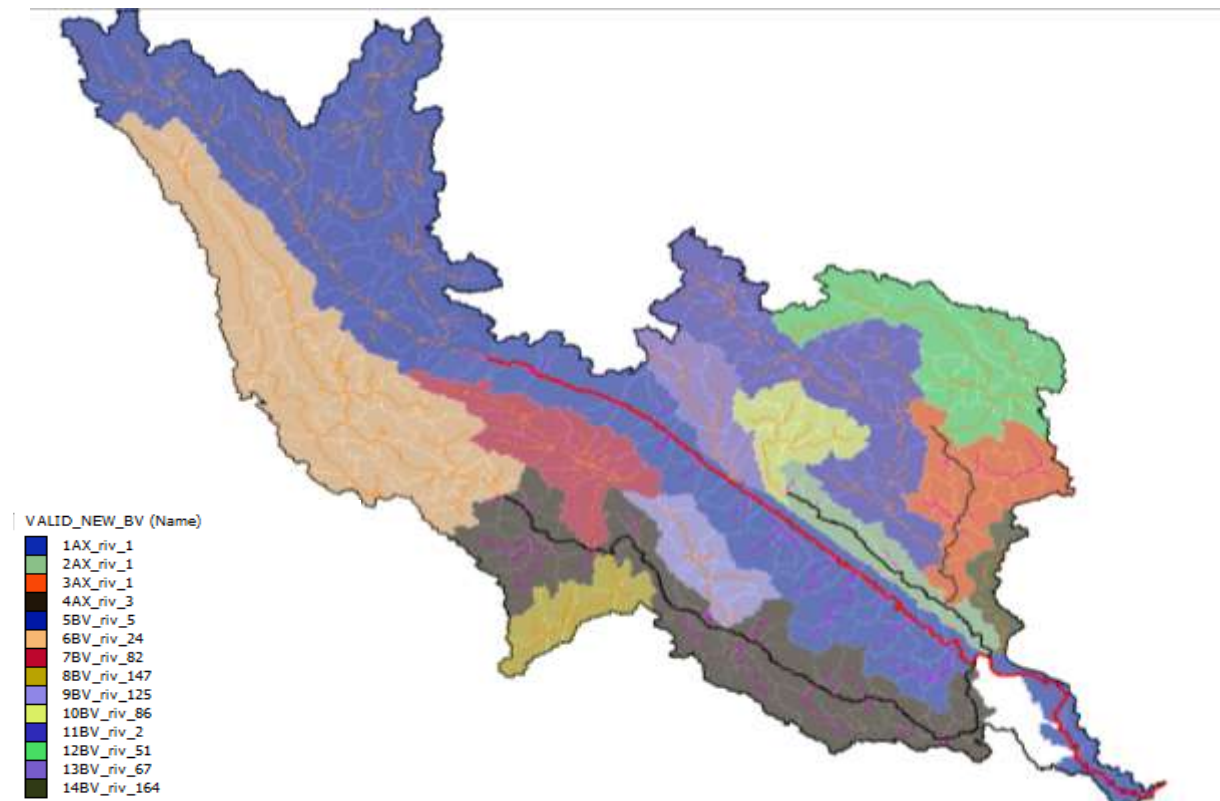


Figure 2.10: ‘Decoupage’ of the Red River drainage network as used for the modeling runs in this thesis.

Table 2.6: Position of the main sampling stations in terms of kilometric points (pK) on the river branches of the Red River system, as represented in the SENEQUE/Riverstrahler model.

Sampling station	pK	Branch name
Yen Bai	402	Thao River
Vu Quang	135	Lo River
Hoa Binh	434	Da River
Son Tay	475	Red River
Hanoi	524	Red River
Ba Lat	652	Red River

3 Faecal indicator bacteria

3.1 Faecal indicator bacteria

3.1.1 Introduction and definition

Microbiological contamination of water supplies is a globally important problem and poor water quality is a major brake on development. The World Health Organization (WHO) estimates that almost 2 million deaths annually are due to the consumption of contaminated water (WHO, 2012). River water subject to wastewater contamination is often used for washing of clothes and food utensils and for bathing and even cooking (Bain *et al.*, 2014). This is true for urban and peri-urban areas where population densities are high (Ashbolt, 2004; Bain *et al.*, 2014) as well as in rural areas where water supplies are often informal and therefore unregulated. In Asia over 40% of rural drinking water sources are contaminated as compared to only 12% in urban areas. Access to clean water is therefore a problem faced by both urban and rural populations in developing countries.

Considering the known risks associated with the consumption of sewage contaminated water, it is critical to identify the factors that control the persistence and dissemination of these microbial pathogens. By increasing the knowledge based on the dynamics of the water borne pathogens in tropical ecosystems we will be able to reduce the risks associated with the use of untreated water. Moreover, understanding the links between human activities, natural process and biogeochemical functioning and their ultimate impacts on human health are prerequisites for efficient water resources management. However, the data required to adequately understand and interpret these links is often missing or of poor quality in many developing countries (Rochelle-Newall *et al.*, 2015).

Developing countries are faced with a double problem. Often no adequate structures exist for long term monitoring of water borne pathogens in the environment due to economic

constraints and secondly, very little knowledge exists on the distribution of these microbes in tropical environments. In order to detect these waterborne pathogens at limited cost, Faecal Indicator Bacteria (FIB) are used as a proxy for pathogenic bacteria. The term FIB describes the range of bacteria that inhabit the gastrointestinal tract of homeothermic animals and includes *Escherichia coli* and the faecal coliforms, *Enterococcus* spp., all of which are found in faecal material.

Faecal coliforms (FC) and *E.coli* (EC) have been used as the standard indicator of recent fecal contamination in temperate regions (e.g. Servais et al., 2007; Ouattarra et al., 2013). In tropical ecosystems they have also been used to monitor contamination levels despite reports that free-living coliforms may be indigenous to some tropical waters and can't be distinguished from those from a fecal source (Carillo et al., 1985; Jiminez et al., 1989). Indeed, Rochelle-Newall et al (2015) noted that our ability to culture FIB (eg, *E.coli* and faecal coliforms) and the ability of these FIB to become naturalized in the environment are two of the most important factors for deciding whether or not we can estimate correctly FIB and the pathogens for which they are indicators. Ideally, methods that monitor faecal contamination without having to rely on culturing techniques are required. The use of specific biomarkers for fecal contamination such as stannols is one such option (Solecki et al., 2011; Jeanneau et al., 2012). However, while these methods have the advantage that they are culture-independent and species specific (chicken, pig or human); they have the disadvantage that they require considerable analytical technologies that are often lacking in developing countries (Rochelle-Newall et al., 2015).

Today, the most commonly measured bacterial indicators are Total coliforms, Faecal Coliforms, and *Escherichia coli*. Total coliforms are a group of bacteria that are widespread in nature, which are found in the soil, in many environments water, in human feces or animal manure, submerged wood and in other places outside the human body. Thus, the usefulness of

total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found (Willden, 2006). Fecal Coliforms are a form of total coliform bacteria, and as its name implies, it originates from fecal matter. *E. coli* is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. Leclerc et al. (2001) proposed that the use of *E. coli* is the best indicator of the presence of pathogenic bacteria and fecal contamination. In environmental waters, several studies have reported significant correlations between indicators of fecal pollution. For example, Donze (2004) reported that there was a significant positive relationship between total coliforms and *E. coli* ($r = 0.59$, $N = 30$, $p < 0.001$). Byamukama et al (2000), in a study from the Nakivubo channel, Uganda, that all microbiological parameters (total, fecal coliforms and *E. coli*) were significantly correlated. Wilkes et al (2009) also found, in a comparative study on the presence and concentration of several pathogenic and indicator bacteria in the surface water of a Canadian river, that significant correlations were found total coliforms and *E. coli*. It therefore seems that although it is preferable to use *E. coli* as an indicator of recent fecal contamination, given the correlations between the coliforms and *E. coli*, that in the absence of *E. coli* numbers, faecal and total coliform numbers can be used to indicate the presence of possible faecal contamination.

3.1.2 Primary sources of FIB

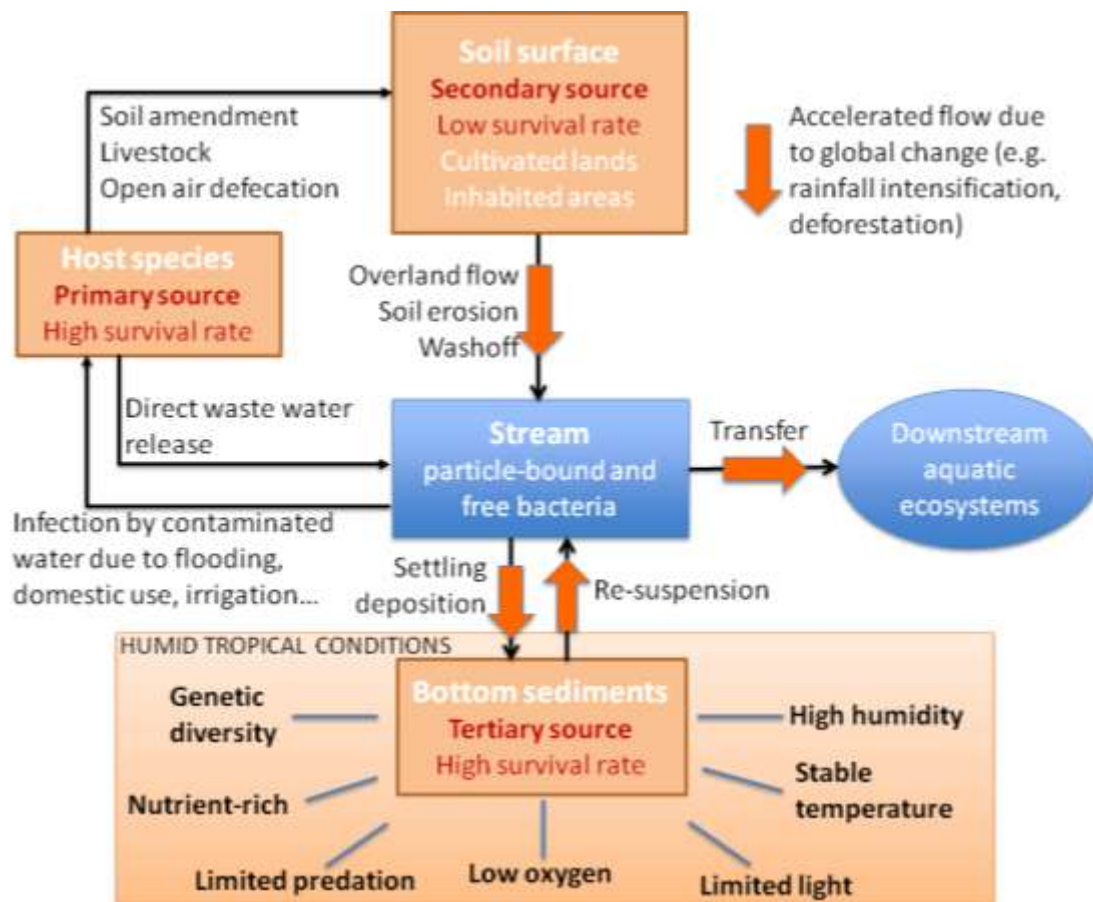


Figure. 3.1: Conceptual diagram of the factors influencing FIB in developing countries. From Rochelle-Newall et al., 2015.

The microbiological quality of rivers is primarily controlled by human and animal activities in the watershed. Humans, livestock and wild animals are all primary sources of faecal contamination (Fig. 3.1) although human faecal waste has the highest risk of waterborne disease, since the probability of human pathogens being present is highest. In urban areas, faecal microorganisms are mainly brought to aquatic environments through the discharge of treated and untreated domestic and industrial wastewaters (Servais *et al.*, 2007b). Point sources (outfall of

wastewater treatment or industrial plants and open sewer outlets) are often a major source of pollution in urbanized and industrialized catchments. Servais et al. (2007b) showed that in a large urbanized watershed (Seine, France), the input of fecal microorganisms from non-point sources is much lower than the inputs from point sources. These authors also examined the links between land use and FIB concentrations. They looked at surface runoff and leaching under three types of land use: forest areas, cultivated areas and grassland areas and found that small streams draining pastures were significantly more contaminated (around 1000 FC 100ml⁻¹) than those draining forests or cultivated areas (around 100 FC 100ml⁻¹).

In temperate regions with industrial scale agriculture, the microbial loading potential from point sources, such as storage facilities and feedlots, and from non-point sources, such as grazed pastures and rangelands, can be substantial (Table 3.1). Muirhead et al. (2005) in an experimental study of surface runoff from cowpats found *E. coli* concentrations of 1 x 10⁶ MPN 100ml⁻¹ and other workers have reported concentrations of up to an order more. High numbers of *E. coli* from animals and wildlife can reach surface waters in agricultural watersheds where direct excretion and runoff of fecal material from manure can enter waterways (Crowther *et al.*, 2002; Kloot, 2007; Vidon *et al.*, 2008a).

In developing countries and particularly in rural areas, agriculture is less intensive, wastewater treatment is often absent and non-point sources tend to predominate and the primary source of FIB is faecal matter generated by domestic and wild animals. For example, Ribolzi et al. (2011) and Causse et al. (2015), working in rural Laos, found that *E. coli* concentrations were below 1 MPN 100ml⁻¹ in the upper areas of the watershed indicating a very low background level of contamination that was probably caused by wildlife. However, as the density of poultry and humans settlements increased in the downstream areas, values of up to 230 MPN 100ml⁻¹ were found. Other non-agricultural sources of microbial pollution in rural watersheds include failing

septic systems and latrine overflows during periods of heavy rain, all of which can create health problems in the downstream human populations. However, as a consequence of their dispersion, these non-point sources of microbial pollution are inherently more difficult to identify and characterize than point sources.

Table 3.1: Faecal coliforms and *E. coli* numbers in some primary sources.

Bacteria	No.Bacteria/ 100ml	Source	Reference
Faecal coliform	10^7	Fresh cow pats	Thelin and Gifford (1983)
	10^5	30 days old cow pats	Kress & Gifford (1984) as cited in Muirhead et al. (2005)
	10^4	100 days old cow pats	
<i>E. coli</i>	10^7	From fresh cow pats	Muirhead et al. (2005)
	$10^3 - 10^7$	Sheep and beef	Collins et al. (2005)

3.1.3 Secondary sources of FIB

FIB and the water borne pathogens for which they are an indicator are particularly susceptible to shifts in hydrology and water quality (Vidon *et al.*, 2008b; Cho *et al.*, 2010; Chu *et al.*, 2011). Stormwater discharges are a major cause of rapid deterioration in surface water quality. Storm events increase turbidity, suspended solids, organic matter and faecal contamination in rivers and streams, although the microbiological quality of stormwater varies widely and reflects human activities in the watershed (Ribolzi *et al.*, 2011; Causse *et al.*, 2015;

Ekklesia *et al.*, 2015). Geldreich (1991) reported that stormwater in combined sewers can have more than 10-fold higher thermotolerant coliform levels than in separate stormwater sewers.

In rural areas and in urban areas without adequate wastewater treatment or stormflow management, one of the major pathways via which faecal contaminants enter waterways is via overland flow. Overland flow occurs when rainfall is unable to infiltrate the soil surface and runs over the ground, normally in rivulets. Overland flow is also the predominant means by which soil particles and faecal contamination in soils are transported from land to surface waters. The concentrations of FIB in overland flow are controlled by many factors such as rainfall duration and intensity, manure application, faecal deposit age and type, adsorption to soil particles, etc. (Blaustein *et al.*, 2015; Rochelle-Newall *et al.*, 2015).

Many authors have highlighted the low contribution of groundwater to FIB concentrations (Jamieson *et al.*, 2004). These low values are probably as a result of efficient soil filtering of microorganisms in infiltrating water (Matthess *et al.*, 1988), in contrast to overland flow characterized by high FIB concentrations. The values of only 4 *E.coli* 100 ml⁻¹ found in groundwater of a village in Laos is far lower than the reported values of 230 000 *E.coli* 100 ml⁻¹ in overland flow during a storm downstream of a small stream (Ribolzi *et al.*, 2011).

In stream sediments have also been identified as a reservoir for *E. coli*. Many studies indicate that sediments harbor much higher populations of both Faecal coliforms (FC) and *E. coli* than the overlying water (e.g. Rehmann and Soupir, 2009; Chu *et al.*, 2011; Pachepsky and Shelton, 2011). Mechanical disturbance of bottom sediments, as occurs during flood events can cause increased *E. coli* concentrations in the overlying waters as a result of their resuspension (Cho *et al.*, 2010). Muirhead *et al.* (2004), during an artificial flood experiment, observed that *E. coli* concentrations peaked ahead of the flow peak, consistent with the entrainment of FC into the water column from underlying contaminated sediments by accelerating currents on the rising

limb of the hydrograph. A two order of magnitude increase was observed during the event. *E.coli* concentrations were correlated with turbidity over the flood event, however, when turbidity returned to base levels between each flood, *E. coli* concentrations remained elevated. A similar dynamic was observed by Ribolzi et al. (2016a), who conducted a detailed examination of *E.coli* dynamics during a flood in an upland stream. By separating the groundwater and overland flow components of the flood they were able to identify the contribution of sediments to the total *E.coli* numbers in the stream. They showed that up to 75% of the *E.coli* in the stream were from the sediments and not from soil runoff from the sloping lands above the stream.

3.1.4 Fate in the aquatic continuum

The fate of FIB in the environment is controlled by the bacteria strain characteristics, the indigenous microbial community and by external environmental variables (Rochelle-Newall et al, 2015; Fig. 3.1). This latter group includes sunlight, nutrient and suspended solids concentrations, sedimentation and resuspension rates, water temperature, pH, predation, and organic matter (OM) concentrations. All of which influence the die-off rates and, potentially, the growth characteristics of FIB in the non-host environment.

Although FIB are considered to be enteric bacteria and therefore adapted to a nutrient and organic matter (OM) rich, low oxygen environment in their host there is some evidence that they can persist in the ecosystem and particularly so in tropical soil environments (Byappanahalli and Fujioka, 1998; Winfield and Groisman, 2003; Ishii *et al.*, 2006; Ishii and Sadowsky, 2008). When they are released from the host FIB are in an environment that is colder, more dilute and has higher oxygen concentrations and much lower OM concentrations. The OM chemical quality is probably also very different from that of the host. Fujioka and Byappanahalli (2001) have shown that FIB have the capacity to degrade a series of carbon sources found in soils.

Table 3.2: Summary of die-off rates for faecal bacteria in aquatic systems.

Bacteria	Die-off rate (per day, d⁻¹)	Environment	Reference
<i>E. coli</i>	14.7–107	Hong Kong coastal waters, under light-exposure	(Chan <i>et al.</i> , 2015)
	0.85 –1.50	Hong Kong coastal waters, in darkness	
	1.3 – 5.1	Freshwater, in-situ	
Faecal coliform <i>E. coli</i>	0.0048 0.0078	Manure ponds	Panhorst et al (2002)
Faecal coliform	0.43	Aquatic environments dark at 10 ⁰ C	Auer and Niehaus (1993)
	0.81	Aquatic environments dark at 35 ⁰ C	
	0.73	Natural aquatic conditions in the dark	
Faecal coliform	4.24	Reservoir, exposed to sunlight	Dewedar and Bahgat (1995)

Bouvy et al (2008) working in Senegal found that it was substrate concentration rather than temperature that controlled FIB persistence in a coastal system subject to high sewage inputs. Garzio-Hadzick et al. (2010) observed that the survival of *E.coli* was higher in soils with high OM contents and Topp et al (2003) found that survival was high in loamy soils and that this survival increased with the addition of manure. This is often the situation in developing countries where fresh manure is frequently used as an economical fertilizer option for both fields and in aquaculture (e.g. Yajima and Kurokura, 2008). It is therefore probable adequate concentrations of bioavailable carbon may contribute to FIB survival in tropical environments.

Light intensity has been identified as one of the most influential factors causing die-off of coliforms in freshwater (Sinton *et al.*, 2002; Chan *et al.*, 2015). Other studies have also reported

that the inactivation of *E.coli* is more rapid in saline waters than in freshwaters (e.g. Troussellier *et al.*, 2004). *E.coli* survive longer in turbid conditions (Alkan *et al.*, 1995) and the presence of suspended solids in the water column has been shown to increase *E.coli* survival rates by limiting the effects of sunlight (Milne *et al.*, 1986).

E.coli survive longer in cold temperatures than in warm temperatures in the environment (Medema *et al.*, 1997). Increased mortality rates at the higher temperatures may be due to damage to the bacterial cell components or due to increased predation (Sinton *et al.*, 2002). However, it is interesting to note that the effects of temperature have been reported to be less important in the presence of light for enteric bacteria (Alkan *et al.*, 1995), although why this might be is not clear. An important question often addressed in the literature is related to the differences in disappearance rates for different bacterial strains. For example, Menon *et al.* (2003) did not find a significant difference in the rates of disappearance of the strains tested (*E. coli*, *S. faecium*, and *S. typhimurium*). Sinton *et al.* (2002), on the other hand, reported a disappearance rate for *E.coli* that is four times higher compared to *Enterococcus* spp.. The die-off rate constant for *E. coli* and faecal streptococci in groundwater ranged from 0.16 to 0.36 day⁻¹ and from 0.03 to 0.23 day⁻¹, respectively (Gerba and Britton, 1984). However, most of the published values have focused on temperate systems and few have published die-off rates from tropical situations.

3.2 Seasonal variability of faecal indicator bacteria numbers and die-off rates in the Red River basin, North Viet Nam (Article 1)

This chapter is published in the journal “Scientific Reports” as:

Nguyen TMH, Le TPQ, Billen G, Garnier J, Janeau J-L, Rochelle-Newall E Seasonal variability of faecal indicator bacteria and die-off rates in the Red River, North Viet Nam. 2016. Scientific Reports: 6, 21644; doi: 10.1038/srep21644.

Author contributions: EJRN, QTPL, JG designed the experiments; HTMN, JLJ and QTPL carried out the work; EJRN, HTMN, QTPL, JG interpreted the results and HTMN and EJRN wrote the manuscript that was revised and improved by all the coauthors

3.2.1 Abstract

The Red River is the second largest river in Viet Nam and constitutes the main water source for a large percentage of the population of North Viet Nam. Here we present the results of an annual survey of *Escherichia coli* (EC) and Total Coliforms (TC) in the Red River basin, North Viet Nam. The objective of this work was to obtain information on faecal indicator bacteria (FIB) numbers over an annual cycle and, secondly, to determine the die-off rates of these bacterial indicators. Monthly observations at 10 stations from July 2013 - June 2014 showed that TC and EC reached as high as 39100 cfu (colony forming units, CFU) 100 ml⁻¹ and 15300 CFU 100 ml⁻¹, respectively. We observed a significant seasonal difference for TC ($p < 0.05$) with numbers being higher during the wet season. In contrast, no significant seasonal difference was found for EC. The FIB die-off rates ranged from 0.01 d⁻¹ to a maximum of 1.13 d⁻¹ for EC and from 0.17 d⁻¹ to 1.33 d⁻¹ for TC. Die-off rates were significantly higher for free bacteria than for total (free + particle attached) bacteria, suggesting that particle attachment provided a certain level of protection to FIB in this system.

3.2.2 Introduction

Biological contamination of aquatic systems by water borne pathogens from untreated wastewater and agricultural effluent is a globally important water quality problem (Ashbolt, 2004). However, it is particularly problematic in tropical regions where a large proportion of the developing world is located. On a global scale, it is estimated that 88% of diarrheal diseases are due to the use of unclean water sources, leading to the deaths of 1.8 million people annually, most of whom are children in developing countries (WHO, 2012). Indeed, in many developing countries, surface water (e.g. rivers and stagnant ponds) subject to wastewater contamination is often used for domestic purposes as access to uncontaminated water is limited (Bain *et al.*, 2014). Therefore, considering the high death rates as well as the large economic burden associated with the construction and maintenance of water treatment plants, having an understanding of the spatial distribution and temporal variability of the microbial pathogens responsible for these diseases is essential. Furthermore, understanding the factors that control their distribution is a prerequisite for reducing the human health risks associated with the use of unclean water. This is particularly important in tropical areas where there is a paucity of data, where population growth is high, and where populations are the most exposed to these contaminants (Ashbolt, 2004; Bain *et al.*, 2014).

Rivers are a major source of fresh water for irrigation, industry and domestic water requirements. However, many tropical rivers have been adversely affected by human activities, such as industrialization, urbanization and agricultural intensification (Broussard and Turner, 2009; Seitzinger *et al.*, 2010). Although the chemical contamination of water bodies has been documented in many tropical systems (Berg *et al.*, 2007; Navarro *et al.*, 2012), the extent of biological contamination from untreated wastewater and animal husbandry is often unknown.

This is despite the fact that detailed knowledge on the range and origin of microbial pollution is required for watershed management in order to provide safe water for human demands.

The Red River is the second largest river in Viet Nam, after the Mekong River, and one of the five largest rivers in East Asia (Vinh *et al.*, 2014). Over 24 million inhabitants live in the Red River basin, including over 17 million people in its delta. This area is also characterized by several large industrial zones and by a large number of craft villages that are considered as hotspots of biological and chemical contamination (Mahanty *et al.*, 2012). The Red River Delta (RRD) is the second most important rice-producing area in Viet Nam and accounts for 20% of the national production. It is also the main freshwater source for the surrounding areas as well as being the major outlet for wastewater (Luu *et al.*, 2010; Luu *et al.*, 2012). According to the Viet Nam Environment Administration Report 2012, the urban area of the RRD concentrates 24% of the national production of domestic wastewater. It also receives the second largest proportion of industrial wastewater in the country after that of the South East region around Ho Chi Minh City. Despite the high proportions of wastewater that are released into the Red River on a daily basis, little information exists in the published literature on microbial or faecal contamination levels in this semi-tropical region.

Faecal indicator bacteria (FIB) are used to monitor faecal contamination levels and hence the possibility of pathogens of faecal origin in soils and water in both tropical and temperate systems (Ishii and Sadowsky, 2008; Pachepsky and Shelton, 2011). FIB is a generic term for a range of bacteria that inhabit the gastrointestinal tract of homeothermic animals. This group includes *Escherichia coli*, *Salmonella spp.*, *Enterococcus spp.*, and the coliforms. We hypothesized that FIB numbers would increase along the river length as a consequence of the increasing industrialization and urbanization in the downstream sections. Here we present the results of an annual survey of FIB at ten stations along the Red River, North Viet Nam. The

objective of this work was to obtain information on FIB concentrations over one annual cycle and to identify the environmental factors controlling FIB numbers and to determine their die-off rates.

3.2.3 Materials and methods

3.2.3.1 Study site:

The Red River basin has an area of about 156 451 km² of which 51.2% is in Viet Nam, 47.9% in China and 0.9% in Laos (Le *et al.*, 2007). The basin is subject to a semi-tropical climate with two clear seasons. The wet season persists from May to October during which 80-90% of the total annual rainfall of 1900 mm occurs (Xuan, 2010). The cooler, dry season persists from November to April. Mean monthly temperatures are lowest in January, with June-August being generally the hottest. In general, temperature is relatively uniform across the basin and the mean relative humidity is greater than 80% (IMH, 1997-2004). Concomitant with the highest rainfall, discharge and suspended load peak during August in the middle of the wet season (Le *et al.*, 2007).

Samples were collected monthly from July 2013 to June 2014 at 10 stations (total of 120 samples) in the Red River Basin. The sample sites are located on different river branches (distributaries) of the Red River system and include, from upstream to downstream, Yen Bai (Thao River), Hoa Binh (Da River), Vu Quang (Lo River), Gian Khau (Day river), Truc Phuong (Ninh Co River), Quyet Chien (Tra Ly River), Nam Dinh (Dao River) and Son Tay, Ha Noi and Ba Lat on the main axis of the Red River (Table 3.3).

3.2.3.2 Sample collection

At each sample site, 1500 ml of river water was collected with a clean plastic bottle before storage in a cooler and return to the laboratory for processing. The sample was used to measure pH, conductivity, temperature, salinity, total suspended solids (TSS), total phosphorus (TP), dissolved inorganic phosphate (PO_4), ammonia-nitrogen ($\text{NH}_3\text{-N}$) and free and attached FIB.

Die-off rates

At four stations, a second series of samples was collected in the same way for the determination of FIB die-off rate over time. These stations were (1) Yen Bai, located in the upstream main branch of the Red River known as the Thao River; (2) Ha Noi, after the confluence of three major upstream tributaries of the Da, Thao and Lo rivers; (3) Gian Khau, a peri-urban river system located in the Red River Delta and, (4) Truc Phuong, located in the downstream Red River on the Ninh Co River. These four stations were chosen to give a good representation of the land uses and population densities in the basin and to provide a good geographical separation over the area. For each station, 750 ml of sample were incubated in duplicate in glass bottles at *in situ* temperature and in the dark for five days. For the estimation of die-off rates, samples were collected from the incubations every day during 5 days (T_0 , T_1 , T_2 , T_3 , T_4 , T_5) to determine the decrease in FIB numbers for both total and free bacteria using the method described below.

Table 3.3: Location and characteristics of the surrounding areas at the 10 stations. River depth (m) at the sampling site is also provided. All samples were collected from the surface layer as grab samples.

Station	Sampling location		River	Characteristics
	Latitude	Longitude		
Yen Bai	21°42'	104°53'	Thao River (upstream Red River)	Opposite a traditional brick factory that uses coal and mud and upstream of the agro-industrial processing zones of Van Yen (40km) and the Tran Yen urban district (14km). The sample was collected at 80 m from the bank. Water depth at this site is 5m.
Vu Quang	21°34'	105°15'	Lo River	Low population area. The sample was collected at 130m from the bank. Water depth is 17m.
Hoa Binh	20°49'	105°19'	Da River	Site is 5.5km upstream of the Hoa Binh hydroelectric dam. Sample was collected at 300 m from the bank. Water depth at this site is 10m and the river banks are formed of weathered rock.
Son Tay	21°09'	105°52'	Red River	Site is 300m upstream of the Son Tay coal ports. Sample was collected at 60 m from the bank, water depth 12m.
Ha Noi	21°02'	105°51'	Red River	Site is 50m downstream of the Chuong Duong bridge in Hanoi city. Sample was collected at 10m from the bank, water depth was 1m. This station is

downstream of the confluence of the Da, Thao and Lo Rivers.

Gian Khau	20°19'	105°55'	Day River	Site is 2km from the Gian Khau industrial zone and Visai coal clinker port. Sample was collected at 38m from the bank. This station is in a peri-urban area of the Red River delta.
Quyet Chien	20°30'	106°15'	Tra Ly River	Site is 2km upstream from poultry and fish farms. Sample was collected at 35m from the left bank, water depth was 7m.
Nam Dinh	20°25'	106°10'	Dao River	Site is opposite a factory that produces construction materials. Sample was collected at 100m from the left bank, water depth was 5m.
Truc Phuong	20°19'	106°16'	Ninh Co River	Site is 7km upstream of Nam Dinh city from which it receives sewage. Traditional silk spinning villages near the river release effluent containing silk chemicals and silkworm cocoon waste. Sample was collected at 100m from the bank.
Ba Lat	19°30'	106°00'	Red River	Site is 7km downstream of the Ba Lat seaport. Sample was collected at 300m from the bank, water depth was 8m. Site is under tidal influence.

3.2.3.3 Analytical methods

Temperature, pH, and total suspended solids (TSS) were measured using a water quality probe WQC-22A (TOA, Japan) and conductivity (Cond) was determined using a conductivity meter (Hach, USA) immediately upon sampling. Nutrients (N, P, Si) were spectrophotometrically determined on a Drell 2800 (HACH, USA) in the laboratory according to APHA (2012) methods.

FIB abundance (free and attached) was measured by a direct count method using 3M Petrifilm™ E.coli/Coliform Count Plate (Petrifilm EC plate), which contain Violet Red Bile (VRB) nutrients. *E.coli* (EC) produces beta-glucuronidase, which produces a blue precipitate and Total coliform (TC) colonies growing on the Petrifilm EC plate produce acid and the colonies are denoted by dark red points. This method has been validated by the APHA and is a technique commonly used for coliform and EC counts (APHA, 2001; Harmon *et al.*, 2014).

For the total counts (free + attached bacteria; EC_{tot} and TC_{tot}), 1ml was removed from each sample (or incubation) after shaking to ensure an even distribution of bacteria, the sample was then aseptically delivered to the center of a Petrifilm EC plate. The water sample was then left to stand for 1h and a second 1ml aliquot was inoculated onto a second Petrifilm EC plate to estimate the number of free EC (EC_{free} or TC_{free}; i.e. non-sedimented). The Petrifilm EC plates were then incubated in triplicate at 37° degrees for 24 hours, using a Fukusima incubator (Japan). The number of colonies (EC and TC) was determined using a Colony Counter CL-560 (Sibata, Japan). To facilitate the comparison of our data with that of previously published data and with water quality limits, we express our data as the number of colony forming units per 100 ml (CFU 100ml⁻¹) of sample.

The number of attached EC or TC (EC_{att} or TC_{att}) is determined from the difference between EC_{tot} (or TC_{tot}) and EC_{free} (or TC_{free}) as:

$$EC_{att} = (EC_{tot} - EC_{free}) \text{ or } TC_{att} = (TC_{tot} - TC_{free});$$

and the percent of attached EC or TC ($\%EC_{att}$ or $\%TC_{att}$) calculated as:

$$\%EC_{att} = [EC_{att}/EC_{tot}] * 100\% \text{ or } \%TC_{att} = [TC_{att}/TC_{tot}] * 100\%$$

The die-off rates of TC and EC were estimated by fitting an exponential equation to bacterial abundances measured over time. The equations were expressed as first order decay in the general form of:

$$C_t = C_0 * e^{(-kt)}$$

Where C_t = is the number of EC or TC at elapsed time t , C_0 is the initial number of EC or TC per ml, k is the decay constant in day^{-1} and t is the elapsed time in days. As in several incubations complete die-off was observed by day 4, k is calculated for the first 4 days for all incubations to ensure comparability between dates and stations. The k value was determined for both the free and total fractions (e.g. EC_{free} , TC_{free} , EC_{tot} , and TC_{tot}).

All statistical analyses were performed with XLSTAT (v. 2014). Pearson's correlation was used to test the relationships between environmental variables and FIB. Wilcoxon's non-parametric test was used to test for significant differences between variables and the Kruskal-Wallis test was used to test differences between stations and season as the data were non-normally distributed even after normalization. When statistical relationships concerning FIB number were tested, $\log(EC)$ or $\log(TC)$ was used. When a significant difference was observed, an *a posteriori* Dunn's all-pairwise test was used and significance is determined as $p < 0.05$.

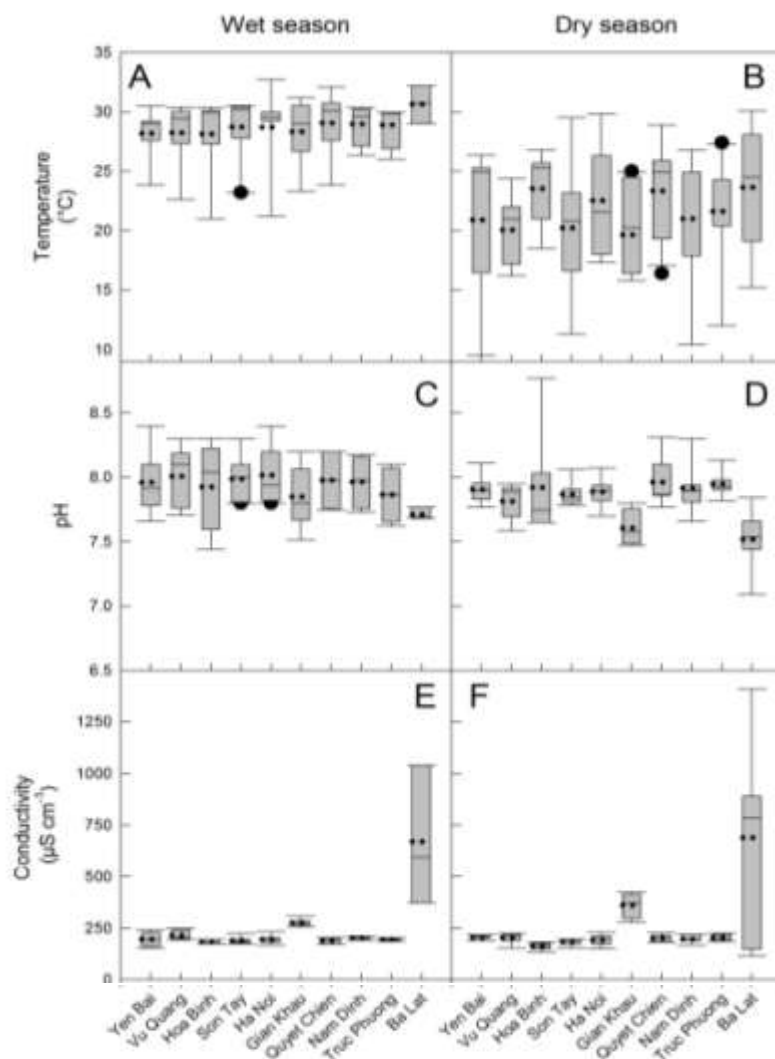


Figure 3.2: Box plots of temperature, pH and conductivity concentrations for each station for the wet (May to October) and the dry (November to April) seasons for the study period (July 2013 to June 2014). Left side panels are for the wet season and the right hand side panels are for the dry season. Panels A and B: Temperature, panels C and D: pH, panels E and F: conductivity. Mean (dotted line), median (solid line) and whiskers (error bars) above and below the box indicating the 90th and 10th percentiles are shown.

3.2.4 Results

3.2.4.1 Physico-chemical variables

The variability of the physico-chemical parameters observed in the ten stations for the wet and dry seasons are presented in Figures 3.2 and 3.3. Temperature varied between 9.5 and 35°C and was significantly higher during the wet season ($p < 0.0001$). Temperature was generally highest at Ba Lat and lowest at Gian Khau.

The pH ranged from 7.1 to 8.8, with the lowest values observed at the downstream Ba Lat station and the highest at upstream Hoa Binh station, however, no seasonal differences were observed. Salinity was almost 0 at all stations during the entire year; the only exception was during March at the most downstream station (Ba Lat, located at the river mouth and under tidal influence) where a salinity of 8.5 was observed (data not shown). The highest conductivities were also observed at this station at this time ($1410 \mu\text{Scm}^{-1}$). At the other stations, conductivity varied between $136\text{--}423 \mu\text{Scm}^{-1}$ (Fig. 3.2) and had no significant seasonal pattern.

Concentrations of NH_4 , PO_4 and TP are shown in Fig. 3.3. Ammonium varied from 0.01 to 0.6 mg N l^{-1} and was significantly higher during the dry season (November – April) at low dilution and in the downstream stations (Gian Khau, Nam Dinh, Truc Phuong and Ba Lat) of the highly populated delta area. Similar to NH_4 , the PO_4 tended to be higher during the dry season however the difference was not significant. TSS was significantly higher during the wet season, when particle load is higher. Over all, concentrations were generally lowest at the most downstream station Ba Lat in July and the highest at in the upstream Yen Bai station. As for TSS, TP was significantly higher during the wet season ($p < 0.012$). Interestingly, high concentrations of TP were found at Yen Bai and Gian Khau during the dry season (January) when values of up to 1.39 and $1.59 \text{ mg PO}_4 \text{ l}^{-1}$ were observed.

Table 3.4: Average (\pm se) die-off rates for EC_{tot} and EC_{free} and TC_{tot} and TC_{free} (k , d^{-1}) in the Red river basin. The values were calculated for the first 4 days of the incubation. Different capital letters indicate a significant difference between station and different lowercase letters indicate a significant difference between decay rates of EC_{tot} , EC_{free} , TC_{tot} and TC_{free} within the same station.

Decay rate (d^{-1})	Yen Bai			Ha Noi			Gian Khau			Truc Phuong		
	ave	se	r^2	ave	se	r^2	Ave	se	r^2	ave	se	r^2
k EC_{tot}	0.59Bab	0.31	0.22	0.31Aa	0.13	0.74	0.36Aab	0.06	0.68	0.60Ba	0.21	0.20
k EC_{free}	0.50BCa	0.28	0.72	0.25Aa	0.10	0.85	0.25Aba	0.09	0.71	0.54Ca	0.229	0.55
k TC_{tot}	0.85Bb	0.21	0.74	0.57ABab	0.21	0.70	0.47Abc	0.18	0.70	0.55Aa	0.35	0.73
k TC_{free}	0.77Aab	0.24	0.85	0.90Ab	0.13	0.65	0.79Ac	0.20	0.86	0.84Aa	0.21	0.74

3.2.4.2 FIB abundance: monthly observation

The EC_{tot} , and TC_{tot} numbers for each season at the ten different stations are shown in Fig. 3.4. The number of TC_{tot} varied between < 100 CFU $100ml^{-1}$ at Hoa Binh in January to over 39100 CFU $100ml^{-1}$ at Yen Bai in September. During the wet season, TC_{tot} at Yen Bai, the most upstream station, was 9783 ± 14431 (mean \pm standard deviation) CFU $100ml^{-1}$ as compared to 4383 ± 3797 CFU $100ml^{-1}$ for this station during the dry season. For the other upstream tributaries, relatively low mean TC_{tot} numbers were observed during the wet season (1850 ± 715 and 800 ± 789 CFU $100ml^{-1}$ for Hoa Binh and Vu Quang, respectively). The highest mean values were found in the downstream delta stations with 6066 ± 4506 , 5408 ± 5379 and 6050 ± 5469 CFU $100ml^{-1}$ for Nam Dinh, Truc Phuong and Ba Lat, respectively. The overall pattern was similar for the dry season, i.e. higher mean values at Yen Bai and in downstream delta stations such as the peri-urban Nam Dinh station where a seasonal average of 5016 ± 5840 CFU $100ml^{-1}$ was found. When the data from all of the stations were combined TC_{tot} exhibited a significant seasonal difference with lower numbers during the dry season as compared to the wet season (Fig. 3.4; $p= 0.042$).

As for TC_{tot} , low numbers of EC_{tot} were found in the upstream tributaries during wet season (283 ± 248 and 150 ± 234 CFU $100ml^{-1}$ at Vu Quang and Hoa Binh, respectively), and high numbers were observed at Yen Bai (3108 ± 5960 CFU $100ml^{-1}$). The more anthropogenically impacted downstream stations of the delta had high values during wet season (e.g. 1733 ± 1632 CFU $100ml^{-1}$ at Nam Dinh). During the dry season, a similar pattern was observed with high mean EC_{tot} numbers at Nam Dinh (2266 ± 3007 CFU $100ml^{-1}$) and low values in the upstream stations (167 ± 122 , 83 ± 75.7 CFU $100ml^{-1}$ for Vu Quang and Hoa Binh, respectively). Regarding the dataset as a whole, there was no significant difference between the wet and dry

seasons for EC_{tot} . EC_{tot} numbers were significantly lower than TC_{tot} ($p < 0.05$) during both seasons at all of the particle attached FIB.

The percentage of EC_{att} and TC_{att} varied greatly between stations (Fig. 3.5). For example, the percentage of EC_{att} varied between 9.7 % and 100 % with a mean (\pm se) of $46.5 \pm 2.9\%$. The percentage of TC_{att} was also highly variable (2.7 – 100 %) with a mean $34.8 \pm 1.8\%$ for the whole data set combined. The number of EC_{att} or TC_{att} was significantly lower than for EC_{free} or TC_{free} when the entire dataset was examined ($p < 0.05$).

The number of EC_{att} differed significantly between stations ($p < 0.05$) and was highest at Yen Bai (4900 CFU 100ml⁻¹) and lowest at Hoa Binh (100 CFU 100ml⁻¹), similar to what was observed for the total number (free + attached). As with EC_{att} , the number of TC_{att} differed significantly between stations. TC_{att} was highest at Yen Bai (13050 CFU 100ml⁻¹) and the lowest at Hoa Binh (< 100 CFU 100ml⁻¹). The numbers of free and attached TC and EC were positively correlated with each other and had correlation coefficients of 0.73 and 0.81, respectively. This indicates that attached and free TC and EC increased concomitantly even though the actual numbers were different.

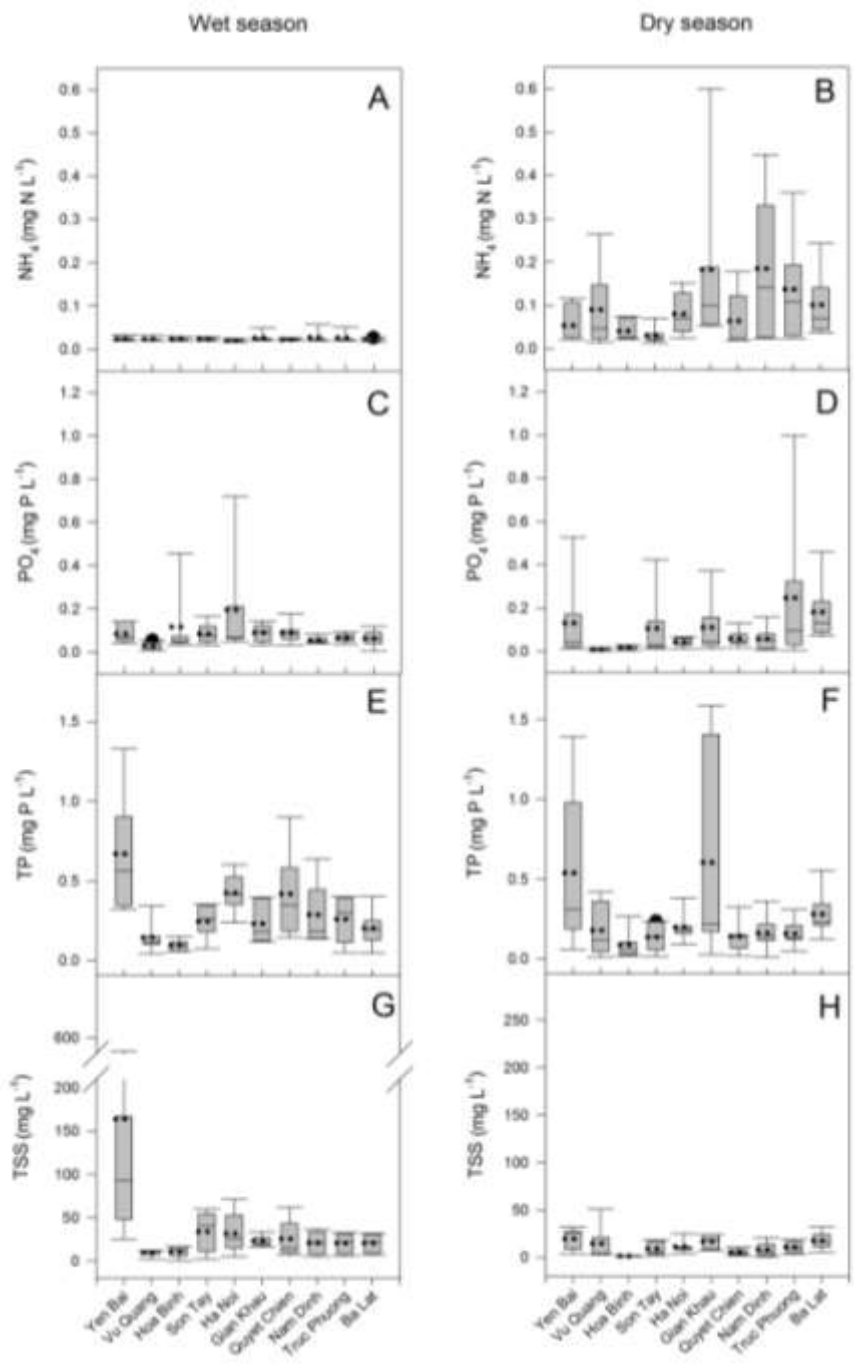


Figure 3.3: Box plots of NH_4 , PO_4 , TP and TSS concentrations for each station for the wet (May to October) and the dry (November to April) seasons for the study period (July 2013 to June 2014). Left side panels are for the wet season and the right hand side panels are for the dry season. Panels A and B: NH_4 , panels C and D: PO_4 , panels E and F: TP and panels G and H: TSS. Mean (dotted line), median (solid line) and whiskers (error bars) above and below the box indicating the 90th and 10th percentiles are shown.

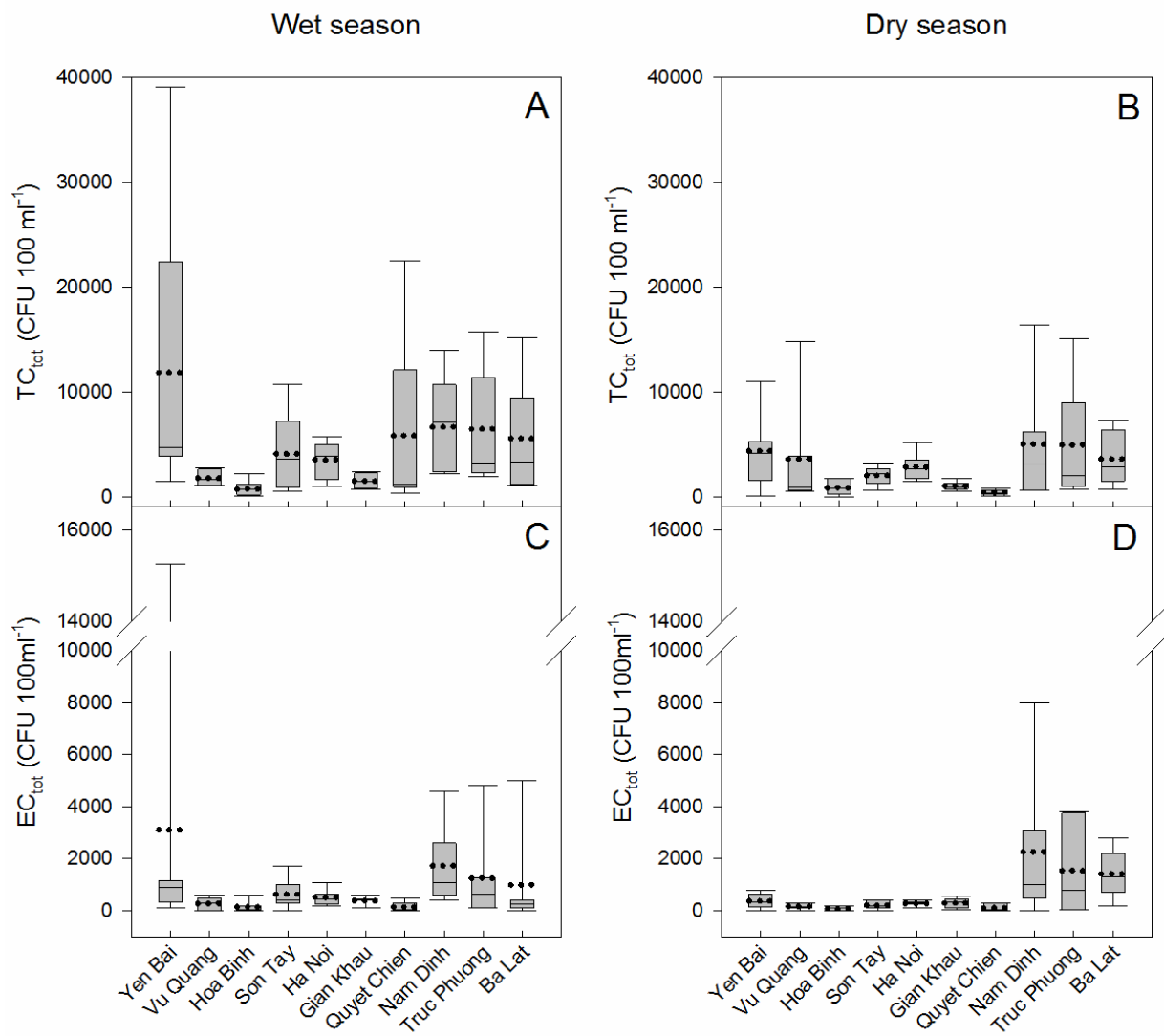


Figure 3.4: Box plots of the number colonies (CFU 100ml⁻¹) of TC_{tot} and EC_{tot} for the wet season (left hand side) and dry season (right hand side) for the ten stations. Panels A and B: TC_{tot}, panels C and D: EC_{tot}. Mean (dotted line), median (solid line) and whiskers (error bars) above and below the box indicating the 90th and 10th percentiles are shown.

3.2.4.3 Die-off rates

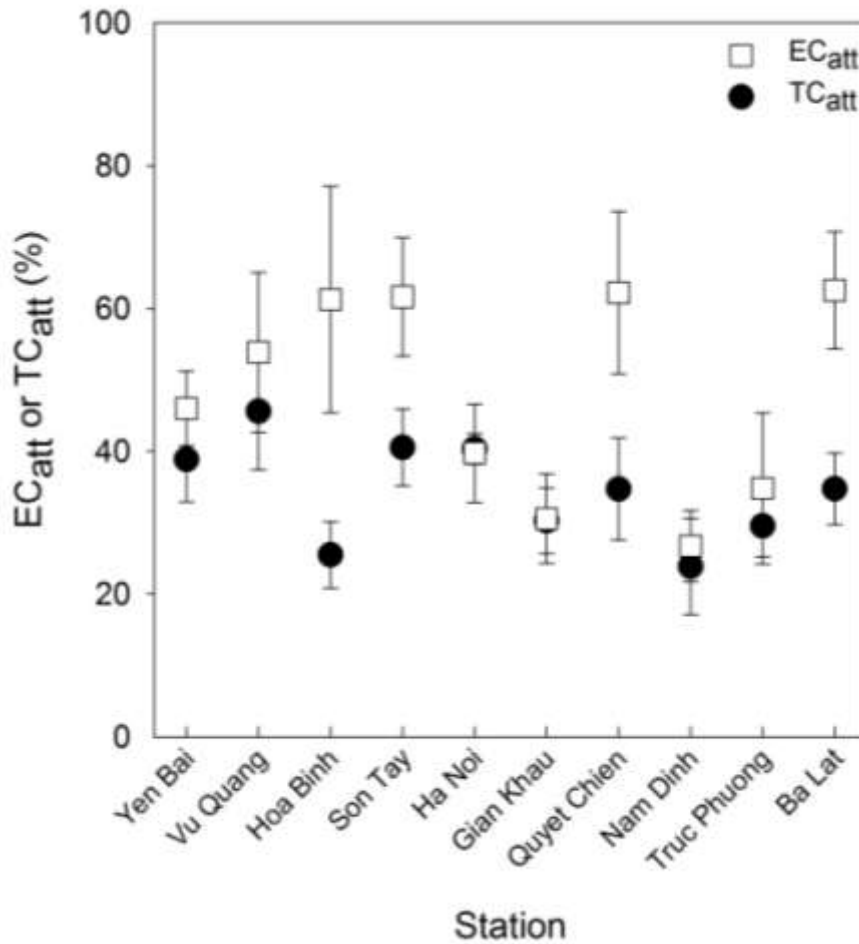


Figure 3.5: Percentage TC_{att} and EC_{att} for each of the 10 stations. The mean and standard error for each station are given. Filled circles: TC_{att}, open squares: EC_{att}.

The die-off rates of EC and TC calculated over 4 days are shown in Table 3.4. In general, die-off rates for TC_{tot} were significantly higher than for EC_{tot}. For example, at Yen Bai in August, the die-off rate for TC_{tot} was 1.33 d⁻¹ compared to 0.50 d⁻¹ for EC_{tot} and 1.03 d⁻¹ and 0.43 d⁻¹ TC_{tot} and EC_{tot} respectively, at Truc Phuong during February. It is interesting to mention again the specific behavior of FIB at Yen Bai. Along with the high TC and EC numbers at this station, TC_{tot} die-off rates were higher than at the other three stations examined e.g. 1.33 d⁻¹ as compared to 0.70 d⁻¹, 1.03 and 0.42 d⁻¹ for Hanoi, Truc Phuong and Gian Khau, respectively. Similarly, EC_{tot} die-off rates at Yen Bai were also significantly higher (p<0.05) than those

observed for the three other stations. However, and in contrast to TC_{free} , we observed a significant station effect for EC_{free} with the rates found at Yen Bai and Truc Phuong being significantly higher than those found at the two other stations, Hanoi and Gian Khau. Overall, die-off rates were significantly higher for TC_{free} than for TC_{tot} ($p < 0.05$) when the whole data set was examined. In contrast, the opposite was true for EC_{tot} and EC_{free} ($p < 0.05$).

3.2.4.4 Relationships between FIB and environmental factors

When the data set was regarded either as a whole or by station, EC and TC (free, attached and total) numbers were only positively correlated with TSS and TP ($p < 0.05$; Table 3.5). No other significant relationship was found between EC or TC and the measured environmental variables (pH, DO, NH_4 , conductivity, or PO_4).

As for TC_{tot} and EC_{tot} numbers, the die-off rates of both TC_{free} and EC_{free} and TC_{tot} and EC_{tot} were uncorrelated with temperature, DO, NH_4 or conductivity (Table 3.6). However, EC_{tot} die-off rate was significantly correlated with TP and TSS. No significant correlations were observed for TC_{tot} and TC_{free} and TSS.

Table 3.5: Pearson's correlation matrix for the environmental variables and FIB. The correlation was performed with the log(TC or EC) values.

Variables	EC _{free} log(CFU 100 ml ⁻¹)	EC _{att} log(CFU 100 ml ⁻¹)	EC _{tot} log(CFU 100 ml ⁻¹)	TC _{free} log(CFU 100 ml ⁻¹)	TC _{att} log(CFU 100 ml ⁻¹)	TC _{tot} log(CFU 100 ml ⁻¹)
pH	-0.112	-0.168	-0.170	-0.017	0.039	0.001
T (°C)	0.147	0.017	0.054	0.196	0.137	0.176
Cond (µs cm ⁻¹)	0.093	0.151	0.123	0.012	0.108	0.038
TSS (mg l ⁻¹)	0.238	0.224	0.216	0.247	0.236	0.293
NH ₄ (mg l ⁻¹)	-0.008	0.026	-0.016	0.100	-0.090	0.098
PO ₄ (mg l ⁻¹)	-0.081	-0.260	-0.169	-0.314	-0.011	-0.251
TP (mg l ⁻¹)	0.252	0.230	0.222	0.270	0.262	0.322

Values in bold indicate significance at $p < 0.05$.

3.2.5 Discussion

3.2.5.1 Distribution of FIB

Land use affects the abundance and distribution of FIB in both tropical (Crowther *et al.*, 2002; Causse *et al.*, 2015) and temperate regions (Boyer and Pasquarell, 1999; Isobe *et al.*, 2004). It is also probably the case in the Red River system. In general, mean FIB numbers (EC, TC) in the upstream stations (e.g. Vu Quang, Hoa Binh, Son Tay) were lower than the downstream stations (Nam Dinh, Truc Phuong and Ba Lat). Of the ten stations investigated, the upstream Hoa Binh station on the Da River had the lowest FIB levels. The Da River sub-basin is primarily forest (74% of total sub-basin area) and population density is sparse (<100 people per km²) and this probably explains the low FIB numbers at this station. In contrast, Yen Bai on the Thao River has the highest and most variable levels of contamination, despite being upstream. The Thao River basin has a relatively high population density (190 people km²) and a large proportion of the total sub-basin area (33%) is used for agriculture and livestock grazing. Livestock are known to be an important source of FIB contamination in streams and rivers (Crowther *et al.*, 2002) and this along with non-treated human wastewater from the surrounding population may well explain the high and variable FIB numbers observed at this site during both seasons. Furthermore, the Van Yen industrial zone which processes agricultural products is located in the upstream Thao River and this is probably also a source of FIB. Indeed, the high and variable FIB numbers observed during the wet season also suggest that FIB originated mostly from diffuse runoff sources.

The higher FIB abundances observed in the downstream stations (Nam Dinh, Ba Lat and Truc Phuong) are also probably related to the surrounding land use. These three sites are in peri-

urban areas of the delta with high population densities (1200 people km⁻² compared to 100-150 people km⁻² in the upstream basin(Le *et al.*, 2007)), agriculture and industry all of which release untreated wastewater into the system. Although the Hanoi station is located in the city and the FIB numbers are relatively low (Fig. 3.4), they are still above the acceptable limits for individual, non-commercial water supplies in Viet Nam (20 EC CFU 100 ml⁻¹ and 150 TC CFU 100 ml⁻¹) (MoH, 2009). Domestic wastewater from Hanoi city is mostly discharged into small urban rivers as shown by the high ammonium concentrations in the To Lich and Nhue Rivers (Luu *et al.*, 2010; Trinh *et al.*, 2012) and other work from the urban rivers in Hanoi city has reported very high TC numbers. For example, TC numbers at Lien Mac, at Phu Van Bridge (Nhue River) and at Thanh Liet Dam (To Lich river) were 7820, 9820 and 148480 MPN 100ml⁻¹, respectively (Cuong, 2012) meaning that the water at these locations is considered as being unfit even for irrigation due to its high FIB load.

3.2.5.2 Free and attached FIB

Understanding the relative numbers of attached and free FIB and their differential fate allows us to better estimate the time these indicator organisms remain viable in the environment. In soils and sediments, bacteria tend to be associated with particles as opposed to in the free-state (e.g. Oliver *et al.*, 2007) whereas in aquatic systems the percentages of particle associated bacteria are highly variable and can range from 10% in clear waters with very low organic particle loads to over 70% in estuaries with high particle loads (Crump *et al.*, 1998; Lemee *et al.*, 2002). Suter *et al.* (2011) have shown that high proportions of FIB are associated with particles (52.9% ± 20.9% and over 90% in some areas) and that, perhaps unsurprisingly, these values were related to turbidity levels.

In our work, free-living bacteria generally predominated (only $36\% \pm 20\%$ of TC were particle attached and $50\% \pm 26.9\%$ of EC particle attached). However, the $\%EC_{att}$ and $\%TC_{att}$ were highly variable (7.8% to 100% and 2.7% to 80% for EC_{att} and TC_{att} , respectively). Previous studies from temperate systems have indicated that the attachment to particles by FIB in the aquatic environment is influenced by various factors, including temperature, bacterial genotype, soil particle size, organic matter, pH, ionic strength, dissolved nutrients and turbidity (Pachepsky *et al.*, 2006; Garcia-Armisen and Servais, 2009). We also found significant correlations among TC_{att} , EC_{att} , TSS and TP. In one of the few articles investigating the factors controlling FIB concentrations in the tropics, Byamukama *et al.* (2005) working in Uganda, also found that FIB were correlated with TSS concentrations as we show here. The Red River system has high turbidity levels (Le *et al.*, 2007; Vinh *et al.*, 2014) and this may explain the relatively high $\%EC_{att}$ and $\%TC_{att}$ observed in this system. Moreover, attachment to particles probably plays a strong role in controlling the transport of FIB in the system as well (Ribolzi *et al.*, 2016a). Particles in aquatic systems are often associated with nutrients and particulate carbon and phosphorus in particular, both of which can be limiting substrates for bacterial growth and in turn, can affect FIB survival in non-host environments (Rochelle-Newall *et al.*, 2015) and in systems with high particle loads, the TP pool is generally dominated by the particulate fraction, as is the case here. This, along with higher carbon concentrations in the particles probably confers a competitive advantage to the bacteria thereby enhancing their survival. However, for the moment, little other information exists on the proportions of attached and free FIB in other tropical water bodies and so it is difficult to compare our results with other tropical ecosystems. Nevertheless it also appears that TSS and TP plays an important role in determining the proportions of attached and free FIB in this highly turbid, tropical riverine system.

3.2.5.3 Die-off rates

Schumacher (2003) in an in-stream incubation in the Upper Shoal Creek Basin, Southwestern Missouri, found that fecal coliform and EC densities decreased more than 90 % from initial densities over a 42h period. Troussellier et al. (2004) also reported rapid die-offs with over 90% of FIB lost over a 128h period. We also observed a rapid decrease in EC and TC over the 5 days incubation, with in many cases up 100% loss after 120h. We therefore chose to calculate the decay constants over the first 4 days. Our TC and EC die-off rate decay constants (k) ranged from a minimum of 0.01 d^{-1} to a maximum of 1.13 d^{-1} for EC and from 0.17 d^{-1} to 1.33 d^{-1} for TC, with a mean of $0.36 \pm 0.21 \text{ d}^{-1}$ for EC_{free} and $0.44 \pm 0.23 \text{ d}^{-1}$ for EC_{tot} and $0.83 \pm 0.19 \text{ d}^{-1}$ for TC_{free} and $0.61 \pm 0.28 \text{ d}^{-1}$ for TC_{tot} . These rates are similar to other studies from temperate aquatic environments. For example, Menon et al.(2003), working in the Seine river, France observed die-off rates of 0.19 to $0.82 \times 10^{-3} \text{ h}^{-1}$ for EC and Blaustein et al. (2013), in a review on EC survival in a range of temperate aquatic environments reported an average rate of $0.725 \pm 0.078 \text{ d}^{-1}$. In one of the few articles dealing with die-off rates in sub-tropical systems, Chan et al (2015) also found values of between 0.85 to 1.50 d^{-1} for the coastal water around Hong Kong, similar to the values we report in this work.

Table 3.6: Pearson's correlation matrix for the environmental variables and $k(d^{-1})$ for the free and total (attached + free) TC and EC. The $k(d^{-1})$ was determined over 4 days.

Variables	EC _{tot} die-off k (d ⁻¹)	EC _{free} die-off k (d ⁻¹)	TC _{tot} die-off k (d ⁻¹)	TC _{free} die-off k (d ⁻¹)
pH	0.078	0.078	0.043	0.096
T (°C)	0.079	0.070	0.046	0.256
DO (mg l ⁻¹)	0.181	0.160	0.014	0.082
Cond (µs cm ⁻¹)	-0.060	-0.213	-0.158	-0.086
TSS (mg l ⁻¹)	0.405	0.213	0.195	0.025
NH ₄ (mg l ⁻¹)	-0.024	-0.072	0.208	-0.208
PO ₄ (mg l ⁻¹)	-0.130	-0.123	-0.112	0.065
TP (mg l ⁻¹)	0.424	0.223	0.272	-0.059

Values in bold indicate significance at $p < 0.05$.

We observed higher die-off rates for TC_{free} than for TC_{tot} , however, this was not the case for EC, where die-off was significantly higher for EC_{tot} than for EC_{free} . Some authors (Davies *et al.*, 1995; Craig *et al.*, 2004) have shown lower decay rates for fecal bacteria attached to sediments as compared to free fecal bacteria, as we show here. Attachment to particles may protect FIB against grazing by small heterotrophic nanoflagellates (the main grazers of bacteria in aquatic systems) as well as providing a micro-environment rich in nutrients. Although, according to Sinton *et al.* (2002) and Chan *et al.* (2015), exposure to solar radiation and predation are among the most important factors controlling die-off, although it seems from our work that attachment to particles can also be an important factor at least for TC.

We observed systematically higher die-off rates for TC_{tot} than for EC_{tot} . Why this might be the case in this system merits some reflection. The appropriateness of using TC and EC as indicators of faecal contamination in tropical systems has been questioned by several authors (Nshimiyimana *et al.*, 2014). Indeed, it has been shown that *E.coli* may be able to persist and even proliferate for some time in tropical freshwaters (Carillo *et al.*, 1985; Jiminez *et al.*, 1989; Winfield and Groisman, 2003) particularly in those with high temperatures and elevated nutrient and organic matter concentrations. This may well explain the differences in die-off rates between EC and TC. It may also be why die-off in EC_{free} was lower than that for EC_{att} . Although the technique used in this work allowed us to determine the presence or absence of TC or EC, it provides no information on the sources of the TC or EC. Therefore, if the FIB are from different origins with different levels of adaptation to the environment, then this may explain some of the differences between the die-off rates of free and attached TC and EC. Nevertheless, this clearly is a hypothesis that merits further investigation *in situ* using some of the newer microbial source tracking techniques (Rochelle-Newall *et al.*, 2015).

Many studies have indicated that seasonal variations can influence FIB die-off rates through modifications of temperature, pH, nutrient concentration, and dissolved oxygen (Burton *et al.*, 1987; Flint, 1987). In this study, die-off rates were determined over the whole year with temperatures varying between 9.5 and 35 °C and we did not observe any significant relationship with *in situ* temperature. Moreover, we only observed a significant correlation between temperature and TC_{free} in contrast to what has been found by other investigators (Crowther *et al.*, 2001; Isobe *et al.*, 2004). We did, however, find significant differences in FIB numbers and die-offs between the wet and dry seasons, emphasizing the dominant role of the hydrology. Why we did not find a clear temperature relationship for FIB number or die-off rate is not clear from the data, however, it is probably related to other environmental and source related factors not investigated in this work.

3.2.6 Conclusions

There have been many studies on the survival of EC and other indicators of faecal pollution in freshwater, marine and estuarine habitats in temperate ecosystems however less information exists for tropical aquatic environments. Crane and Moore (1986) observed that the identification of relationships between environmental and physical parameters and FIB survival was a fundamental topic for future research in the field. Although their work dates, and much work has been published since on temperate ecosystems, it is still the case that very little information is available on die-off rates in tropical and sub-tropical ecosystems where the management of FIB concentration is most needed and where the risks to human populations are highest (Rochelle-Newall *et al.*, 2015). In our work from the sub-tropical Red River system in Viet Nam, we found that FIB numbers exceed Viet Nameese water quality guidelines of 20 and 150 CFU 100ml⁻¹ for EC and TC, respectively, throughout the whole

year at almost all of the 10 stations investigated. Moreover, many values exceeded 500 colonies 100 ml^{-1} above which the World Health Organization considers that there is a 10% risk of gastro-intestinal illness after one single exposure. Therefore, the use of water from sites with high FIB numbers such as those in the downstream sites pose a real risk to public health. We also found a significant correlation between particles (as TSS or TP) and FIB as has been observed in temperate riverine ecosystems. However, in contrast to other studies we did not find a significant correlation between FIB and temperature for this subtropical environment, but instead a correlation with discharge (wet vs. dry season). Indeed, the highest TSS concentrations and FIB numbers were found during the wet season at high discharge. It is therefore probable that in developing countries where sanitation facilities are deficient and where people and their livestock live in close proximity to the river, FIB contamination mostly originates from diffuse sources (Causse *et al.*, 2015). Nevertheless, the data presented here, notably that of FIB numbers and their respective die-off rates, provides a base for the application of a model that will allow the parameterization of FIB dynamics in Red River and delta and potentially in other large river basins of the sub-tropical and tropical belt.

3.3 Modeling of Faecal Indicator Bacteria (FIB) in the Red River basin, Viet Nam (Article 2):

This chapter is in submission to the “Environmental Monitoring and Assessment” as:

Huong Thi Mai Nguyen, Gilles Billen, Josette Garnier, Emma Rochelle-Newall, Olivier Ribolzi, Quynh Thi Phuong Le. Modeling of Faecal Indicator Bacteria (FIB) in the Red River basin (Viet Nam).

Author contributions: GB, JG, QTPL designed the experiments; HTMN carried out the work; HTMN, GB, JG interpreted the results and HTMN wrote the manuscript that was revised and improved by all the coauthors.

Keywords: sub-tropical watershed modeling, future scenarios, water quality, faecal coliforms, point and non-point sources

3.3.1 Abstract

Many studies have been published on the use of models to assess water quality through fecal contamination levels. However, the vast majority of this work has been conducted in developed countries and similar studies from developing countries in tropical regions are lacking. Here we use the Seneque/Riverstrahler model to investigate the dynamics and seasonal distribution of FIB in the Red River (Northern Viet Nam) and its upstream tributaries. The results of the model show that, in general, the overall correlations between the simulated and observed values of fecal indicator bacteria (FIB) follow a 1: 1 relationship at all examined stations. They also show that FIB numbers are affected by both land use in terms of human and livestock populations and by hydrology (river discharge). We also developed a possible scenario based on the predicted changes in future demographics and land use in the Red River system for the 2050 horizon. Interestingly, the results show only a limited increase of FIB numbers compared with the present situation at all stations, especially in the upstream Vu Quang station and in the urban Ha Noi station. This is probably due to the dominance of diffuse sources of contamination relative to point sources.

3.3.2 Introduction

Access to clean water is one of the primordial necessities for humans. Water used for drinking, washing, and recreation needs to be free from biological, chemical, and physical contaminations in order to protect human health (Palaniappan *et al.*, 2010). However, poor water quality continues to pose a significant threat to human health worldwide. Over twenty years ago Tebbutt (1992) reported that in tropical regions some of the most common human diseases are due to the use and consumption of contaminated water, and this is still the case. Inadequate water, sanitation and hygiene are estimated to be responsible for 842,000 deaths every year, including the death of 361,000 children under five years of age, many of which occur in developing countries (WHO, 2014).

Contamination of surface water by faecal matter is the major mechanism by which pathogens attain high concentrations in aquatic systems. Faecal contaminants originate from many different sources, including wastewater from sewage treatment plants, industrial parks and residential areas. Animal husbandry and runoff from grazing pastures are also important sources (Crowther *et al.*, 2002), particularly when the animals have unrestricted access to waterways (Collins *et al.*, 2005; Wilcock *et al.*, 2006). In rural areas in developing countries wash-off from outside latrines during rain events may also be an important source of faecal contamination to streams (Causse *et al.*, 2015).

Fecal indicator bacteria (FIB) are used as a measurement of the sanitary quality of water that is used for domestic, industrial, agriculture and leisure activities. The term FIB is used to describe the bacterial community that is released in faecal matter of homeothermic animals (e.g. humans and other mammals). The group includes *Escherichia coli* and coliforms, *Salmonella spp.*, *Enterococcus spp.*, all of which are used as a proxy for detecting the presence

of other pathogenic bacteria in environmental samples such as soil and water (Ishii and Sadowsky, 2008; Byappanahalli *et al.*, 2012).

Although the initial presence of these bacteria in water is due to faecal contamination, their survival and transfer varies as a function of environmental influences such as sunlight, temperature, competition with other bacteria, predation, and other pollutants such as agrochemicals (Chan *et al.*, 2015; Rochelle-Newall *et al.*, 2015). Moreover, mobilization and transfer of FIB and other pathogens in an ecosystem is strongly affected by hydrology, soil characteristics and land use in catchments (Chu *et al.*, 2011; Ribolzi *et al.*, 2016a; Ribolzi *et al.*, 2016b).

Given the strong links between hydrology and the transfer of FIB in aquatic systems, there is a growing demand for modeling methods that allow the assessment of water quality. A variety of models have been used to predict the distribution of FIB in streams and rivers. Generally, they are divided into two types: the first uses a multivariate linear regression model approach to create a link between the input variables (meteorological, hydrological, physical chemistry, land use) and the output of FIB concentrations to predict water quality (e.g. Eleria and Vogel, 2005; Frick *et al.*, 2008; Nevers and Whitman, 2011; Tilburg *et al.*, 2015). This type of model has to be calibrated on a large set of observations and they lose their predictive capacity outside the range of conditions covered by the calibration situations. The second type are mechanistic (or process-based) models (e.g. Wilkinson *et al.*, 1995; Brauwere *et al.*, 2014). They tend to describe the dynamics of FIB within the environment based on the detailed kinetics of the processes involved. Observation data are used for validating such models (or assessing their degree of accuracy), however, they are usually not used to calibrate the kinetic parameters, which are separately determined. These models have therefore a much higher capacity to predict the impact of potential future changes in land use and human activities in the watershed on water quality and FIB concentrations.

The Senéque/Riverstrahler model is of this second type. It was developed for the study of ecological function and nutrient transfer in large drainage networks in France (Billen *et al.*, 2007), central Europe (Garnier *et al.*, 2002) and in France (Servais *et al.*, 2007a). Le *et al.* (2010) applied the model to the Red River, North Viet Nam and showed how land use affects the hydrology and transport of carbon, nitrogen and phosphorus in the basin. Recently, the model has been adapted to investigate the transport of FIB in Belgium (Ouattara *et al.*, 2013) and in Laos (Causse *et al.*, 2015). Indeed, this latter article is one of the few that discusses the modeling of FIB in developing countries.

Recently, Nguyen *et al.* (2016) showed that concentrations of FIB in the Red River (Viet Nam) varied between 0 and 39100 colonies 100ml⁻¹, with significantly higher values found during the wet season as opposed to the dry season. Throughout the year the FIB numbers were above the acceptable limits for individual, non-commercial water supplies in Viet Nam of 20 *E.coli* colonies 100 ml⁻¹ and 150 total coliforms colonies 100 ml⁻¹ (MoH, 2009) at most of the 10 stations examined. Here we use the latest version of the FIB dynamics module developed by Servais *et al.*, (2007b) and Ouattara *et al.*, (2013) to model the distribution and seasonal dynamics of FIB in the main branch and upstream tributaries of the Red River. To do this we supplied the model with data on the possible factors driving the input and transfer of FIB in the Red River flowpath through agricultural and rural areas as well as across densely populated conurbations (e.g., Ha Noi) where domestic effluents are only partially treated. We then compared the results with monthly field measurements collected over a three years period from January 2012 to December 2014. After validation of the model, we tested a scenario based on predicted future changes in demographics and land use in order to evaluate their impact on FIB concentrations in the Red River system in 2050.

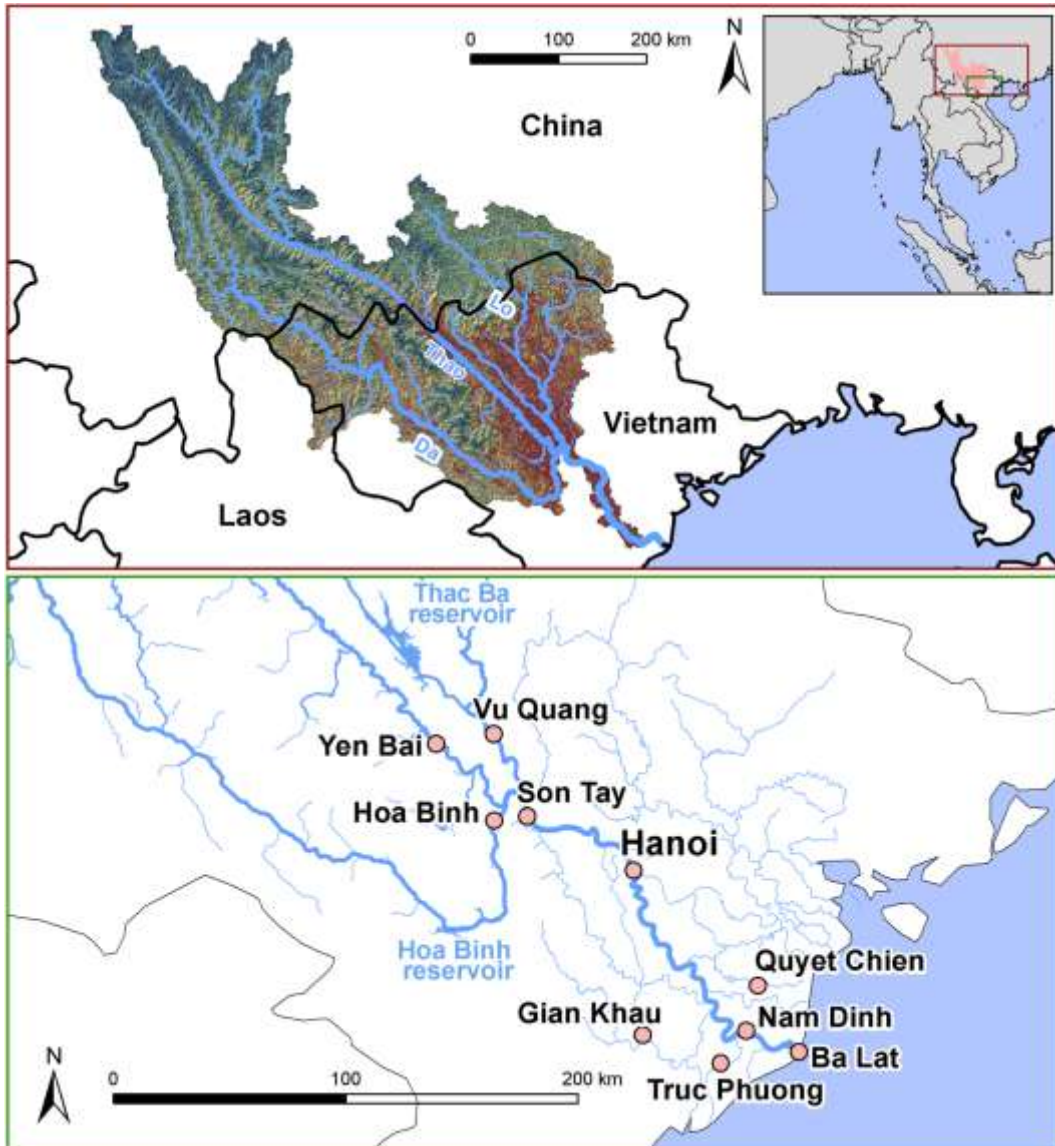


Figure 3.6: Map of the Red river and localization of the main hydrological and water quality stations.

3.3.3 Material and methods

3.3.3.1 Study area

The Red River (RR; in Viet Nameese: *Đồng bằng sông Hồng*, or *Châu thổ sông Hồng*) is trans-national and the catchment covers parts of China, Viet Nam and Laos (Fig. 3.6). The Red

River Delta itself (RRD) is located in the sub-tropical monsoon zone of the western coastal Gulf of Tonkin. The Northeast monsoon occurs from November to late April and dryer, colder weather dominates. From May to October the Southwest monsoon occurs and warmer, wetter weather dominates. The area is subject to high rainfall that varies from about 1200 – 4800 mm per year with an average of 1900 mm, of which 80% falls from May to October (Xuan, 2010). From July to October, the coastal areas of the RRD are affected by low pressure systems of varying intensity from the South China Sea that bring tropical storms and typhoons. The coldest month is January, when temperatures can reach 10°C.

The Red River itself is the second largest river system in Viet Nam with an average rate of flow of 3389 m³s⁻¹ (for the 7 years period 1997 – 2004 as quoted by Le *et al.*, 2007). It is formed by three large river branches (Da, Lo and Thao Rivers) and enters into the sea at Ba Lat. The Da River has the highest discharge (about 42% of the total) and Thao River has the lowest with only about 19 % (Le *et al.*, 2007). Discharge of the Lo River is intermediate (25.4 %), however, this river has the smallest catchment of the three. Peak flow occurs during July (32% frequency), August (55%), September (7.3%), and October (4.4%) (Kok, 2006).

The RRD has a very high population density (average population density is over 1,250 people per km²) and industry and agriculture are the dominant economic activities. In the upstream section, Yen Bai province has 19 industrial and craft production zones and the Hoa Binh province has 8 economic and industrial zones. In the downstream part (e.g., Viet Tri, Ha Noi, Hai Phong and Nam Dinh), there are many small and large industrial zones and urbanisation is increasing. The delta region also hosts other important economic activities such as fisheries, aquaculture, land reclamation for agriculture, harbour construction mangrove forestry all of which can negatively affect water quality (Navarro *et al.*, 2012; Trinh *et al.*, 2012; Chu *et al.*, 2014).

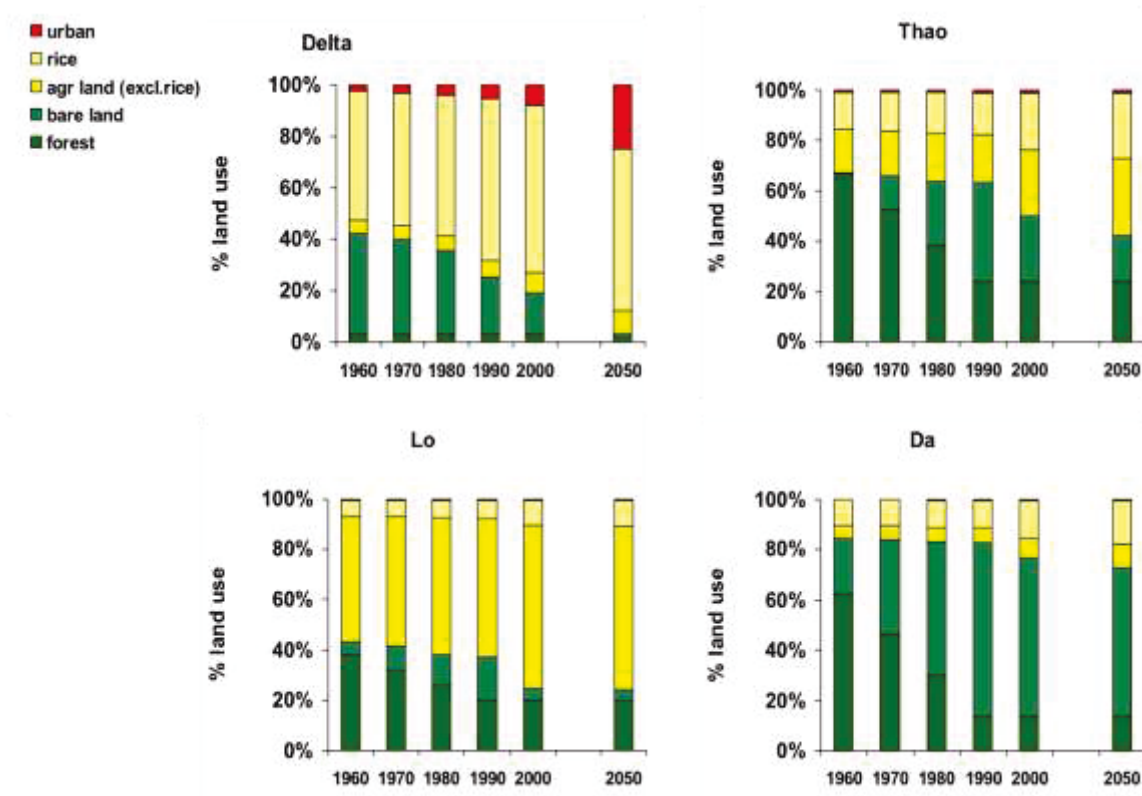


Figure 3.7: Trends of land use changes in the 4 major sub-basins of the Red River (compilation by Le et al (2010) of data from DeFries et al., (1998); De Koninck et al. (1998); Hansen et al (2000); GSO (1997); Poffenberger et al. (1998)).

3.3.3.2 Model description

The water quality and ecological functions of the basin that are affected by climate and human activities were calculated using the Seneque/Riverstrahler model (Billen *et al.*, 1994; Garnier *et al.*, 2002). In the model, water flows are calculated for each sub-basin from the sum of specific surface runoff and base flow from groundwater. These two components of runoff are determined from daily measurements of discharge at 5 hydrometric stations (see Fig. 3.6) using the recursive filter of Eckhardt (2005, 2008).

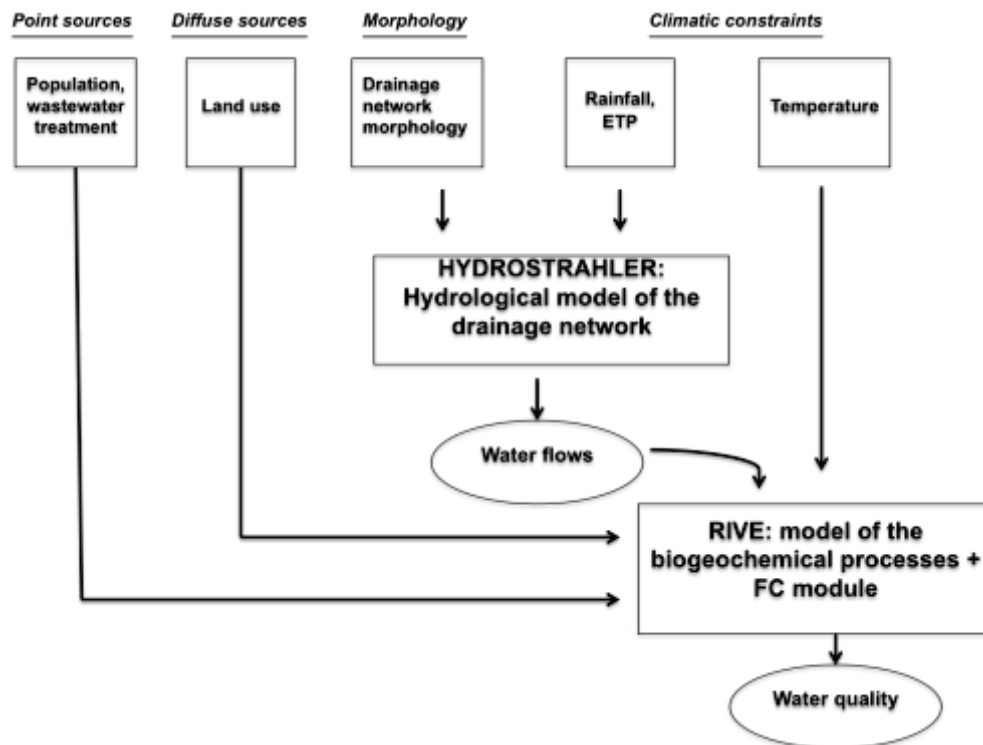


Figure 3.8: Schematic description of the Seneque/riverstrahler model including the module of the dynamics of fecal bacteria (Servais *et al.*, 2007a).

Seneque/Riverstrahler includes a module describing the dynamics of FIB (Servais *et al.*, 2007a; Ouattara *et al.*, 2013). Three classes of faecal bacteria are considered: free bacteria, bacteria attached to suspended solids and bacteria in the sediments. Each of the three stocks is affected by a mortality rate and attached bacteria can settle to the sediment. The release of FC by WWTPs effluents (Table 3.7) and the diffuse sources are calculated on the basis of land use (Table 3.8).

The necessity of having a long term dataset on FIB in the Red River for the application of the model and given the significant correlations that have previously been observed between Total Coliforms and *E. coli* (Leclerc *et al.*, 2001; Byamukama *et al.*, 2000; Wilkes *et al.*, 2009) (see chapter 3, section 3.1), we used data on TC for the model application as a relatively large

data set on TC exists for the Red River, whereas much less data is available on *E. coli* numbers in this system. Many factors impact FIB die-off rates in tropical environments (Rochelle-Newall *et al.*, 2015) and some of the processes affecting the survival of bacteria in the drainage network of the RRD have recently been studied (Nguyen *et al.*, 2016). We used a FIB mortality rate of 0.045 h^{-1} for the FIB *E. coli*, as proposed by Servais *et al.* (2007a). This value also falls within the range observed during one year at four stations in the Red River (Nguyen *et al.*, 2016). Sedimentation of attached bacteria (considered to represent half the FIB inputs by point and diffuse sources) is taken into account in the model through a sedimentation velocity of 0.02 m h^{-1} . The mortality rate of attached FIB in the sediment compartment is set as half of that for water column organisms (Ouattara *et al.*, 2013) and attached FIB in the sediment are subject to resuspension when water velocity allows sediment erosion.

3.3.3.3 Point and diffuse sources evaluation

For running the FIB module in the Seneque/Riverstrahler model, inputs of FIB from the watershed into the drainage network have to be provided. The model distinguishes between (1) point sources such as the discharge of domestic wastewater that is either directly released or is treated in wastewater treatment plants (WWTPs) and (2) diffuse sources of FIB from forested lands, croplands, grasslands, paddy rice fields and urban surfaces.

The point sources are taken into account in the model by considering all potential sources of wastewater in the watershed based on the urban population census and industrial activities (Le *et al.*, 2005). A portion of the wastewater from the Ha Noi urban area is now collected and partially treated by the Yen So infrastructures. The point sources of FIB were determined using either the urban population (in the case of no wastewater treatment) or the treatment capacity (in number of inhabitant equivalents) and the type of treatment for each WWTP in the Red River watershed.

Table 3.7. Total coliform (TC) numbers for different wastewater types (10^3 nb l⁻¹: 10^3 number of TC l⁻¹) estimated from data reported by HAWACO (2012)

Type of treatment	Median (10^3 nb l ⁻¹)	(min-max)
Non-treated domestic wastewater	40,000	(1,500 – 85,000)
Basic activated sludge process	315	(10 – 4,400)
UV treated effluents	11	(1 – 220)

Table 3.8. Total coliform concentration for surface runoff and base flow assigned to each landuse class (nb l⁻¹: number of TC l⁻¹).

Land use	Surface runoff, nb l ⁻¹	Base flow, nb l ⁻¹
Forest	360	250
Degraded forest, bare land, grassland	25 000	250
Agricultural land exl rice	11 000	250
Rice fields	25 850	250
Urbanized area	68 750 000	720

The capacity of each WWTP was then multiplied by the corresponding specific load of TC per inhabitant and per day according to the type of treatment applied to obtain the flux of TC released to rivers. Table 3.7 gathers the values of TC loads for the different types of wastewater considered. Loading values were calculated from the concentration of TC in domestic water and water flux, by considering an average specific water consumption of 250 L inhab⁻¹ day⁻¹. This was obtained by dividing the value of the daily sewage discharge volume (m³ day⁻¹) by the number of inhabitants served (Chu *et al.*, 2010). The Yen So WWTP complex has a median total coliform concentration of 40,000,000 l⁻¹ before treatment, this value is

therefore considered as representative of raw wastewater. The flux of TC from each WWTP was set to 50% as attached bacteria and 50% as free FIB in the model (Ouattara *et al.*, 2013).

The diffuse sources of TC are calculated on the basis of land use in each sub-basin of the watershed. The GIS land use coverage information, taken from the data of Le *et al.*(2005), is summarized in Fig. 3.7 and also shows the trends of land use changes over the last 50 years. Data from the literature and from available monitoring programs on the concentration of FIB in small rivers, streams and ditches draining homogeneous land cover types were used to characterize TC numbers in surface runoff and in base flow as a function of land used (e.g. Nguyen, 2006; VIWRR, 2008 ; Nguyen, 2012; Vu, 2012). The TC concentration assigned to each of the land use classes was determined as the median value of all data compiled from these empirical surveys (Table 3.8).

An alternative way of defining diffuse sources of FIB was recently proposed by Causse *et al.* (2015). These authors showed a robust relationship between the total flux of EC to the watershed soil reservoir (through grazing livestock excretion, application of manure and human outside excretion, flux EC (FlxEC)) and the FIB concentration in surface runoff, this relationship is of general validity whatever the dies off used:

$$\text{FIB (nb l}^{-1}\text{)} = 14.6 \times 10^{-6} * [\text{FlxFB (nb km}^{-2}\text{ day}^{-1}\text{)}]^{0.78}$$

Where FlxFIB is flux FIB to the soil reservoir.

The work of Causse *et al.* (2015) was conducted in the Nam Khan drainage network in Laos. Given the strong similarities between the two study sites in Laos and Vietnam (both are tropical, developing countries in Southeast Asia), it is assumed in this work that the relationship above is the same in the two systems. Moreover, and as noted by Causse *et al.* (2015), it is probable that the equation above for the estimation *E. coli* concentration in

overland works well in other similar systems, particularly those with similar landuse and sanitation practices. The application of this relationship to the 4 main sub-watersheds of the Red River system, considering the rate of FIB inputs to the soil through livestock and rural human population, provides values in the same order of magnitude, ($\pm 20\%$ on average) of the FIB concentration as those obtained from a weighted mean of the data by land use of Table 3.8, taking into account the distribution of land use (Table 3.9). This difference is probably due to differences in land use between the Da and Thao Rivers.

3.3.3.4 Validation data

In order to validate the modelling results, a monthly monitoring survey was carried out from January 2012 to July 2015 with water samples collected from the ten stations along the Red River (see Fig. 3.6). Details on this monitoring survey and the data for the first twelve months can be found in Nguyen et al.(2016).

3.3.3.5 Construction of the 2050 scenario

One of the objectives of this work was to examine the impact of changes in the basin on FIB numbers under a future of global and local change in 2050. The FAO (2011) evaluated the effects of climate change on the Red River Delta region and they predict that the annual average temperature is expected to increase by 1.2°C to 1.3°C and precipitation increase by 3.9 to 4.1%. However, in terms of bacterial contamination of the river system, the direct effects of the anticipated changes on diffuse and point sources of FIB are likely to be much larger than climate change effects. Population in the Red River basin has increased rapidly from 5×10^6 inhabitants in 1950 up to 20×10^6 inhabitants in 2005 and this number is expected to have doubled by 2050 (FAO, 2004). Moreover, most of this increase will occur in urban areas where population will triple while the rural population, which currently represents about 50% of the total population, is expected to stay roughly constant. For the upstream part of the watershed,

we therefore considered that the number of urban inhabitants (discharging their wastewater into the drainage network) will increase by a factor of 3. We assumed that only the agglomerations larger than 50,000 inhabitants will have a wastewater treatment plant with a basic activated sludge process, while the smaller villages will release their wastewater without treatment with 75% of that wastewater being recycled in agriculture (Le *et al.*, 2005).

In the Ha Noi area, the population is predicted to reach 14,000,000 inhabitants in 2050, which means a 4.7 fold increase with respect to the current population. According to the Prime Ministers office (PMOV, 2013), the treatment capacity of the Ha Noi district sector will reach 1,896,000 m³day⁻¹, representing 10,600,000 inhabitants.equivalent i.e., 75% of the total population. This value takes into account a 30% reduction with respect to current values in per capita water consumption (reduced to 180 L day⁻¹), as has been observed over the past decades in Europe). We considered that 50% of this (i.e., 37.5% of the total population) will be treated by standard activated sludge process and 50% (37.5% of the total) with further treatment including UV disinfection. The remaining 25% of the Ha Noi population will release its wastewater without treatment.

Table 3.9. Results from both approaches used to assess the average TC concentration in surface runoff in the three major sub-basins of the Red River system due to diffuse sources. In the land use approach, an average TC concentration in surface runoff was assigned to each land use class based on a survey of empirical data from small streams draining homogeneous watersheds. In the input approach, TC concentration is estimated based on the total input of TC to the soil from animal and human excretion. (Cap.: capita, Ag. land: agricultural land).

Land use approach

Land use class*	Da %	Lo %	Thao %	Delta %	Land use specific TC conc nb l ⁻¹
Forest	74.4	22.4	54.2	17.8	360
Bare land and Grassland	9.8	10.3	11.3	7.5	25,000
Ag land excluding rice	3	58.6	14.4	6.5	11,000
Rice fields	12.5	8.1	18.7	63	25,850
Urbanized area	0.3	0.6	1.4	5.2	6,880,000
TC conc, nb l⁻¹	26,919	52,475	105,758	376,700	

FIB input approach

	Da	Lo	Thao	Delta	Spec TC release rate
Livestock	cap.km ⁻²	cap. km ⁻²	cap.km ⁻²	cap km ⁻²	nb cap ⁻¹ day ⁻¹
Poultry	423	415	733	4,173	2.4 x10 ⁹
Buffalo	13	16	17	7	2.6 x10 ¹⁰
Cattle	9	10	5	24	1.4 x10 ¹⁰
Pig	43	56	79	321	1.4 x10 ¹⁰
Rural human Population	32	24	140	196	5.0 x10 ⁹
TC conc in runoff water**, nb l⁻¹	62,685	67,709	99,973	290,636	

* from Le et al (2005)

**based on the Causse et al (2015) relationship

Regarding diffuse sources on the 2050 horizon, we took into account both the predicted land use changes and the expected increase in livestock numbers. The former consists of a significant increase of agricultural and urbanized surfaces at the expense of rangeland and forested areas (Fig. 3.7). Livestock numbers are expected to increase considerably based on the figures for livestock density (Nguyen *et al.*, 2012) and General Statistics Office of Viet Nam in the Red River basin (GSO, 2013).

Considering the projected increase in livestock density together with a constant rural human population (Pham, 2014), we estimate that the flux of FIB inputs to soils will be multiplied by a factor 12 at the 2050 horizon. Based on the approach illustrated in Table 3.10, we recalculated the FIB concentrations assigned to surface runoff from agricultural land use classes (Table 3.10). The concentration assigned to base flow is assumed to remain unchanged.

3.3.4 Results and discussion

3.3.4.1 Model simulation of seasonal and geographical variations in the Red River system under current conditions

The model was run over a period of three years with the hydrological forcing of 2012-2014. The results are presented either as the temporal changes in FIB concentration at a given sampling station (Fig. 3.9) or as a spatial distribution of FIB in the river branches in terms of kilometric point (kp) at particular time of the seasonal cycle (see Fig. 3.11). The model simulations of the temporal variations generally follow the dynamic trend of FIB concentration in the water when the output data is compared with the *in situ* measurements at each of the selected stations (Fig. 3.9). The results of the model show that, in general, the overall correlation between the simulated and observed values follow a 1:1 diagonal at all stations with some degree of scatter (Fig. 3.10a).

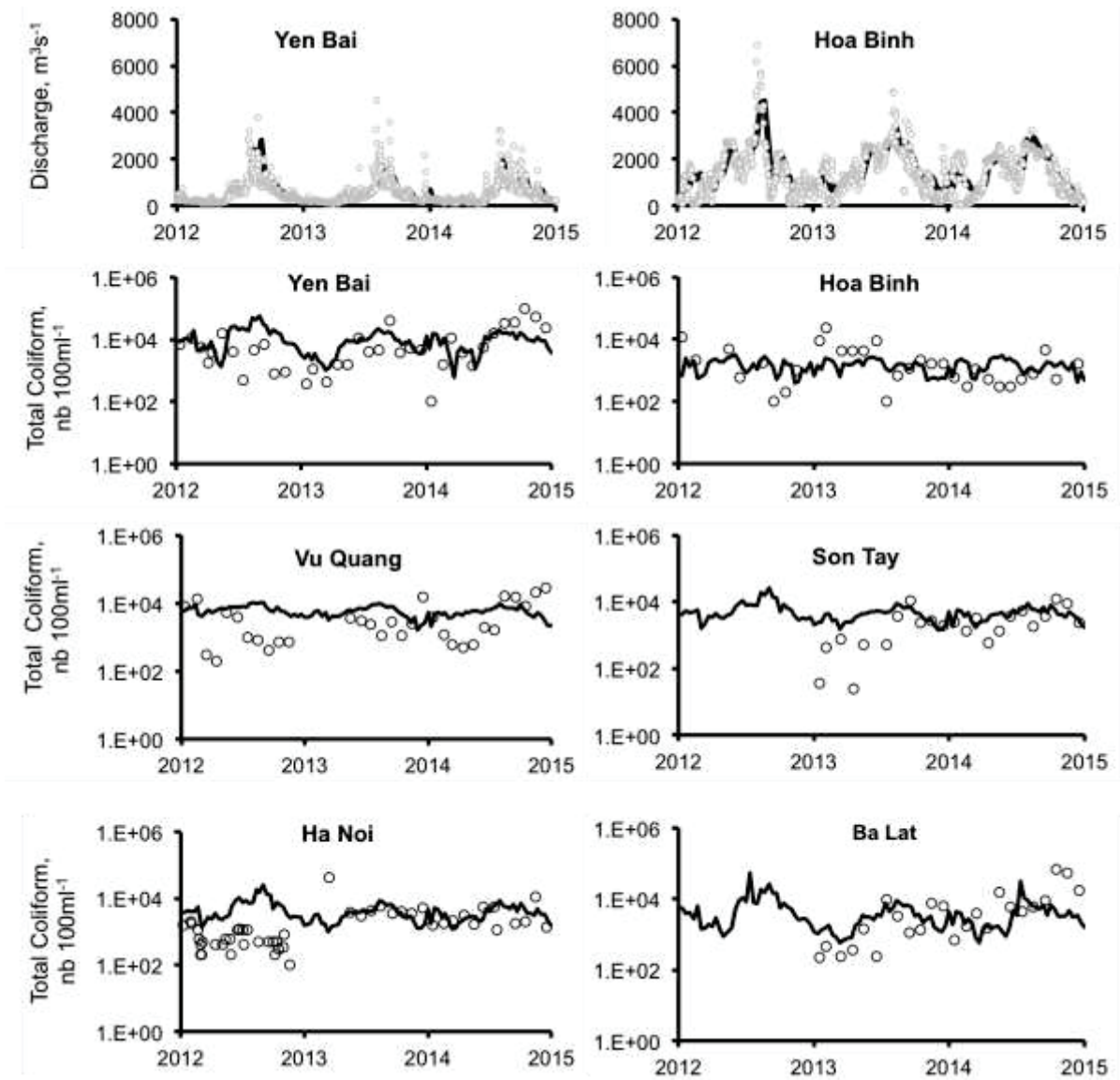


Figure 3.9: Model simulated (black line) seasonal variations of total coliform (TC) concentration compared with observations (dots) at different stations in the Red river system for the years 2012 - 2014.

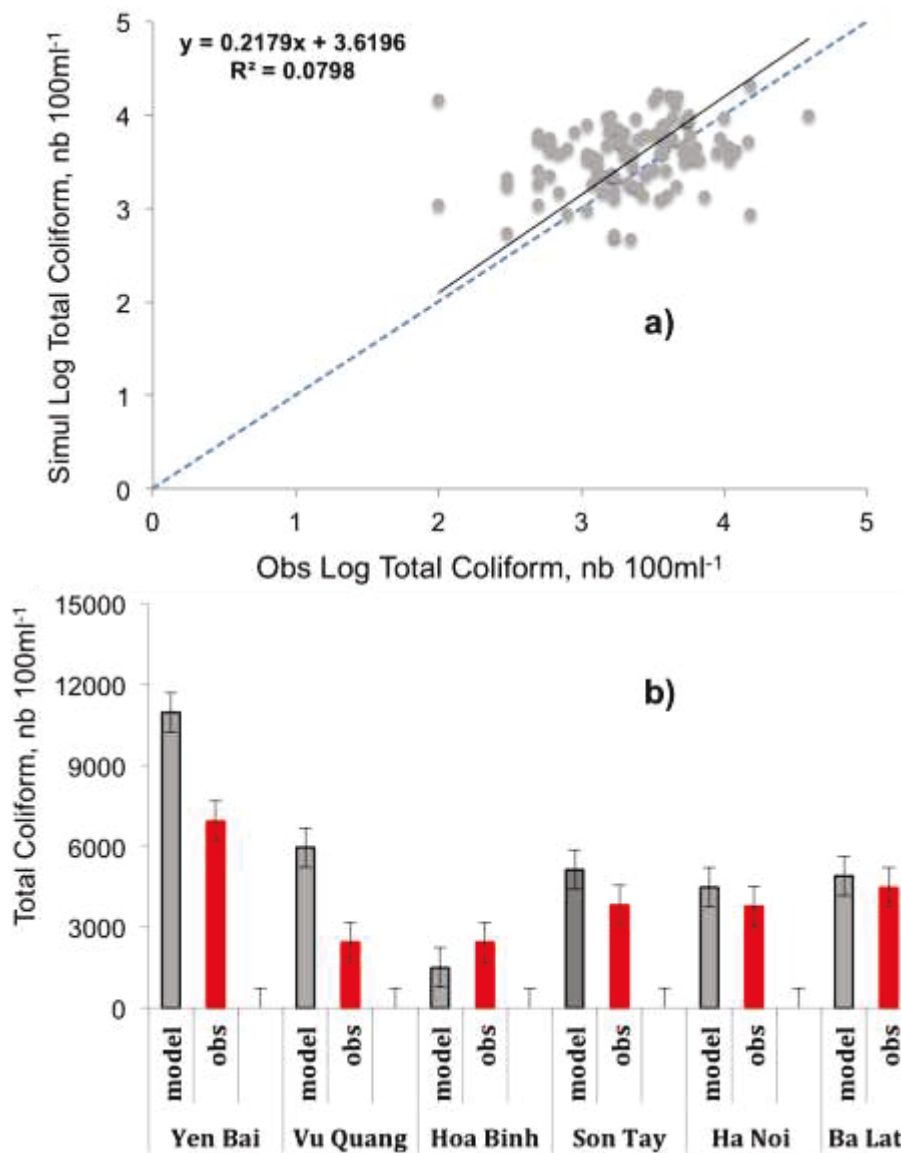


Figure 3.10: Relationship between the average value of the model and observations for all stations for the years 2012 - 2014 (a) and comparison of the average values of this period at 6 stations from upstream to downstream (b).

To further evaluate the results, we compared the average observations and the simulation data for all stations (Fig. 3.10b). Although the averaged simulated FIB concentrations were lower than those of the observations at Hoa Binh, and higher at Yen Bai and Vu Quang, the differences were only statistically significant at Yen Bai and Vu Quang ($p < 0.05$).

Spatially, the observed and simulated data along the main branches of the three main tributaries were also compared (Fig. 3.11). Field FIB numbers were averaged for low (December - March) and high (May - October) discharge periods at each station for the 2011-2014 period as were the model calculations. In all cases, higher FIB values were observed at higher rather than low discharge, showing the dominance of diffuse over point sources. In the Thao River, a sharp decrease of FIB concentration occurs at the confluence of the Da River. This is due to the much lower FIB concentrations found in the Da River than in the Thao River which is characterized by a larger proportion of agricultural land (Fig. 3.11).

The much lower FIB concentrations in the Da River may also be due to the Hoa Binh reservoir which considerably increases the residence time of the water masses, leading to a strong decrease of FIB through mortality (Fig. 3.11). The same self purification of FIB in reservoirs is observed in the Lo River downstream of the Dai Thi dam (Fig. 3.6), despite a much larger proportion of agricultural land use in this basin (Fig. 3.11). A sharp decrease is also observed at the confluence with the Chay river (at kp 145) on which Thac Ba reservoir is located (Fig. 3.6; Fig. 3.11).

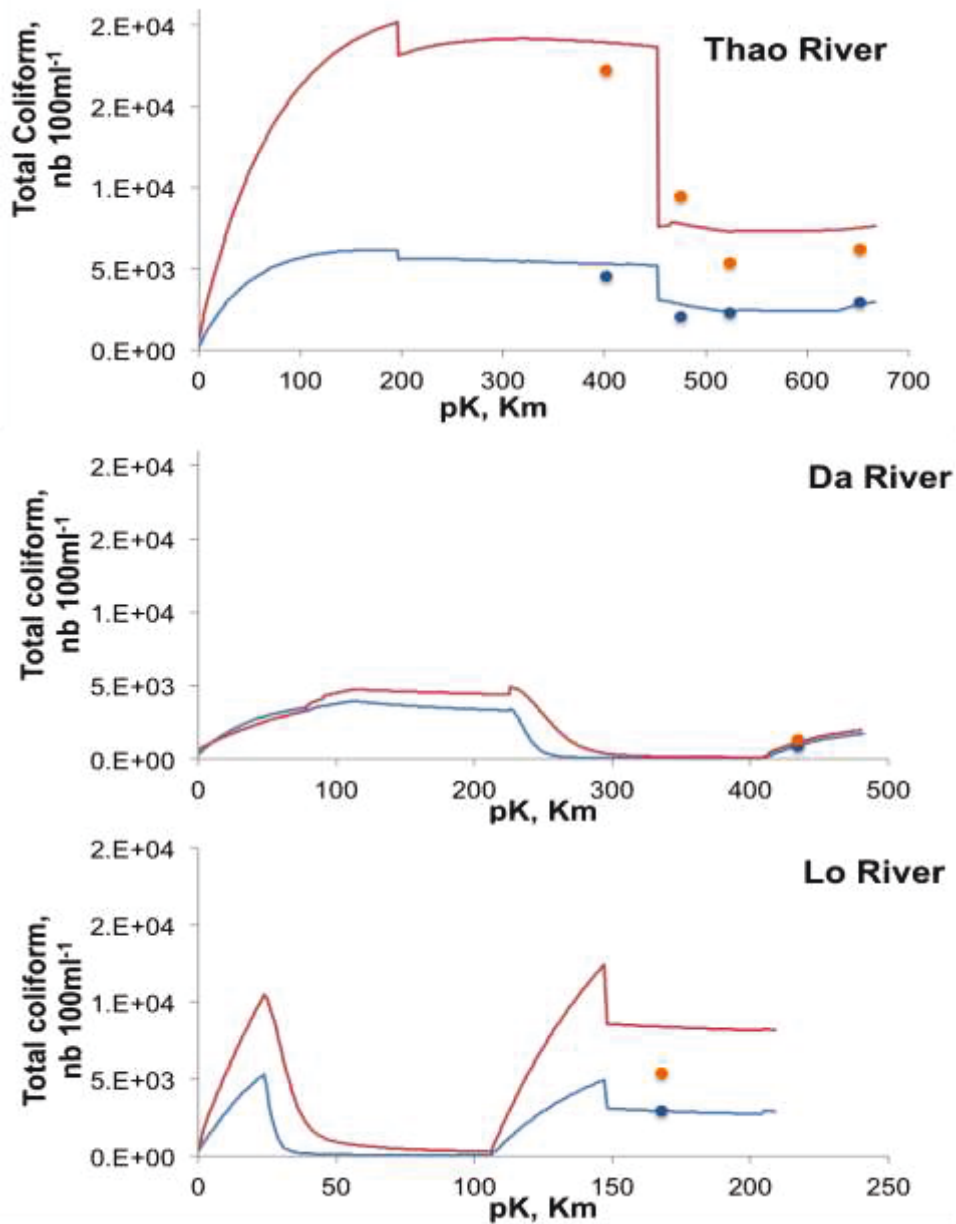


Figure 3.11: Longitudinal variations of FIB concentrations in the Red River calculated by the Seneque/Riverstrahler model for the years 2011-2014. The red line and blue lines are the median of the calculated values over the 3 years at high (January-March) and low (June-August) discharge, respectively. The x-axis is a kilometric unit along the upstream-downstream course of each tributary.

As a result of the mixing of upstream Thao River water with the much less contaminated waters of the Da and Lo Rivers, a sharp decrease FIB number occurs in the downstream sector of the Thao (also called Hong) River, despite the importance of wastewater release from the Greater Ha Noi urban area in this sector. The low impact of urban discharges on FIB numbers in the lower Red River, as well as the higher concentrations observed and modelled at higher flow highlights the dominance of diffuse over point sources of FIB in this system as has been observed elsewhere (e.g. McDonald and Kay, 1981; Collins *et al.*, 2005; Causse *et al.*, 2015).

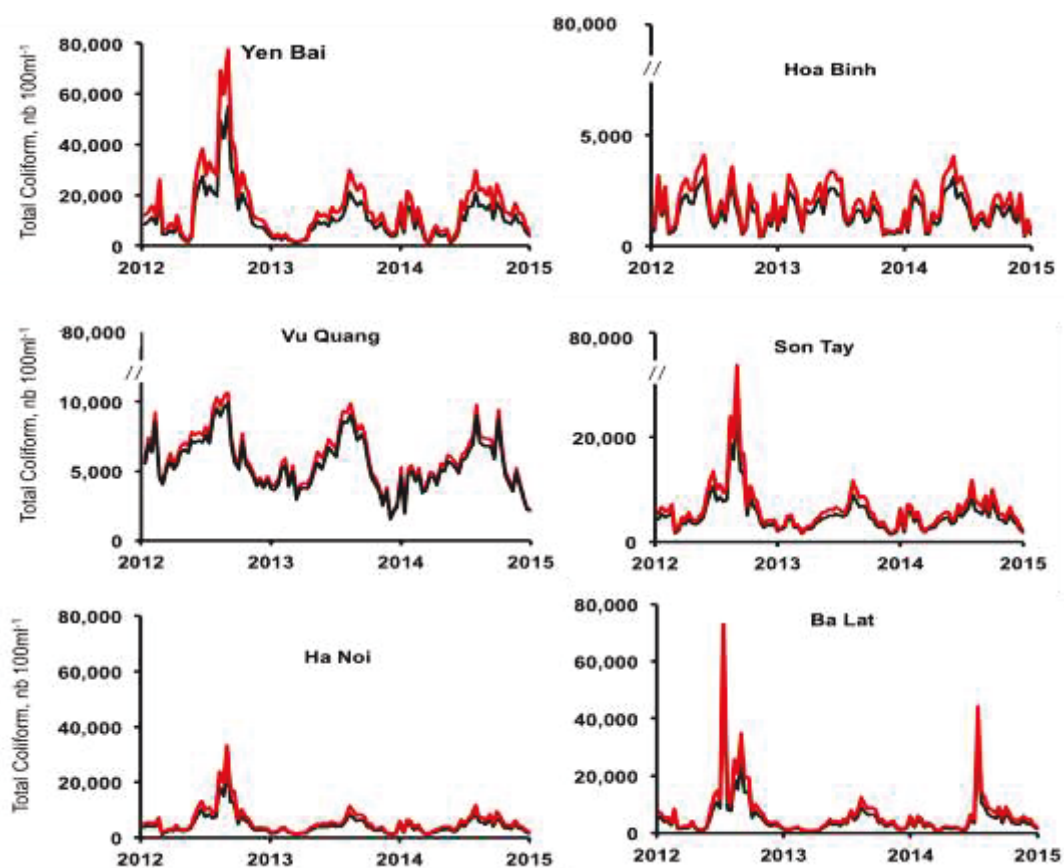


Figure 3.12: Simulation results for six stations along the Red river for the ‘2050’ scenario (red line) as compared with simulations for present (black line).

3.3.4.2 Model Simulation of FIB contamination at 2050 horizon

As explained above, we used the model to predict the future level of FIB for 2050. For this scenario, FIB within the entire drainage system was calculated using the reconstructed point and diffuse sources for the predicted 2050 situations and the hydrological constraints of the 2012-2014 period. The result shows only a limited increase of FIB compared with the present situation at all stations, especially in the Vu Quang and Ha Noi stations (Fig. 3.11). This might appear surprising in view of the predicted increase of point and diffuse sources of FIB at the 2050 horizon. However, the limited model response can be explained by two reasons.

Regarding point sources, the predicted increase in urban population is for a large part compensated in the scenario by an increase in wastewater treatment. For instance, although the total population of Greater Ha Noi is expected to increase by a factor 4.7, only 25% of the wastewater produced is expected to be discharged without treatment (whereas 100% was discharged before the implementation of the Yen So facilities in 2014). Regarding diffuse sources, the predicted increase in livestock numbers led us to predict a 12 folds increase of the flux of FIB deposited on pasture and agricultural soils. Although this results in a considerable increase of the FIB concentration of surface runoff issued from these land use classes (Table 3.11), it affects neither the contamination from forested and urbanized areas, nor the FIB concentration from baseflow. It is therefore the combination of these two factors that explains the lack of increase in FIB on the 2050 horizon in the Red River system.

Table 3.10: TC concentrations (nb l^{-1}) assigned to surface runoff in each land use class for the 2050 scenario.

Land Class	TC , nb l^{-1}
Forest	360
Degraded forest and bare land	30,0000
Agri land excluding rice	132,000
Rice fields	310,200
Urbanized area	68,750,000

3.3.4.3 Respective role of point and non point sources of FIB

Overall, our model results and our observational data, show a initial positive linear relationship between FIB levels with river discharge, with however very different slopes according to the subbasins (Fig. 3.12). This is quite different from the expected decreasing hyperbolic relationship with discharge, often observed in case of point source dominated systems. The fact that such a pattern is not observed any where in the Red River system, even in the populated area of the Delta, further indicates the dominance of diffuse pathways over point sources of FIB contamination.

This is probably a consequence of the very high discharge regime of the Red River, even compared with the high population of the watershed. The calculation of the respective inputs of FIB from surface runoff of the different land use classes and from urban point sources (Table 3.8) illustrates this point very well. The budget of FIB inputs and outputs also underlines the importance of self purification in this system, as the average FIB flux at Son Tay amounts less than 1% of the total FIB input to the upstream river system from diffuse and point sources.

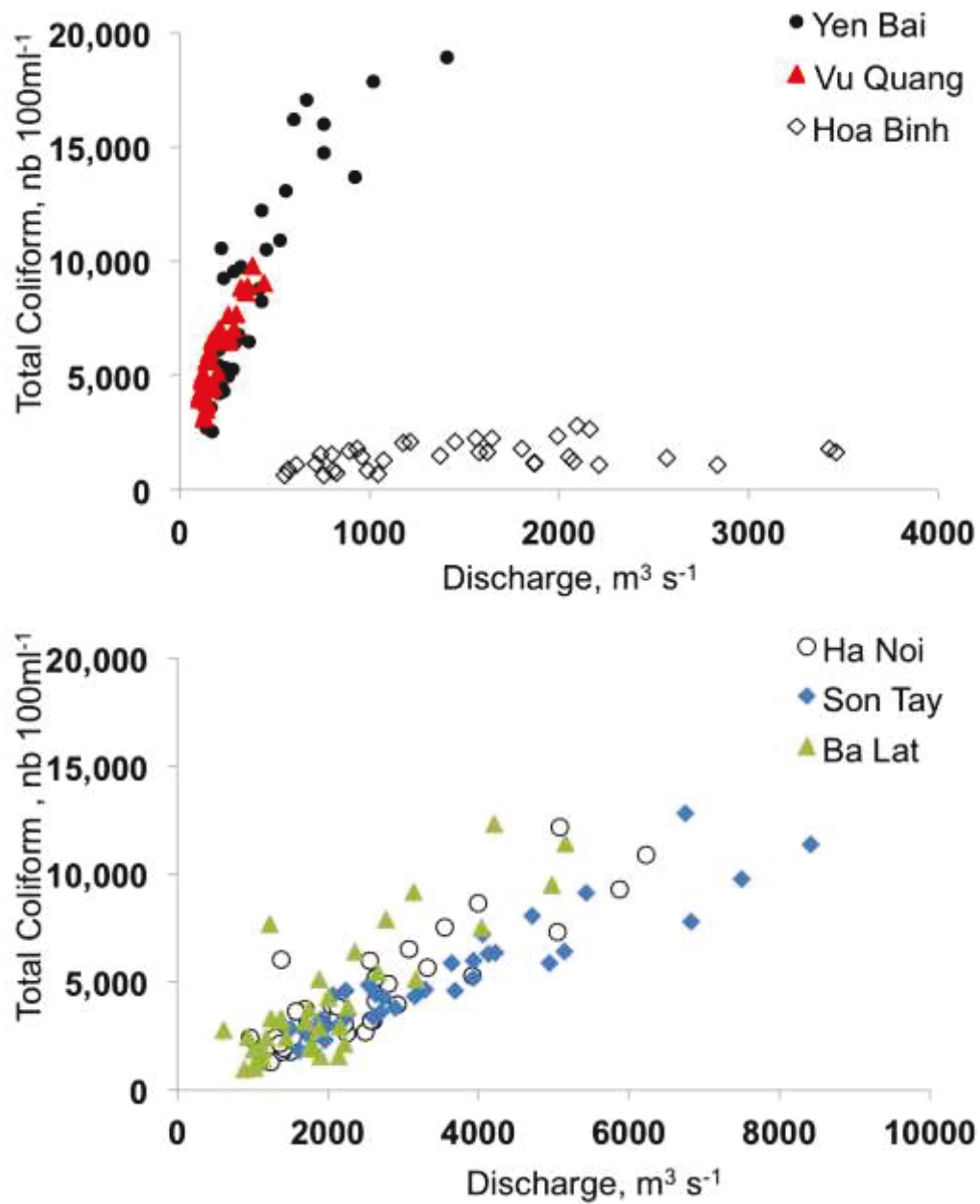


Figure 3.13: Relationship between TC concentrations (calculated by the model) and discharge values at three upstream stations (a) and three downstream stations (b) of the Red River system

Table 3.11: Budget of TC inputs calculated by the model for diffuse and point sources to the Red River system upstream from Son Tay in the current (2012) and future (2050) situations (Fluxes, in 10^{15} FIB day⁻¹).

	2012 situation	2050 situation
Input from diffuse sources	130	176
Input from point sources	0.8	10.4
Output at Son Tay	1.1	1.4

3.3.4.4 Comparison with other river systems

Figure 3.13 summarises the literature values of FIB contamination downstream of large urban areas of varying population in rivers of different mean discharge. The ratio of population to mean river discharge (together with the presence of wastewater treatment facilities) explains the major trends of the distribution of faecal contamination. Above a ratio of about 10 000 inhabitants per m^3s^{-1} , FIB contamination increases rapidly to concentrations higher than 10^6 FIB L^{-1} , which are dominated by point sources. This is the case for cities like Paris, Reims and Troyes (Seine River, France; Servais *et al.*, 2007a), Brussels (Zenne river, Belgium; Ouattara *et al.*, 2013), Nairobi (Nairobi river, Kenya; Musyoki *et al.*, 2013), Hong Kong (DongJiang river, China; Hong *et al.*, 2010). Below this ratio, the contamination is much lower (although often above sanitary thresholds) and is controlled by diffuse sources of FIB. This is the case for Ha Noi city (as shown in this study) but also for Luang Prabang (Nam Khan River, tributary of the Mekong River, Laos; Causse *et al.*, 2015) and Vienna (Danube River, Austria; Hoch *et al.*, 1996). The ratio of population to average river discharge can vary over a very large range according to the size of the river and with that of the river draining its wastewater. It is therefore a useful indicator of whether point or diffuse sources are the likely dominant factor controlling FIB contamination. Note the case of Meriden (on the Quinnipiac river, USA; Mitch

et al., 2010) which is characterized by low values of FIB contamination owing to the good performance of the wastewater treatment.

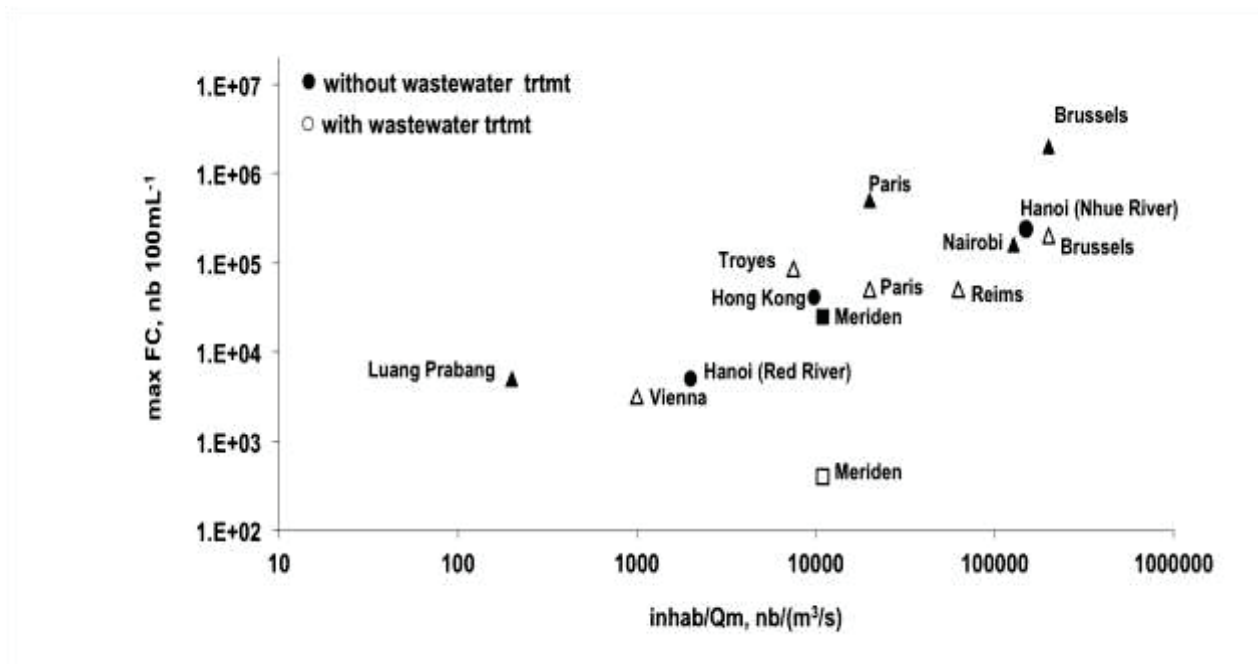


Figure 3.14: Observations on FIB contamination (Square: Fecal coliforms; Circle: total coliforms; Triangles: *E.coli*) downstream from some large cities as a function of the ratio between total population (inhab) and annual mean discharge of the river (Qm). This index allows distinguishing between point source dominated systems and diffuse source dominated ones.

Although the implementation of wastewater treatment is an important step to mitigate the impact of human waste on bacterial contamination of water resources, our analysis shows that reductions in point source inputs are not sufficient to prevent bacterial contamination of surface waters (Meyers et al., 1998). This is because of the predominance of diffuse sources in situations where point sources have been eliminated and/or in situations where there is an efficient dilution as a consequence of river high discharge.

3.3.5 Conclusions

Our study is one of the few on faecal contamination of surface water in a large river basin that covers both urban and rural areas of a tropical, developing country. The Seneque/Riverstrahler model, which combines a module describing the dynamics of FIB in a hydro-ecological representation of the whole drainage network of a large regional river basin is to our knowledge one of the first mechanistic models able to simulate spatial and seasonal variations of microbial contamination (FIB concentration). The simulations of our model on FIB concentration compare rather well with field data collected over a 3 years period in different stretches of the hydrographic network. The Seneque/Riverstrahler model has been shown a useful tool to explore the impact of wastewater management strategies on microbial contamination in the rivers of the whole drainage network. We have revealed that fecal contamination in the Red River is in the lowest range of the values found in their literature, thanks to its very high dilution capacity. In such river systems where sanitation is poor, diffuse sources of contamination predominate over point ones. If the future plans at the horizon 2050 are achieved, the fecal contamination would not increase considerably, despite a doubling of the population and a planned considerable increase of the livestock.

4 Organic carbon

4.1 Organic carbon in aquatic systems

4.1.1 Introduction and definition

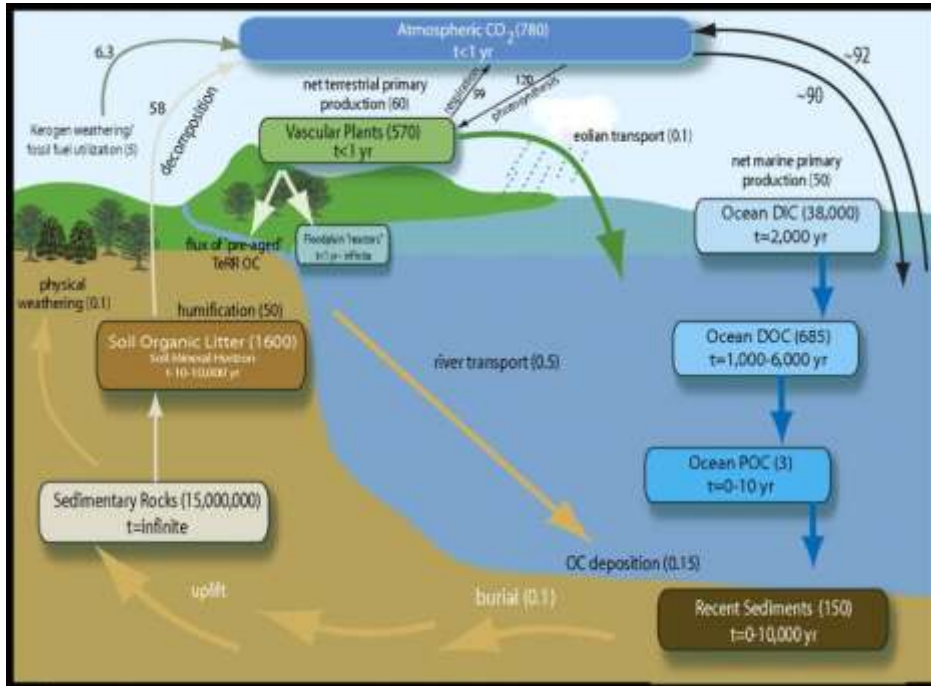


Figure. 4.1: Global carbon cycle. The stocks (PgC) are noted in “()”, the fluxes are in black (PgCyr⁻¹) and the turnover times are provided as “t”. (Bianchi, 2011).

Particulate organic carbon (POC) and dissolved organic carbon (DOC) make up the total organic carbon (TOC) pool. TOC is ubiquitous to aquatic and terrestrial ecosystems and forms a large pool of organic carbon. TOC is the largest pool of reactive carbon in the Earth's crust (Fig. 4.1; Bianchi, 2011). Overall, soils contain 1,600 Pg C, sediments 1,000 Pg C and the dissolved fraction (DOM) represents 685 Pg C in the oceans which is comparable to the atmospheric CO₂ pool. The global estimate of average POC produced as biomass is 1.34 – 6.41 g C m⁻² yr⁻¹ (Stramska, 2009) and the transport of POC and DOC from the terrestrial environment to the oceans forms a significant link between the terrestrial pool and the oceanic

sink with over 0.9 Pg yr⁻¹ of C being transferred from the terrestrial environment to the oceans via rivers and streams (Cole *et al.*, 2007).

TOC plays an important role in ecological processes as its presence in the water column affects light penetration, water pH, nutrient availability and the toxicity of metals (Dalva and Moore, 1991; Kraepiel *et al.*, 2003; Roulet and Moore, 2006). Moreover, as OC is the main nutrition source for aquatic microbes, it also plays a fundamental role at the base of the foodweb (Azam *et al.*, 1983; del Giorgio and Davis, 2003; Strayer *et al.*, 2008; Findlay, 2010).

4.1.2 Sources

Even clear water, such as that from deep lakes or from the open ocean, contains organic carbon. DOC, the soluble fraction of the TOC pool can vary in concentration by several orders of magnitude (0.5 – 50 mgL⁻¹) with concentrations being higher in freshwaters than in the oceans (Benner, 2002; Mulholland, 2003). In rivers, DOC concentrations, tend to be lowest in arid regions (1 mgL⁻¹), intermediate in tundra, temperate and dry tropical regions (2 - 4 mgL⁻¹) and the highest are found in wet tropical and taiga climates (7 - 8 mgL⁻¹) with the global mean being 6.3 mgL⁻¹ (Meybeck, 1988 as cited in Mulholland, 2003).

Each year, the rivers of the world export 0.9 Pg of C annually of which 50-60% is DOC (Cole *et al.*, 2007; Jiao *et al.*, 2010). This organic carbon originates from a multitude of sources, including primary production by freshwater and marine phytoplankton, benthic vegetation and riverine transport of eroded soils (Mulholland, 2003). Agricultural runoff and industrial and human wastewater can also bring an important contribution to TOC in aquatic systems. This can include domestic sewage, pulp mill effluent, discharge from sewage treatment works, as well as other industrial waste that can contribute a significant anthropogenic allochthonous input to the water column and sediments (Swinarski *et al.*, 2009;

Renjith *et al.*, 2013; Thevenon *et al.*, 2013).

Organic matter that originates from terrestrial sources (e.g. soils, vegetation) makes up an important fraction of the total OM especially in small continental water bodies (Cole and Caraco, 2001; Cole *et al.*, 2007). In soils, TOC is most concentrated in the surface layer (1 to 20 cm depth). Under natural conditions the content of organic matter in soil is constant, however, this equilibrium is disturbed when forests are cleared and the land is used for agriculture (Wu *et al.*, 2003). There is also a decline in soil organic matter when grassland in the tropics and subtropics is transformed into cropland, or when savannahs are burned (Lal, 2000; Paustian *et al.*, 2000; Wu *et al.*, 2003).

Terrestrially production TOC can be transferred from the soil into the hydrosphere. Transport of soluble and particulate organic carbon to streams, lakes, and oceans or into groundwater usually occurs in runoff or via percolation through the soil column in the case of groundwater (Chaplot *et al.*, 2006; Clay *et al.*, 2010; Dhillon and Inamdar, 2013). DOC concentrations along overland flow paths can be an order of magnitude higher than groundwater. For example, in the Amazon overland flow and shallow subsurface flow had $19.6 \pm 1.7 \text{ mg C l}^{-1}$ DOC and $8.8 \pm 0.7 \text{ mg C l}^{-1}$, respectively, versus $0.50 \pm 0.04 \text{ mg C l}^{-1}$ DOC in emergent groundwater (Johnson *et al.*, 2006). A similar pattern was observed in Laos where Chaplot and Ribolzi (2014) observed $11.9 \pm 0.8 \text{ mg C l}^{-1}$ in overland flow (runoff) compared to $2.3 \pm 0.6 \text{ mg C l}^{-1}$ in groundwater.

Most DOC in free-flowing rivers without wastewater inputs is derived from vegetation and soil organic matter, whereas in dammed rivers phytoplankton primary production can provide a significant proportion of TOC. This autochthonous production can be the major source of OM in lakes and is principal source of OM in the open ocean (Baines and Pace, 1991). The amount of phytoplankton production in surface water can vary depending on latitude, climate, lake depth, clarity and nutrient levels (Lewis, 2011). However, the global

estimated global net primary production per unit area for lakes is estimated to be $260 \text{ gC m}^{-2}\text{y}^{-1}$ with a large proportion of this production coming from the tropical and subtropical latitudes where a large number of lakes are found (Lewis, 2011).

4.1.3 Role of climate

Climate plays an important role in controlling OC concentration in rivers (Thurman, 1985). Precipitation and temperature independently control terrestrial climate and vegetation in a watershed. Areas of high precipitation, temperatures, and net primary productivity (NPP) and extensively weathered soils as are often found in tropical regions, tropical rivers tend to have high DOC concentrations (Schlesinger, 1997).

Climate change is predicted to decrease the number of rainy days but increase the average volume of each rainfall event (Bates *et al.*, 2008). The anticipated increases in rainfall and rainfall intensity will increase erosion, runoff and solid material transportation from land as well as increasing discharge in rivers and streams thereby altering water quality. Moreover, these effects on the timing and magnitude of river discharge will probably alter the delivery of terrigenous organic carbon to the ocean (Nohara *et al.*, 2006; Dai *et al.*, 2009; Dai *et al.*, 2012). Consequently, the relative importance of the fluxes of terrestrial and marine organic carbon to rivers and to the oceans will likely change, as will the processing and preservation of organic carbon in sediments (Katsev *et al.*, 2006). In areas where droughts are predicted to increase, the drought–rewetting cycles may impact water quality as it enhances decomposition and flushing of organic matter into streams (Evans *et al.*, 2005).

During storm flow, DOC concentration in a river typically rises. Studies attribute this rise to flushing of DOC-rich soil water, leaching of leaf litter from runoff, and flow of shallow groundwater through the soil. Kaplan *et al.* (2006) reported a positive correlation between DOC

concentration and baseflow discharge, suggesting that changes from high to low baseflow conditions were accompanied by subtle shifts in flow paths along hydrologic gradients. DOC concentrations on the order of 10 mg C l^{-1} are transported by approximately 4% of stream flow during storm events, while the remaining 96% of stream flow originates as low-DOC ($\sim 0.5 \text{ mg C l}^{-1}$) base flow (Kaplan *et al.*, 2006).

Tropical rivers have a critical role in the total global fluvial carbon flux (Huang *et al.*, 2012). Indeed, this region contributes 66.2% of freshwater outflow, 73.2% of sediment loads and approximately 61% of terrestrial net primary production (Huang *et al.* 2012). However, much of the Asian region has experienced dramatic changes in water discharge and sediments fluxes over the past decades as a consequence of changes in land use and the damming of rivers (Le *et al.*, 2007; Luu *et al.*, 2010; Vinh *et al.*, 2014; Le *et al.*, 2015). Forest conversion and other changes in land use have resulted in increases in soil erosion (Valentin *et al.*, 2008), which have contributed to changing the inputs of carbon and nutrients to rivers and streams (Quinn and Stroud, 2002; Williams *et al.*, 2010). Although there is evidence that reforestation can reduce inputs of sediments to rivers and streams (e.g. Zhang *et al.*, 2009). Changes in temperature and rainfall in the region have also probably impacted the rivers systems (Nguyen *et al.*, 2014) affecting carbon fluxes in these river systems as well as those of the associated estuaries, coastal regions and even continental shelf areas (Chen, 2000).

4.1.4 Biodegradability of DOC

In aquatic systems, the fate of organic carbon is controlled in part by its biodegradability (Findlay, 2003). The biodegradability of DOC in water environments is controlled by a variety of interacting physical, chemical and biological factors and processes and is generally defined as a function of the time required by bacteria to degrade organic carbon. The most rapidly used

fraction, the labile fraction, is often produced during photosynthesis and has a turnover time of minutes to a few days (Fuhrman, 1987; Carlson *et al.*, 1998). The remaining DOC pool is then divided up into: semi-labile (1.5 years), semi-refractory (20 years), refractory (16,000 years) and ultra-refractory (40,000 years) (Hansell *et al.*, 2012).

Although understanding the factors that control bioavailability is justifiable purely on an ecological basis, in practical terms such studies are also useful as a quantitative measure of pollution in aquatic systems. Many studies have investigated the biodegradability of organic carbon in marine, estuarine and lacustrine ecosystems as well as in treated and untreated wastewaters (Søndergaard and Middelboe, 1995; Stets and Cotner, 2008). For example, Fellman *et al.* (2009a) found that BDOC (biodegradable DOC) in a coastal temperate rainforest watershed ranged between 0.6 – 8.1 mgC l⁻¹. In contrast, BDOC in Lake Taihu (China) ranged from 0.012 ± 0.001 to 1.23 ± 0.05 mgC l⁻¹ (Yea *et al.*, 2015). In general, as a consequence of microbial and photodegradation processes and to the presence of humic and fulvic acids, BDOC in lake waters are lower than other environments, from 12.4% (Søndergaard and Worm, 2000) to 29% (Lønborg *et al.*, 2009). In unpolluted river and sea water showed values of 20% to 35% for an incubation time of 30 days (Servais *et al.*, 1987). Given the strong links between BDOC, OC, microbial respiration and CO₂ fluxes, it is therefore interesting to look at bioavailability in a wide range of environments and particularly in tropical areas where less is known at present.

4.2 Organic carbon transfers in the subtropical Red River system (Viet Nam). Insights on CO₂ sources and sinks (Article 3)

This chapter is in preparation for submission in spring 2016 to the journal “Biogeochemistry” as:

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Author contributions: GB, JG, QTPL designed the experiments; HTMN and GB carried out the work; HTMN, GB, JG interpreted the results and HTMN wrote the manuscript that was revised and improved by all the coauthors.

Keywords: sub-tropical watershed modeling, organic carbon, point and non-point sources, net source

4.2.1 Abstract

The Red River, draining a 169,000km² wide watershed, is the second largest river in Viet Nam and constitutes the main source of water for a large percentage of the population of North Viet Nam. Here we present the results of an investigation on particulate organic carbon (POC) and dissolved organic carbon (DOC) dynamics in the Red River basin, based on measurements in the main branches of the river as well as on the application of a model of OC dynamics. POC concentrations ranged from 0.24 - 5.8 mg C L⁻¹ and DOC concentrations ranged from 0.26 - 5.39 mg C l⁻¹. The model RIVERSTRAHLER / SENEQUE was used to investigate the dynamics and seasonal distribution of OC in the river. The results of the model show that, in general, the model simulations of the temporal variations and spatial distribution in OC concentration followed the observed trends. They also show the importance of the high population in the watershed on the inputs of OC from surface runoff from the different land use classes and from urban point sources. A budget of the main fluxes of OC in the whole river network, including diffuse inputs from soil leaching and runoff, point sources from urban centers, algal primary production and heterotrophic respiration is established based on the model results. It shows the predominantly heterotrophic character of the river system, and allows evaluating the flux of CO₂ across the river-atmosphere interface to 330 GgCyr⁻¹, in reasonable agreement with a few available direct measurements of CO₂ fluxes in the downstream part of the river network.

4.2.2 Introduction

The conversion of natural landscapes to agriculture and urban or industrial areas has altered the quantity and composition of organic matter delivered to rivers with adverse effects on ecosystems and society (Aitkenhead-Peterson *et al.*, 2003; 2013). Inputs of dissolved organic carbon (DOC) from terrestrial sources have increased through the erosion of soil organic carbon (OC) following the introduction of agriculture, while particulate organic carbon (POC) from internal sources such as phytoplankton primary have also changed (Jassby *et al.*, 2002; Beman *et al.*, 2005; Stanley *et al.*, 2012). Moreover, in areas where runoff from agricultural lands has high concentrations of nitrogen (N) or phosphorus (P), primary production increases, leading to further increases in POC and DOC concentrations (Howarth and Paerl, 2008; Howarth *et al.*, 2012).

The fate of this OC in an aquatic system is determined, in part, by its biodegradability (Findlay, 2003). In general, biodegradability is defined as a function of the time required by bacteria to degrade OC. Several fractions based on an estimate of the time required to remineralise the OC pool have been proposed. The most rapidly used fraction, the labile fraction, is often produced during photosynthesis and has a turnover time of minutes to a few days (Fuhrman, 1987; Carlson *et al.*, 1998). The remaining DOC pool is then divided up into: semi-labile (1.5 years), semi-refractory (20 years), refractory (16,000 years) and ultra-refractory (40,000 years) (Hansell *et al.*, 2012).

The biodegradability of OC in aquatic environments has been broadly attributed to a variety of interacting physical, chemical and biological factors and processes (Findlay, 2003). Although understanding these factors and their relative importance is justifiable purely on an ecological basis, in practical terms such studies are also useful as a quantitative measure of pollution or of carbon stocks and fluxes in aquatic systems. Indeed, many studies have

investigated the biodegradability of OC in marine, estuarine and lacustrine ecosystems as well as treated and untreated wastewaters (Sondergaard and Middelboe, 1995; Katsoyiannis and Samara, 2007; Stets and Cotner, 2008).

The biodegradability of OC in aquatic environments also impacts the potential carbon dioxide (CO₂) fluxes across the water-air interface as microbial respiration is a major source of CO₂ to the atmosphere (Richey *et al.*, 2002; Farjalla *et al.*, 2009). When respiratory rates exceed CO₂ fixation in aquatic environments, the system is considered to become a net source of CO₂ to the atmosphere, thereby contributing to the increase of this constituent in the atmosphere (Raymond *et al.*, 2013). The inverse is also true: when net uptake by primary production exceeds respiration, the system is considered to be a net sink of CO₂. The proportions of each process (production and respiration) depend on many factors, including the size of the river (Findlay, 2003), its geology and hydrology (Lapierre and del Giorgio, 2012; Maberly *et al.*, 2013), temperature and light penetration (Lomas *et al.*, 2002; Rochelle-Newall *et al.*, 2007), the relative abundances of the autotrophs and heterotrophs present (Smith and Kemp, 1995) as well as the sources and bioavailability of the OC (Raymond *et al.*, 2000; Bouillon *et al.*, 2012).

The use of models provides an interesting strategy for studying the stocks and fluxes of OC in an ecosystem. There have been several studies on OC based on empirical relationships between OC and attributes of the watershed. For example, authors have examined the importance of soil characteristics (Aitkenhead-Peterson *et al.*, 2003; Aitkenhead *et al.*, 2007), watershed dynamics (Band *et al.*, 2001; Raymond and Saiers, 2010), size and slope of basins (Clair and Ehrman, 1996). However, these models often contain a large number of environmental variables, so their application to future scenarios of climate change and human activities is difficult.

There are also models that take into account streamflow characteristics and mechanisms of particle transport making it possible to identify and assess the behavior and the interaction between different compartments and how they change in space and time in the river (Knapik *et al.*, 2015). However, many of the water quality models do not consider OC as a state variable (Shanahan *et al.*, 1998). This lack of representation of the various components of OC may affect the way that the decomposition process is calculated either through a direct impact or through an indirect impact on the overall understanding of the stocks and fluxes of OC in the water column. In addition, the existence of incomplete databases, particularly in developing countries, on OC stocks and fluxes in aquatic ecosystems also further hinders the development and validation of models of water quality.

The Red River is the second largest river in Viet Nam (after the Mekong River) and one of the five largest rivers on the coast of East Asia. The Red River Delta (RRD) is the economic hub of Northern Viet Nam and the majority of the region's population is concentrated in this basin. Over 30% (24 million) of the total population of Viet Nam (78 million) lives in the Red River basin, including over 17 million people in the delta (Thanh, 2003). The Red River also constitutes the main water source for a large percentage of the population of North Viet Nam as well as being the major outlet for wastewater. However the rapidly increasing urbanization and the transition of land towards more intensive agriculture and plantation forestry have all been linked to decreasing water quality in the Red River system (Luu *et al.*, 2010; Le *et al.*, 2015).

The objective of this work was to identify the seasonal variations of organic carbon (DOC, POC) and to determine the biodegradable fraction of DOC (BDOC) and POC (BPOC) in the Red river over an annual cycle. Then the SENEQUE/Riverstrahler model was applied to quantify riverine organic C dynamics in this large, subtropical river basin over the period 2009-2014 allowing giving insight on CO₂ respiration, and hence balancing between autotrophy and

heterotrophy in the system.

4.2.3 Material and methods

4.2.3.1 Study area

The Red River is a transboundary river basin that flows through Viet Nam, China and Laos. The total basin area is around 169.000 km² of which around 86 660 km² (51.35 % of the entire basin) are in Viet Nam. To the North, 81,240 km² (48%) is located in China with the remaining small section (1,100 km² or 0.65%) in Laos (Fig.4.2).

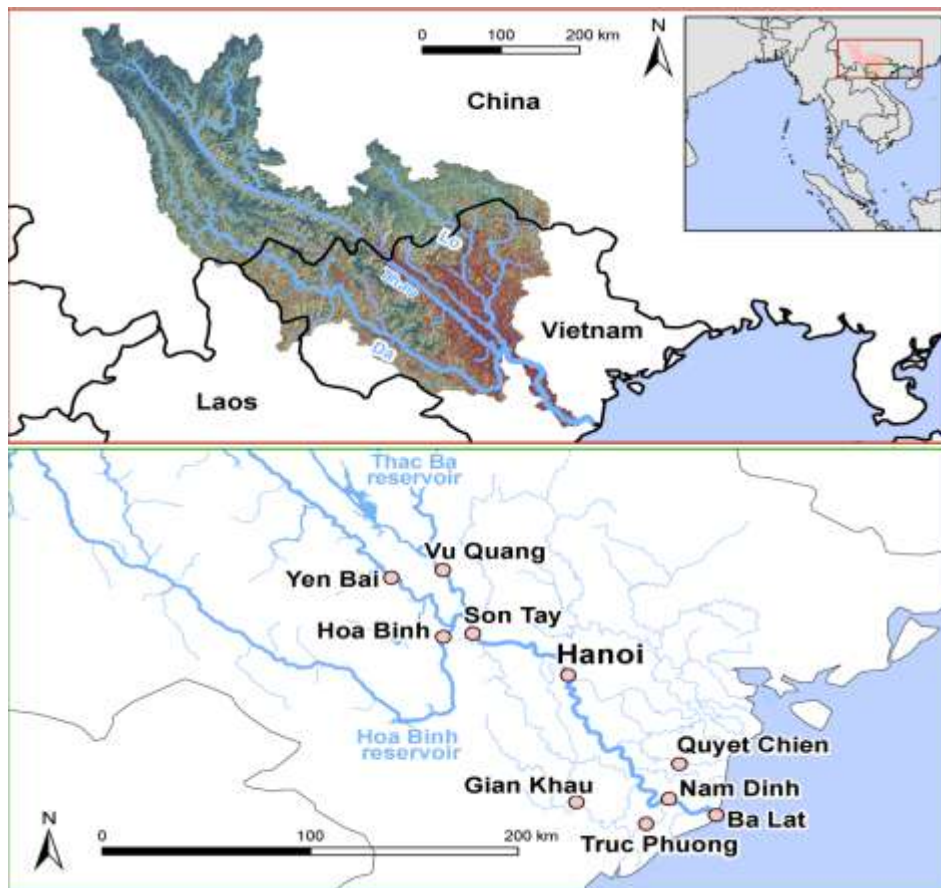


Figure 4.2: Map of the Red River and localization of the main hydrological and water quality stations.

The Red River basin is delimited between latitudes 20°23' to 25°30' North and longitudes 100°00' to 107°10' East. The RRD is located in the sub-tropical monsoon zone of the western coastal Gulf of Tonkin. During the year, the northeast monsoon occurs from November to late April when dryer, colder weather dominates. From May to October the South-West monsoon occurs and warmer, wetter weather dominates. The area is subject to high rainfall that varies from about 1,200 – 4,800 mm per year (IMH, 1997-2004) with an average of 1,900 mm of which 80% falls from May to October. From July to October, the coastal areas of the RRD are affected by low pressure systems of varying intensity that bring tropical storms and typhoons from the South China Sea.

The Red River itself is the second largest river system in Viet Nam with an average rate of flow of 3,389 m³s⁻¹ (averaged over the period 1997 - 2004). It is formed by 3 large river branches (Da, Lo and Thao Rivers) and enters into the sea at Ba Lat. The Da River has the highest discharge (about 42% of the total) and Thao River has the lowest, just about 19 % (IMRR, 2010). Discharge of the Lo River is intermediate (25.4 %), however, this river has the smallest catchment of the three. Peak flow occurs during July (32%), August (55%), September (7.3%), and October (4.4%).

Samples for DOC, POC and TSS were collected monthly from July 2013 to July 2015 at 10 gauging stations in the Red River Basin (Fig. 4.2). The sample sites are located on different river branches (distributaries) of the Red River system and include, from upstream to downstream : Yen Bai (YB) on the Thao River, Hoa Binh (HB) on the Da River, Vu Quang (VQ) on the Lo River, Gian Khau (GK) on the Day River, Truc Phuong (TP) on the Ninh Co River, Quyet Chien (QC) on the Tra Ly River, Nam Dinh (ND) on the Dao River and Son Tay (ST), Ha Noi (HN) and Ba Lat (BL) on the main axe of the Red River (Fig. 4.2). After collection, the samples were returned to the laboratory in a cooler for analysis.

4.2.3.2 Biodegradability

At four stations a second series of samples was collected for the determination of biodegradable OC over time from July 2013 to July 2014. These stations were (1) Yen Bai, located in the upstream main branch of the Red River known as the Thao River; (2) Ha Noi, after the confluence of three major upstream tributaries of the Da, Thao and Lo rivers; (3) Gian Khau, a peri-urban river system located in the Red River Delta and, (4) Truc Phuong, located in the downstream Red River on the Ninh Co River. At each station, 750 ml of sample were incubated in duplicate in glass bottles at initial *in situ* temperature and in the dark for 40 days. The samples were shaken frequently throughout the incubation. Replicate samples from each duplicate incubation were collected at 0h, 6h, 18h, 32h, 60h, 120h and 960h (40d in total) for DOC and POC determination. The biodegradable fraction of DOC and POC (BDOC and BPOC, respectively) was calculated by subtracting the concentrations of DOC and POC during the incubations at time t_{40d} from their respective concentrations at t_0 (Servais *et al.*, 1995; Garnier *et al.*, 2010).

The annual OC flux ($10^6 \text{ kg year}^{-1}$) was determined for the year from July 2013 to June 2014 using the formula of Verhoff (1980):

$$\text{FluxOC (} 10^6 \text{ kg year}^{-1}\text{)} = \frac{[\sum C_i Q_i]}{\sum Q_i} * Q_m * 3600 * 24 * 365 * 10^{-9} \quad \text{eqn. 1}$$

Where FluxOC is the OC flux in $10^6 \text{ kg year}^{-1}$, C_i is the discrete instantaneous concentration (mgC l^{-1}), Q_i is the corresponding instantaneous discharge ($\text{m}^3 \text{s}^{-1}$) and Q_m is the mean discharge for period of record ($\text{m}^3 \text{s}^{-1}$).

4.2.3.3 Analytical methods

Suspended Solids (SS) were determined after filtration of a known volume of sample on

to Whatman GF/F glass fibre filters precalcinated at 550 °C for 4h and preweighed. The filters were then dried for 2 h at 120°C and the weight of the material retained on the filter was recorded. DOC was determined on the filtrate using a Shimadzu TOC-V_E analyzer after acidification of 30ml of sample with 35µl 85% H₃PO₄. POC concentrations were determined on the same filters as for the TSS determination. Filters were then weighed after calcination at 550°C for 4 h. The difference in weight after the 2h at 120°C and after calcination was multiplied by 0.4 to provide an estimation of the POC content (Garnier *et al.*, 2010).

4.2.3.4 Model description

The SENEQUE/Riverstrahler model was developed for application in large drainage networks such as the Seine River, France (Billen *et al.*, 1994; Billen and Garnier, 1999), Scheldt River, Belgium and the Netherlands (Billen *et al.*, 2009; Thieu *et al.*, 2009) and the Danube (Garnier *et al.*, 2002). It has also been adapted to the Red River in North Viet Nam (Luu *et al.*, 2010; Le *et al.*, 2015). Although initially developed to investigate water quality and biogeochemical functioning, it has also been adapted to take into account faecal indicator bacteria, an important marker of wastewater inputs (Ouattara *et al.*, 2012; Ouattara *et al.*, 2013; Causse *et al.*, 2015; Nguyen *et al.*, sub). The model uses drainage network morphology, meteorological conditions, and land-use, point and non-point sources, to calculate geographical and seasonal variations of the main water quality variables at a 10-day time step. Here the model was applied to the Red River to simulate the fluxes of OC and to estimate the C metabolism of this large river system.

The model includes a module describing the dynamics of OC as represented in Fig. 4.3 (Garnier *et al.*, 2008). In the model, organic matter has two fractions: particulate (HP) and dissolved (HD). Each of which is sub-divided into 3 classes of biodegradability that are indicated as HP1, HP2 (rapidly and slowly biodegradable, respectively) and HP3 (refractory)

for particulate stocks, and as HD1, HD2, HD3 for the dissolved pool. The parameters used in the model were those determined experimentally and shown to be generic by Garnier et al. (1992), Servais et al. (1999), Menon et al. (2003), Billen and Servais (1989), Billen (1991). Our measurements of organic matter biodegradability (see below) confirm the coherency of these values.

The three major tributaries (the Da, the Thao and Lo Rivers) of the Red River are taken in account explicitly in the model. Each station is denoted as a function of the distance (pK in km) from an upstream station, namely Lao Cai, Son La, Tuyen Quang cities in the upstream section of the Thao, Da and Lo rivers, respectively. The main stations on the Thao River are Yen Bai, Ha Noi, Ba Lat at pK 402, 524, 652, respectively; in the Da River Hoa Binh is at pK 434; and in Lo River Vu Quang is at pK 135. The remaining part of the drainage network is considered as idealised Strahler basins according to the methodology of the Riverstrahler model (Ruelland *et al.*, 2007).

4.2.3.5 Point and diffuse sources evaluation

For running the OC module in the SENEQUE/Riverstrahler model, inputs of OC from the watershed into the drainage network have to be provided. The model distinguishes between (1) point sources such as the discharge of domestic wastewater (either directly released or treated in wastewater treatment plants - WWTPs) (Table 4.1) Le et al. (2005) and Servais et al., (2007b) and (2) diffuse sources of OC from forested, crop or grassland (considering paddy rice fields separately) and urban surfaces (Table 4.2) (Baric and Sigvardsson, 2007; Chu *et al.*, 2010; Stevenson *et al.*, 2013).

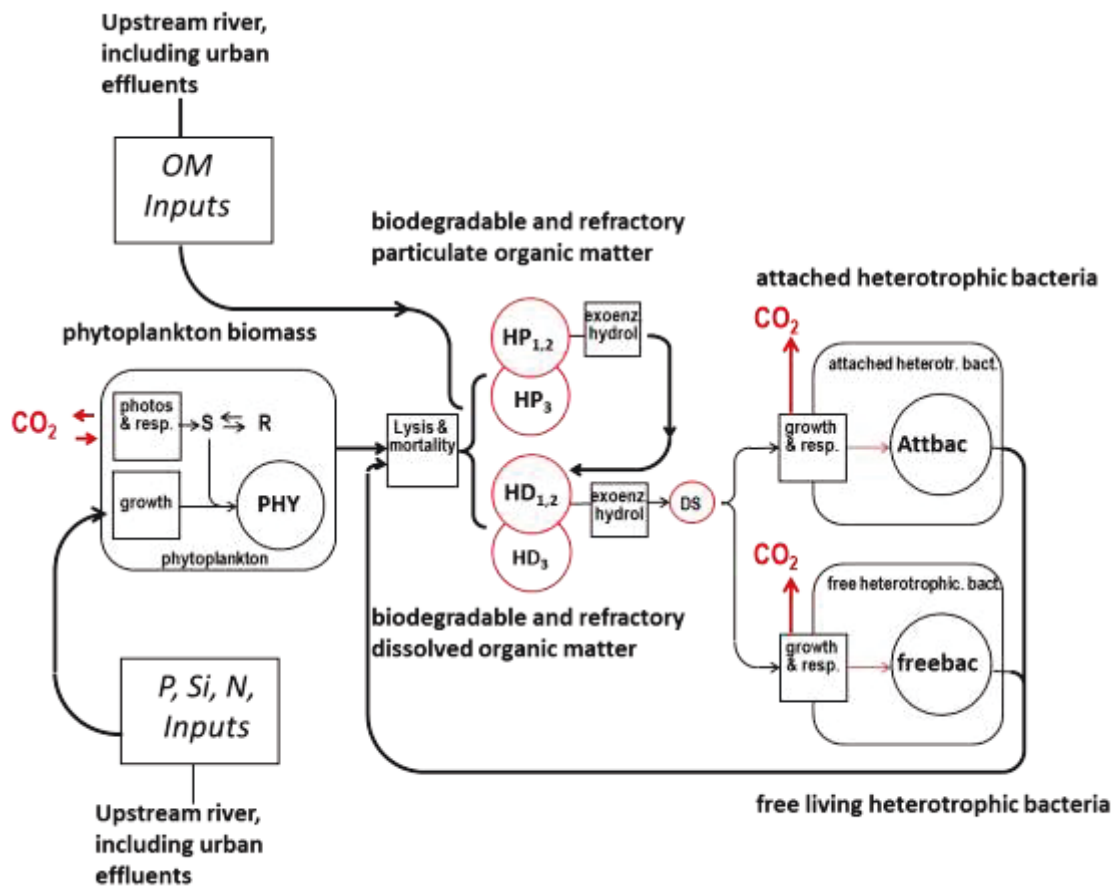


Figure 4.3: Schematic description of the SENEQUE / RIVERSTRAHLER model (adapted from Garnier *et al.*, 2008).

The point sources are taken into account in the model by considering all potential sources of wastewater in the watershed, based on the census of urban population and industrial activities (Le *et al.*, 2005). The point sources of OC were determined using either the urban population (in the case of no wastewater treatment) or the capacity (in number of inhabitant equivalent) and the type of treatment for each WWTP in the Red River watershed. The capacity of each point source was then multiplied by the corresponding specific load of OC per inhabitant and per day according to the type of treatment applied, to obtain the flux of OC released to rivers (Table 4.1). Loading values were calculated from data on the concentration of OC in domestic water and water flux, by considering an average specific water consumption of

250 L inhab⁻¹day⁻¹ that was obtained by dividing the value of the daily sewage volume 88,750 m³ day⁻¹ discharged by the corresponding population served (Chu *et al.*, 2010).

Table 4.1: The flux of OM released to rivers according to the type of treatment taken into account (gC hab⁻¹day⁻¹). From (Servais *et al.*, 1999; 2007b)

Type of treatment	OM, gC hab ⁻¹ day ⁻¹					
	HD1	HD2	HD3	HP1	HP2	HP3
Non-treated	2.4	2.4	1.75	9.6	9.6	4.8
Decantation	3.8	3.8	1.75	5.18	5.18	3.6
Biological-UV	1.04	1.04	1.75	0.96	0.96	0.80

The diffuse sources of OC were calculated on the basis of land use in all elementary sub-basins of the watershed. The GIS land use coverage information was taken from the data of Le et al. (2005). The data from literature and from available monitoring programs on the concentration of OC in small rivers, streams and ditches draining homogeneous land cover types were used to characterize OC concentrations in surface runoff and in base flow as a function of land use (Baric and Sigvardsson, 2007; Eiche, 2009; Pham *et al.*, 2010; Nguyen *et al.*, 2013). The OC concentration assigned to each of the land use classes was determined as the median value of all data compiled from these empirical surveys (Table 4.2).

Table 4.2: The median organic carbon (OC) concentration assigned to each of the land use classes for surface runoff (SR) and base flow (BF) (mgC l⁻¹). Data from a compilation of values cited in Stevenson et al. (2013); Baric and Sigvardsson (2007); Pham et al., (2010).

Type of land use	OC, mgC l ⁻¹											
	HD1		HD2		HD3		HP1		HP2		HP3	
	SR	BF	SR	BF	SR	BF	SR	BF	SR	BF	SR	BF
Forest	0.02		0.05		0.6	0.5	0.01		0.05		0.9	
Degraded forest and bare land	0.02		0.05		0.6	0.5	0.01		0.05		0.9	
Agri. land excluding rice	0.05	0.01	0.4	0.05	3	1.5	0.01	0	0.2		0.9	
Rice fields	0.1		0.4		4	1.5	0.05		0.1	0	1.3	0
Urbanized area	0.5		4	0.5	10	2	0.1	0.01	0.5	0.1	4	1

4.2.3.6 Validation data

In order to validate the modelling results, data from the monthly monitoring surveys (January 2009 to December 2014) at the ten stations along the Red River (see Fig. 4.2) were used.

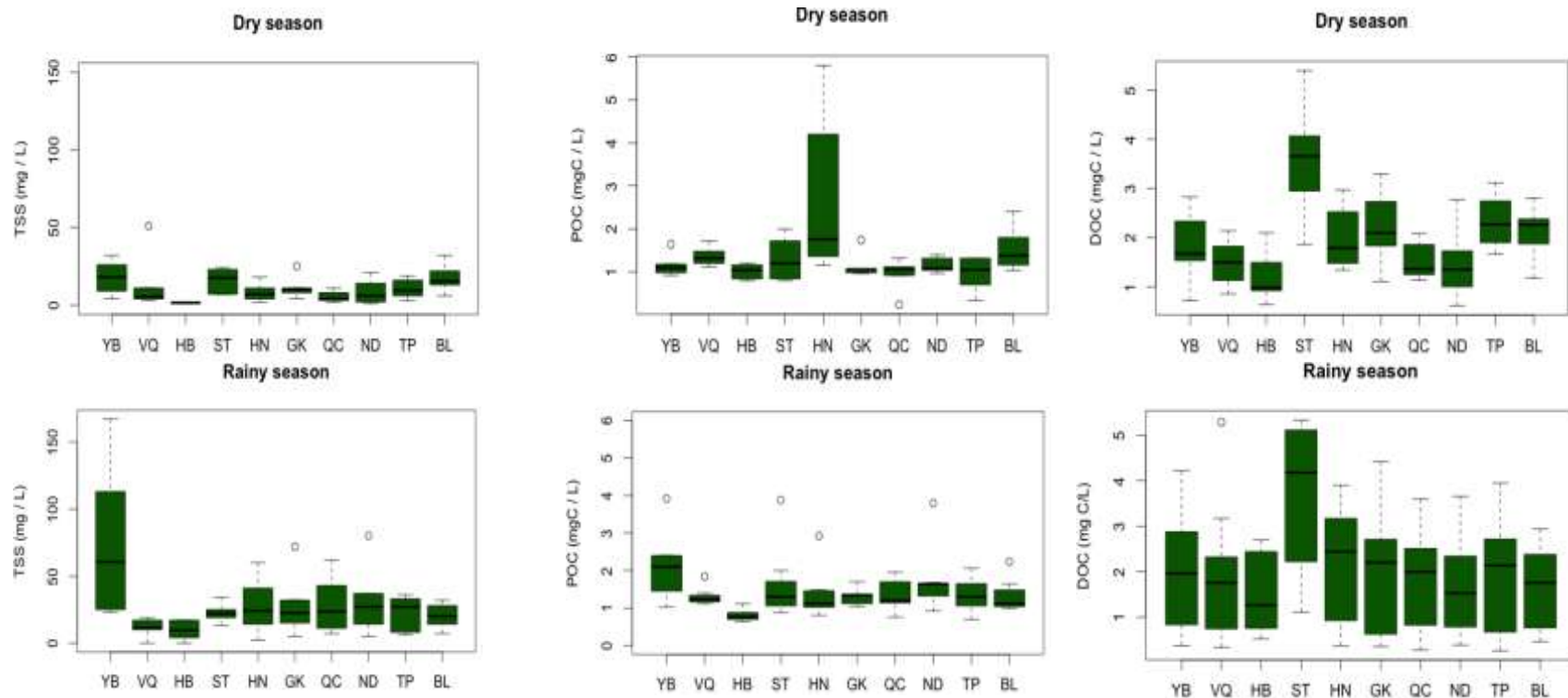
4.2.4 Results

4.2.4.1 Distributions of TSS, POC and DOC

TSS, POC and DOC concentration for the 10 sampling stations for the rainy and dry seasons are presented in Fig. 4.4. TSS concentrations differed significantly between seasons and stations over the sampling period ($p < 0.05$; Fig. 4.4) with concentrations up to three times higher during the rainy as compared to the dry season.

POC concentrations also varied between stations and seasons with a tendency towards higher values during the dry season when the stations were compared individually. POC and DOC concentrations were not statistically different between seasons, although the concentrations were more variable during the rainy season (Fig. 4.4). Overall, as TSS increased, POC also increased, reflecting the importance of POC in TSS.

Figure 4.4: Median concentrations for TSS, DOC and POC (mgC L^{-1}) during the rainy season and the dry season at the ten measurement stations. The black line represents the median value of concentration, the boxes are the upper and lower 25th and 75th percentile, the tails represent the smallest or largest values within 1.5 times the size of the box, and the dots circles are indicated outliers as they exceed 1.5 times from the 25th or 75th percentile.



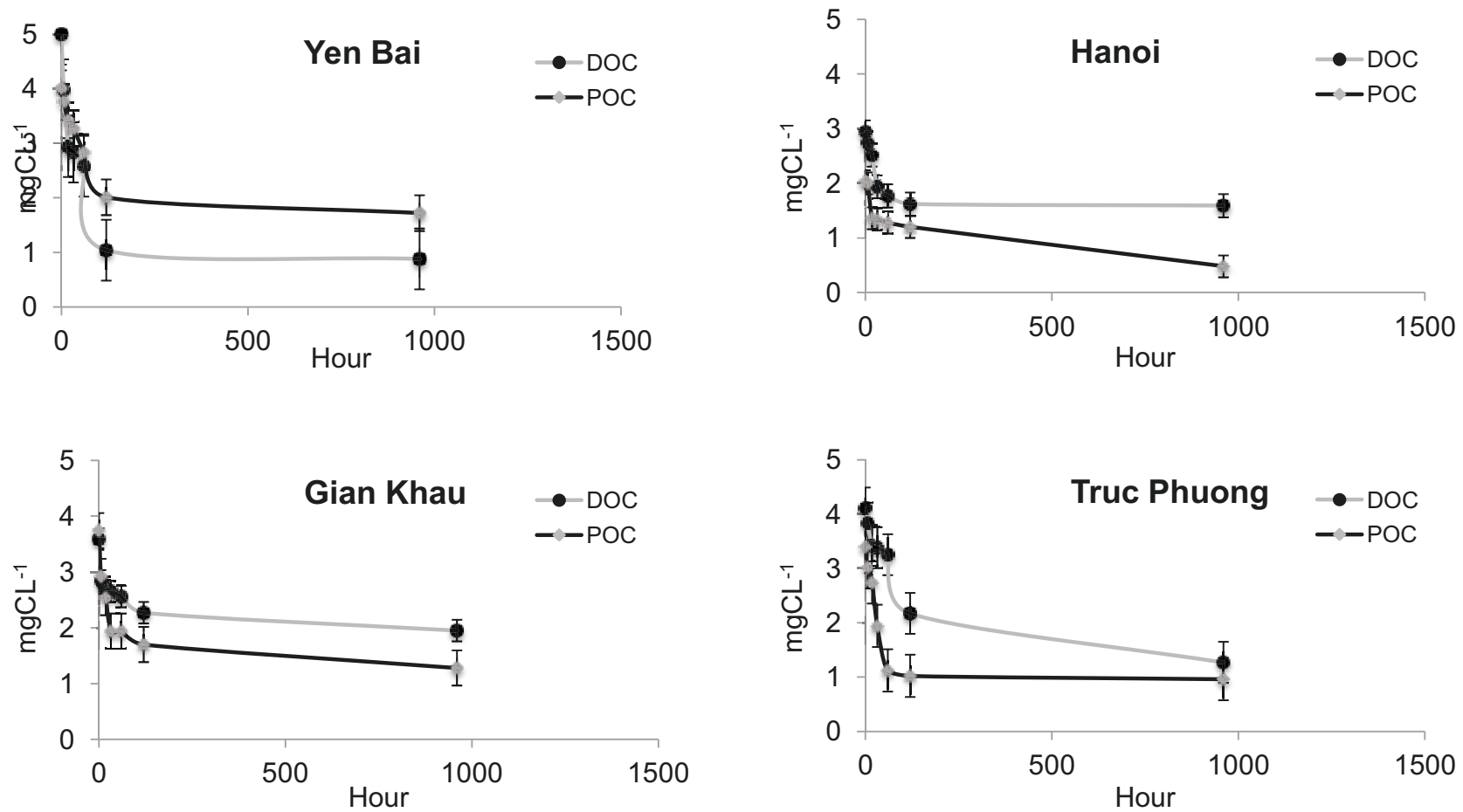


Figure 4.5: Degradation of DOC (mg C L^{-1}) and POC (mg C L^{-1}) concentrations over the 960h incubation period in rainy season (July 2013) for each of the four measured stations (Yen Bai, Ha Noi, Gian Khau and Truc Phuong).

4.2.4.2 Biodegradability of DOC and POC

At all four stations, the initial concentrations of DOC and POC sampled decreased during the biodegradation incubations with a significant effect of season (Fig 4.5). The concentration of biodegradable DOC (BDOC) was lowest (0.2 mgCl^{-1}) at Yen Bai during dry season (January 2014) and was highest (2.84 mgCl^{-1}) at Truc Phuong during rainy season (July 2014). BPOC, the concentration of biodegradable POC was lowest (0.02 mgCl^{-1}) at Ha Noi during dry season (January 2014) was highest (2.6 mgCl^{-1}) at Gian Khau during rainy season. Overall, the biodegradable fraction of TOC (BTOC = BPOC + BDOC) was $1.54 \pm 1.14 \text{ mgCl}^{-1}$ (33% % of the initial TOC), $1.4 \pm 0.49 \text{ mgCl}^{-1}$ (35 % of the initial TOC), $2.43 \pm 1.46 \text{ mgCl}^{-1}$ (36 % of the initial TOC), $1.92 \pm 0.88 \text{ mgCl}^{-1}$ (44 % of the initial TOC) at Yen Bai, Ha Noi, Gian Khau, Truc Phuong, respectively.

Flux calculation

The POC and DOC flux were calculated from the measured concentration at each station in Red River over the period 2013-2014. The results are summarized in Table 4.3.

Table 4.3: OC fluxes in 2013 - 2014 for the each station in the RRD (GgC yr^{-1}), calculated from observed concentration values and daily measurement of discharge.

OC flux	Yen Bai	Vu Quang	Hoa Binh	Son Tay	Ha Noi	Ba Lat
(10^6kgC yr^{-1})						
DOC flux	36	60	77	189	108	36
POC flux	42	58	50	135	76	28

They show that the flux of the particulate form (POC) is equal or slightly larger than the dissolved flux at the outlet of the Lo and Thao Rivers, while the

opposite is true at the outlet of the Da River, probably as a result of sedimentation in the Hoa Binh reservoir. In the main Hong branch, downstream from Son Tay, DOC dominates the flux of OC.

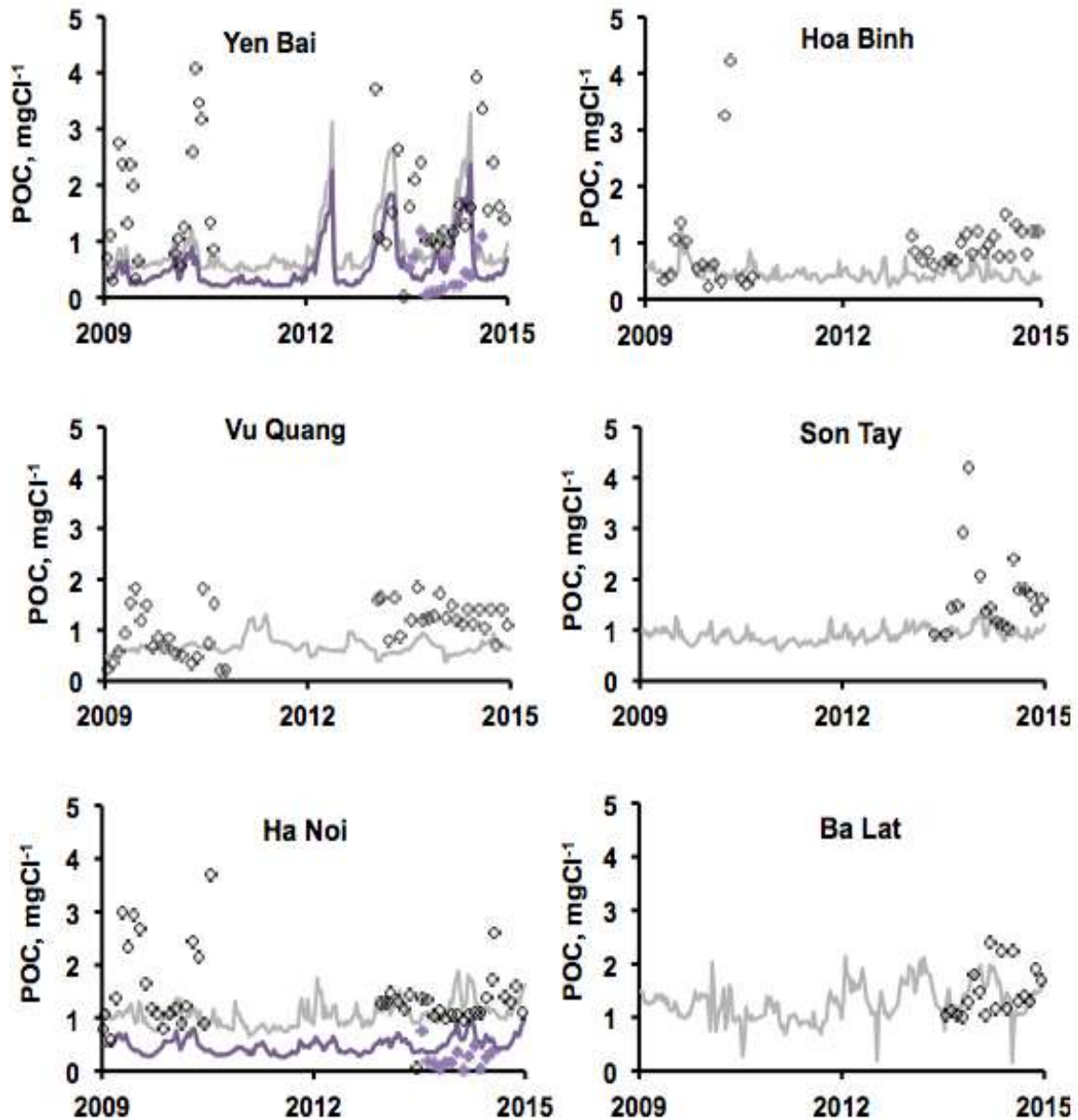
4.2.4.3 Model simulation of seasonal and geographical variations in the Red River system under current conditions

The model was run for the five years (2009 – 2014) and compared with the observed temporal changes in bulk OC concentration and that of its biodegradable fraction for both POC (Fig. 4.6) and DOC (Fig. 4.7).

In general, the model simulations of the temporal variations in OC concentrations followed the same levels as that of the *in situ* data measurements, although it was often lower for POC and higher for DOC.

The spatial distribution of OC in the river branches in terms of kilometric point (pk) at particular time of the seasonal cycle (Fig. 4.8) was also well represented by the model. For both the Da and Thao Rivers, DOC concentrations were higher at low discharge than at high discharge (Fig. 4.8). However, when POC concentrations were simulated; concentrations were highest during high discharge in the Da River, whereas in the Thao River, the opposite was found e.g. POC concentrations were highest during low discharge (Fig. 4.8). In the downstream sector of the Thao (or Hong) River, DOC and POC concentrations and C respiration rates increased in response to the elevated inputs of wastewater from this densely populated sector (Fig. 4.9). In the Da River, a strong reduction of DOC and POC concentration occurred in the region of the Hoa Binh lake (Fig 4.10) concomitant with increased C respiration rates.

Figure 4.6: Model simulated (gray line) seasonal variations of POC (mg C L^{-1}) and biodegradable POC (BPOC, mg C L^{-1} ; violet line) for six stations in the Red river system for the years 2009 - 2014. The points represent the measured values (observations) of POC and BPOC at the different stations. BPOC was only determined at Yen Bai and Hanoi.



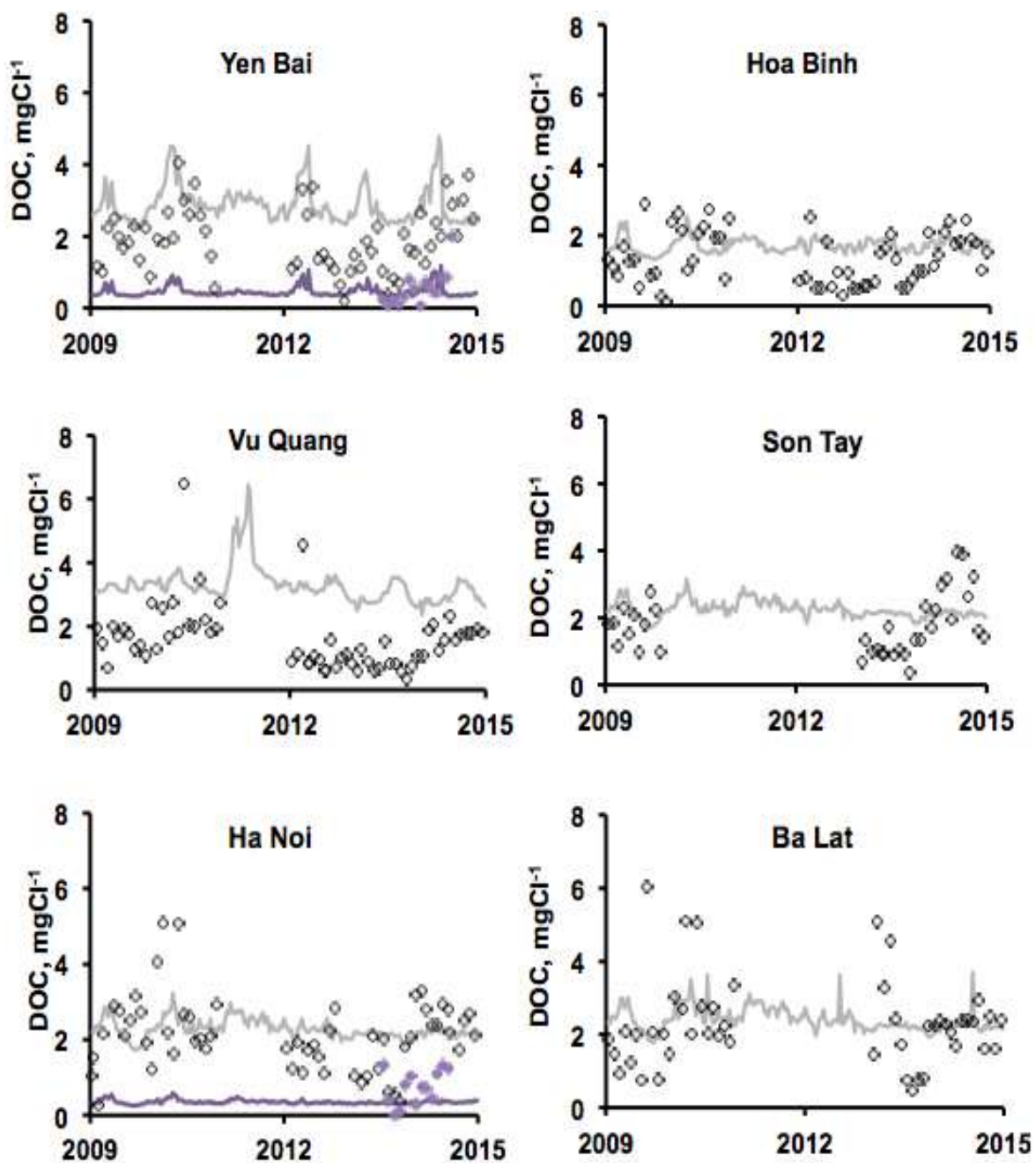


Figure 4.7: Model simulated (gray line) seasonal variations of DOC (mgC L^{-1}) and biodegradable DOC (BDOC, mg C L^{-1} ; violet line) for six stations in the Red river system for the years 2009 - 2014. The points represent the measured values (observations) of DOC and BDOC at the different stations. BDOC was only determined at Yen Bai and Hanoi.

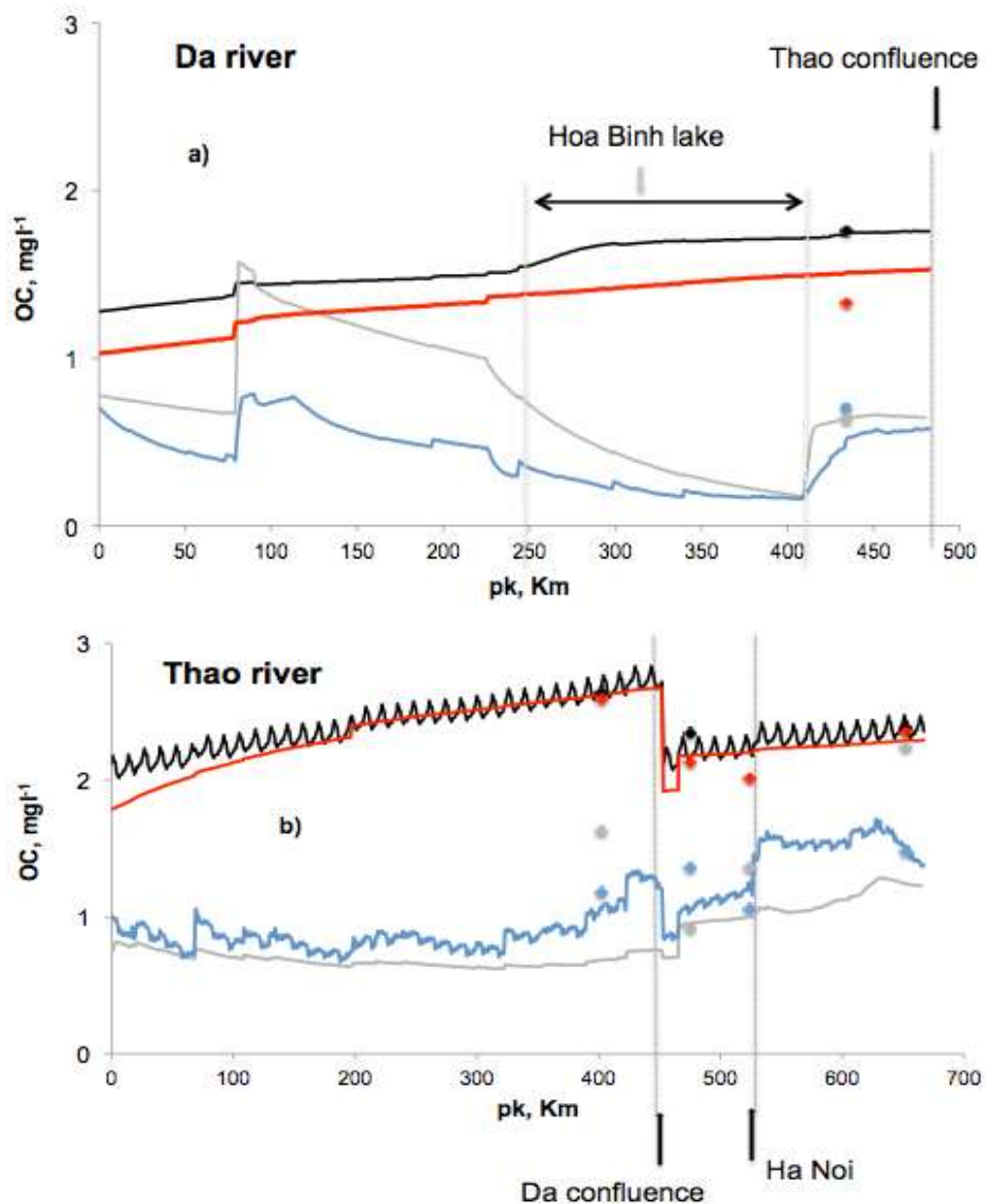


Figure 4.8: Longitudinal variations of POC and DOC concentration in the Da (a) and Thao (b) Rivers as calculated by the SENEQUE / RIVERSTRAHLER model for the years 2009-2014. The simulated DOC and POC concentrations, represented as lines on the graphs, were averaged for low (December - March) and high (May - October) discharge periods. The observations, presented as dots on the graph, are the measured values of DOC and POC in the rivers. The x-axis is a kilometeric unit (pk) along the upstream-downstream course of each tributary. The location of different features along the river courses is also noted. POC low and high water (blue and grey, respectively), DOC low and high water (black and red, respectively).

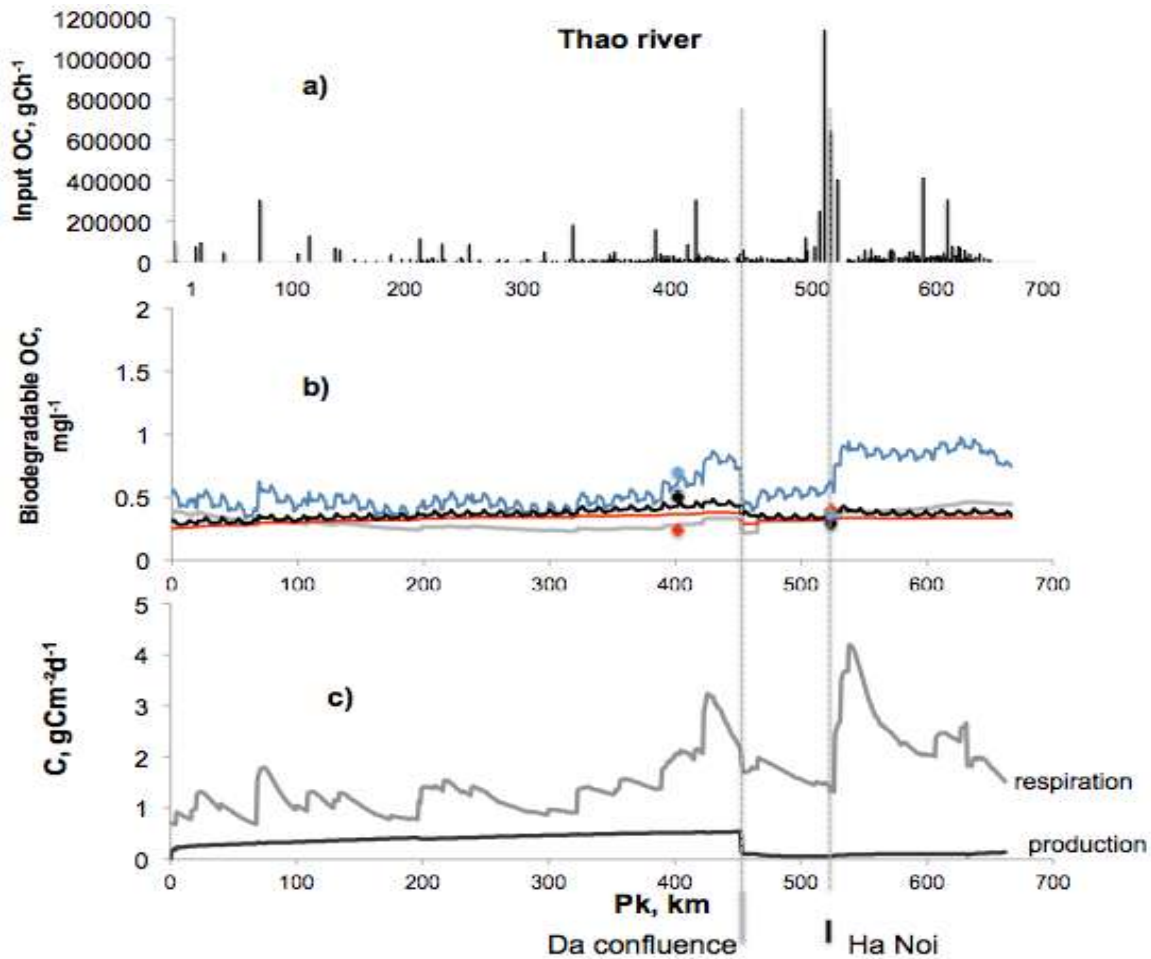


Figure 4.9: Longitudinal variations of OC inputs (a), the median biodegradability (b); respiration and production (c) in the Thao River for high and low flow situations averages over the period 2009-2014. Lines presented the model simulation and the points are the measured values. The x-axis is a kilometric unit (pk) along the upstream-downstream course of each tributary. The location of different features along the river courses is also noted. POC low and high water are blue and grey, respectively, DOC low and high water are black and red, respectively.

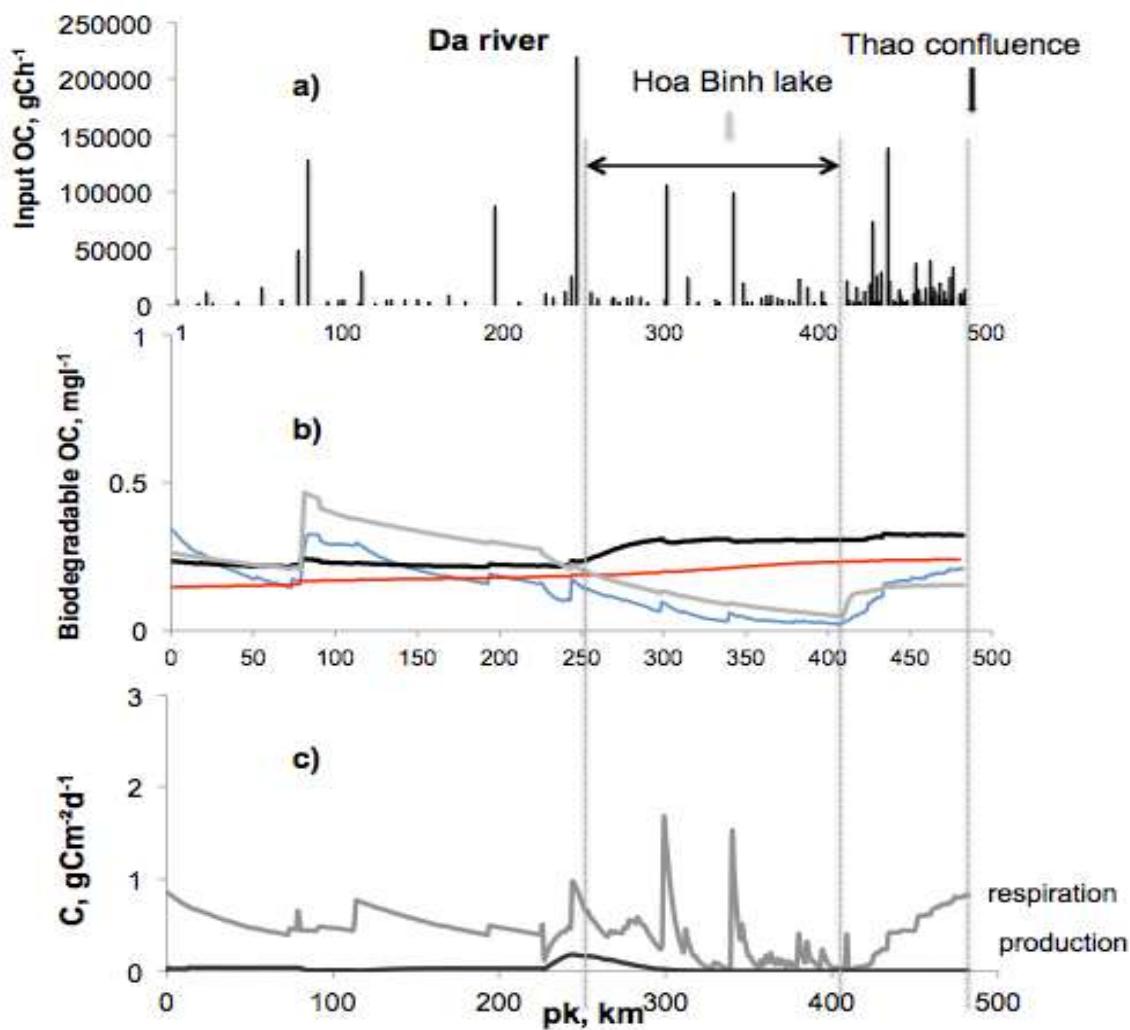


Figure 4.10: Longitudinal variations of OC inputs (a), the median biodegradability (b); respiration and production (c) in the Da River for high and low flow situations averages over the period 2009-2014. Lines presented the model simulation and the points are the measured values. The x-axis is a kilometric unit (pk) along the upstream-downstream course of each tributary. The location of different features along the river courses is also noted. POC low and high water are blue and grey, respectively, DOC low and high water are black and red, respectively.

4.2.4.4 Carbon metabolism in Red River

The modelling approach used offers the possibility to calculate the complete carbon metabolism in all river stretches of the drainage network. The spatial variations of the mean annual respiration and photosynthesis within the two main rivers of the Red River basin, the Da River and Thao River are shown in Fig. 4.9 and 4.10, respectively. The model calculations of mean net primary production ranged from zero to $1.44 \text{ gCm}^{-2}\text{d}^{-1}$ (Fig. 4.9c; Fig. 4.10c), with higher values found during spring ($0.19 \pm 0.31 \text{ gCm}^{-2}\text{d}^{-1}$, range -0.00 to $1.17 \text{ gCm}^{-2}\text{d}^{-1}$) than during summer ($1.44 \pm 0.26 \text{ gCm}^{-2}\text{d}^{-1}$, range from -0.1 to $1.45 \text{ gCm}^{-2}\text{d}^{-1}$). The values of respiration (R) were much higher and ranged from $0.34 \text{ gCm}^{-2}\text{d}^{-1}$ at Hoa Binh to $2.28 \text{ gCm}^{-2}\text{d}^{-1}$ at Yen Bai. The highest R rates ($4.82 \text{ gCm}^{-2}\text{d}^{-1}$) were observed at Yen Bai during the summer. Overall, there is a general longitudinal trend in both Thao and Da River of increasing R in the downstream sections. The ratio of production to respiration (P: R) varied from 0.00 to 0.28, reflecting the dominance of R over P in this system.

Taking into account the morphology (depth, width and length) of each river stretch, we calculated the annual values of net primary production and heterotrophic respiration from the model calculated rates for the period 2009-2014 (Table 4.4). Primary production varied between 40.9 and 57.5 GgCyr^{-1} around an average annual value of 49.1 GgCyr^{-1} . Respiration varies from 208.5 to 270 GgCyr^{-1} , with an average of 274.5 GgCyr^{-1} . This shows the predominance of heterotrophic to autotrophic processes in this system.

The average inputs of OC from point sources (e.g. urban discharge of wastewater) represented 233 GgCyr^{-1} . Diffuse sources of OC, calculated from annual runoff and base flow and the associated concentrations for each land use classes, varied from 230

to 322 GgCyr⁻¹ and average 275 GgC y⁻¹ over the 5 year period. River export of OC at Son Tay was estimated from calculated concentrations and discharge. It varied from 282 to 359 GgCyr⁻¹ and was 309 GgCyr⁻¹ on average for the 5 years period. Table 4.4 gathers the model estimates of these different fluxes of the C cycle in the Red River system up stream from Son Tay, which is represented in Figure 4.11.

Table 4.4: Model calculated C budget of the Red River system at Son Tay (in GgCyr⁻¹)

GgC yr⁻¹	2009	2010	2011	2012	2013	2014	Mean
Respiration	238.5	230.3	208.5	255.7	263.3	270	247.5
Primary production	49.6	43.7	40.9	50.3	52.3	57.5	49.1
Diffuse sources	263	258	230	322	295	280	275
Point sources	233	233	233	233	233	233	233
River export (Son Tay)	293	282	258	359	336	327	309
Ave discharge (m ³ s ⁻¹)	3112.6	2811.7	2514.4	3568.8	3453.6	3348.2	3134.9

During 2011, a dry year (mean discharge was 2514 m³s⁻¹), point sources provide a significant contribution to total OC budget (Figure 4.11b). However, during 2012, a wet year (mean discharge was 3569 m³s⁻¹), the contribution of diffuse pathways dominated over point sources of OC in the network (Figure 4.11c).

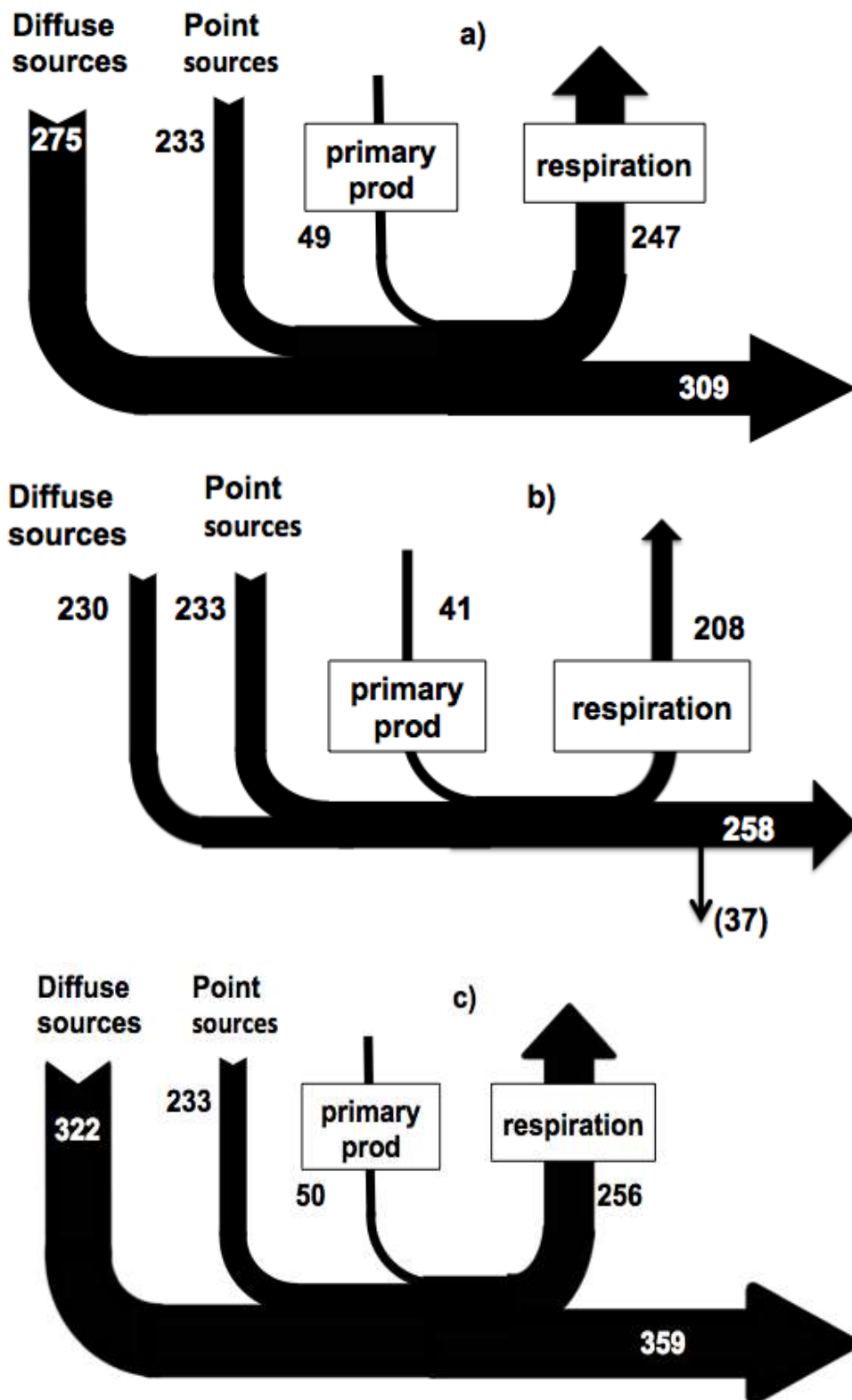


Figure 4 .11: The C budget of the Red River system at Son Tay (a: average for the period 2009-2014; b: dry year; c: wet year).

4.2.5 Discussion

4.2.5.1 Organic carbon concentration levels

The results found here are similar than those previously found for the Red River in 2008-2009 by Dang focusing more on the upstream part of the basin (Dang, 2011). The observed and modelled DOC and POC concentrations are similar to other data from tropical rivers (Table 4.5). For example, Ni et al. (2008) reported means DOC concentrations of 1.67 mgC L^{-1} in the Pearl River and Wang et al (2012) in the Yangtze River ($1.6 - 3.3 \text{ mgC L}^{-1}$). However, our values are lower than the regional means of Huang et al. (2012) ($4.93, 5.17, 4.79 \text{ mg C l}^{-1}$) for the tropical and sub-tropical rivers of the Americas, Asia and Oceania, respectively. Our POC values were also within the range of those reported by Coynel et al (2005) for the Congo River, although they are much lower than in the Mississippi River in the U.S. and the Godavari River in India.

In general, mean DOC and POC concentrations followed a similar trend in both the dry and rainy seasons with lower concentrations in the upstream stations (Yen Bai, Vu Quang, Hoa Binh), increases in the upper delta (Son Tay, Ha Noi, Gian Khau) and then lower values at the downstream stations (Quyét Chien, Nam Dinh, Truc Phuong) and at Ba Lat where there is a dilution by sea water. The low mean POC concentrations at Hoa Binh and Vu Quang can be attributed to the impact of reservoirs (Son La, Hoa Binh and Thac Ba reservoirs upstream Hoa Binh and Vu Quang stations) which are known to lead to reduced TSS and POC concentrations in the downstream reaches (Kim *et al.*, 2000). Similar variations in DOC and POC concentrations have also been observed in temperate systems where it has been proposed that the impoundment of reservoirs along rivers increases the trapping of POC (Downing et al. 2008; Tranvik et al. 2009). Reservoirs increase the residence time of the water, which may lead to increased biodegradation.

However, during high discharge, residence time is reduced within the dammed sector and biodegradation and sedimentation is also potentially reduced. The presence of a number of dams along the Red River may well explain why POC and DOC values are lower than the global averages and support the seasonal differences in the downstream waters.

In contrast, the elevated concentrations observed in the mid-reaches are probably due to the high levels of urbanization and industrialization in this river section. The main rivers (To Lich, Nhue, Day) run through several densely populated regions such as Ha Noi and Son Tay where large amounts of industrial effluent, agricultural runoff, and domestic sewage are discharged into the surface water, resulting in increased levels of TOC in riverine runoff (Ho and Hui, 2001). Finally, some of the highest DOC concentrations were observed at Gian Khau. This station is located downstream of the Van Long nature reserve which forms the largest natural wetland reserve in North of Viet Nam. This may explain the particularly high DOC concentrations and relatively low POC concentrations observed, as wetlands are known to play an important role in catchment carbon dynamics (Twilley *et al.*, 1992; Laudon *et al.*, 2011).

Table 4.5. DOC and POC concentrations from some world rivers

Environment	DOC (mgCl ⁻¹)	POC (mgC ⁻¹) b)	BDOC (%)	Ref
Pearl River	1.67	1-3.8		Ni et al. (2008)
Yangtze River	1.6-3.3			Wang et al. (2012)
Americas	4.93			Huang et al. (2012)
Asia	5.17			Huang et al. (2012)
Oceania	4.79			Huang et al. (2012)
Congo River		0.7-2.3		Coynel et al. (2005)
Mississippi River		16.9 ± 5.8		Bianchi et al. (2007)
Godavari River		20		Sarin et al. (2002)
Alaskan rivers			20 – 40	Holmes et al. (2008), Mann et al. (2012)
Forest rivers (France)			11	Servais et al. (1987)
Tongass National Forest			20–23	Fellman et al. (2009a)
Arbutus Lake			6 -18	Kang and Mitchell (2013)
Red River	0.7 – 10.9	0.91 – 6.24	-	Dang (2011)
Red River	1.58 – 3.62	0.62 – 5.8	32.9 - 54	This study

4.2.5.2 Biodegradability of DOC and POC

The range of BDOC values measured in this study were 35–45 %, higher than in another study from a headwater lake in this area (12-27% over 72h; Pommier *et al.*, 2014), however, this latter work only looked at mineralisation over 72h. The values for the Red River were also higher than the BDOC values reported for Randersfjord (Denmark; Rochelle-Newall *et al.*, 2004), the Scheldt estuary (Belgium and the

Netherlands; Bonilla-Findji *et al.*, 2009) or at the Arbutus Lake watershed in New York State (6-18 %; Kang and Mitchell, 2013). The difference between BDOC in the Red River and those of the other sites is probable related to the sources of organic matter (allochthonous – soils and domestic effluents - versus autochthonous-phytoplankton primary production-) and other processes that influence their concentration in the water column (Ribes *et al.*, 1999). Due to the tight coupling between terrestrial and aquatic ecosystems (Fellman *et al.*, 2009b; Wang *et al.*, 2012), and to the high suspended solid concentration in the Red River, the majority of its organic matter is thought to be of terrestrial rather than phytoplanktonic origin (Dixon *et al.*, 2014). The highly significant correlation between POC and TSS concentration in the Red River ($p < 0.0001$) and the lower DOC:POC ratios points towards soil organic material as being the dominant source of POC in riverine runoff of the Red River as opposed to being of autochthonous origin (i.e. from *in situ* primary production).

The sub-tropical climate of the Red River also may play a role as it is known that the remineralisation of DOC is strongly related to temperature due to the tight relationship between bacterial respiration and temperature (Rivkin and Legendre, 2001). We observed a statistically significant seasonal difference in degradability of DOC and POC between seasons, with higher rates during the warmer, rainy season. This was despite the fact that there was no significant seasonal difference in DOC or POC bulk concentrations. Further variable organic matter sources or of differing hydrological flowpaths may explain these seasonal differences (Schiff *et al.*, 1997; Fenner *et al.*, 2005).

In our biodegradation experiments, it is possible that given the high rates of carbon remineralisation in some of the incubations, that the concentration of oxygen in the incubations became depleted. However, the water samples were shaken during the

incubation and this could have allowed oxygen to diffuse into the sample. Nevertheless, this should be borne in mind. In the Red River itself, oxygen concentrations vary greatly between season and river stage. Indeed, (LE, 2005) reported that during the rainy season, oxygen concentrations averaged $5.5 \text{ mgO}_2\text{l}^{-1}$ in 2003 versus $4.5 \text{ mgO}_2\text{l}^{-1}$ in 2004 for the upstream tributaries, and $6.5 \text{ mgO}_2\text{l}^{-1}$ in 2003 against $4.5 \text{ mgO}_2\text{l}^{-1}$ in 2004 for the main branch. (DUONG, 2006) also report oxygen concentrations higher between 7.3 (6.9 – 8.1) $\text{mgO}_2\text{l}^{-1}$. These differences might be explained by the difference in SS that besides limiting photosynthesis and algal growth are known to be a support for heterotrophic bacteria which consume oxygen (e.g (LE, 2005). Moreover, our model results show that the Red River system is a strongly heterotrophic system, which explains strong biodegradation is indicated by low DO measured during monitoring.

4.2.5.3 Carbon budget and metabolism

Our model results, as well as our observation data, show negative linear relationship of OC content with river discharge, although with some seasonal variability. This is consistent with the model calculated budget of organic carbon (Fig 4.11, Table 4.4) which shows a similar contribution of diffuse pathways over OC point sources in the drainage network as a whole. There is a quite significant contribution of point sources from wastewater release, particularly in the populated area of the Delta and in the Great Ha Noi urban area, but diffuse sources linked to soil runoff and leaching bring again similar amounts of organic matter, although likely with a lower biodegradability. On the other hand, primary production is only a modest contributor of organic carbon to the system.

The differential relationships between DOC and POC and discharge in the Thao and Da Rivers are probably due to shifts in the dominance of point over diffuse sources.

In the Da river, the sharp increase in POC concentration occurs at the confluence with the Nam Na River (pk 70, Fig. 4.9). Conversely, in the Thao River, a sharp decrease of DOC and POC concentrations occurs at the confluence of the Da River (pk 450, Fig. 4.10). This is due to the much lower OC concentrations that are found in the Da River, which is characterized by the presence of the Hoa Binh reservoir (pk 250 – pk 420, Fig. 4.10) than in the Thao River. Reservoirs, as well as trapping POC, also increase the potential for phytoplankton blooms which can lead to increased DOC concentrations through the production of exudates during photosynthesis (Downing *et al.*, 2008; Tranvik *et al.*, 2009; Vinh *et al.*, 2014). The model calculates however no increased photosynthetic activity in the reservoirs due to increased depth and low light penetration. Therefore, it is probable that DOC is originating from allochthonous, terrestrial sources rather than from autochthonous sources.

Rivers are not just conduits for the transfer of OC from terrestrial systems to the environment; they also can be a significant source of CO₂ to the atmosphere from outgassing across the water-air interface (Richey *et al.*, 2002; Cole *et al.*, 2007; Farjalla *et al.*, 2009). Using the model, we determined the rates of P and R in the Red River over the 2009-2014 periods. Our calculated P rates are higher than those observed in some other tropical rivers. For example, Davies *et al.* (2008) reported that in less contaminated river systems rates of 0.01 gC m⁻² d⁻¹ to 0.20 gC m⁻² d⁻¹ are common. However, in tropical and subtropical rivers with high nutrient inputs, primary production may exceed 3.84 gC m⁻² d⁻¹. The values reported here are within the range reported by Rochelle-Newall *et al.* (2011) for the Bach Dang estuary, one of the distributaries of the Red River. These low values of primary production are most probably due to the high turbidities in the Red River as it is known that turbidity, along with nutrient concentrations are known to be a major factor controlling primary production rates in this and other aquatic

systems (Fisher *et al.*, 1988; Rochelle-Newall *et al.*, 2011).

The model calculations of median respiration rates (R) $1.44 \pm 0.864 \text{ gC m}^{-2} \text{ d}^{-1}$ are consistently higher than the rates of primary production. Moreover, the ratio of production to respiration (P:R) varied from 0.00 to 0.28. This imbalance between P and R, also observed in other river systems and estuaries, and particularly in tropical ones (Richey *et al.*, 2002; Borges *et al.*, 2015), is explained by the high respiration rates reflecting a high degree of allochthonous inputs to the Red River system (Sarma *et al.*, 2009).

We compared our respiration rates with the results of direct CO₂ flux measurements that were made with floating chambers at several stations in the Red River during the wet season in 2014. Values were in the range 0.48 to 5.52 gC m⁻² d⁻¹ (Le, unpublished data). Considering a total area of 600 km² for the river-atmosphere interface of the whole drainage network (about 430 km² for the main branches and 170 km² for basins), this range corresponds to 100-1200 GgC yr⁻¹. Our model estimated respiration rate for the whole drainage network is thus in the middle of the range of the few available measurements of CO₂ efflux from Le (unpublished data). This confirms the coherency of our model of OC of the whole Red River drainage network.

4.2.6 Conclusion

Overall, the Red River system appears to be a strongly heterotrophic system, receiving high amounts of allochthonous organic carbon from diffuse sources (soil leaching and erosion, with only limited biodegradability), and from wastewater inputs (with high biodegradability). In contrast, autochthonous primary production only

contributes about 10% of the total OC inputs. About half the total inputs of OC to the system are respired and lost to the atmosphere, while the remaining part is exported to the estuarine and coastal zone. These results further underline the importance of tropical rivers and estuaries in the global carbon budget and highlight the need for more research on these systems that represent 66.2 % of the total global freshwater flow (Huang *et al.*, 2012).

5 General conclusions and perspectives

5.1 General conclusions

The work presented in this thesis focuses on the observation and modeling of organic carbon (OC) and faecal indicator bacteria (FIB) in urban and rural areas of the Red River basin, Viet Nam. Achieving and maintaining good water quality is a challenging problem for both developing and developed countries. Untreated domestic, agricultural and industrial wastewaters are significant sources of OC and faecal contaminants in aquatic ecosystems, both of which degrade water quality. This is particularly problematic in developing countries where efficient wastewater treatment is lacking and where human populations are rapidly increasing, becoming more urban and increasingly industrialized. Moreover, waterborne pathogens and OC from wastewater are particularly susceptible to shifts in water flow and quality and the predicted increases in rainfall and floods due to climate change will only exacerbate the problems of contamination. It is therefore imperative that we have an understanding of the distribution and the factors that control the distribution and dispersion of water borne pathogens.

This PhD thesis research examined the FIB and OC in the Red River system. The Red River basin in Northern Viet Nam, is the country's most densely populated region (GSO, 2013). Red River Delta is also a major economic area and is considered the industrial heart of Viet Nam. This data, along with a large dataset of collected data points from a range of sources was then used to model OC and FIB in the 4 main sub- basins (Da, Lo, Thao and Delta) of the Red River system. Indeed, although many studies have been published on the use of models to assess water quality through faecal contamination levels, the vast majority of this work has been conducted in developed countries and similar studies from developing countries in tropical regions are lacking. A schematic of the work is presented in Figure 1.

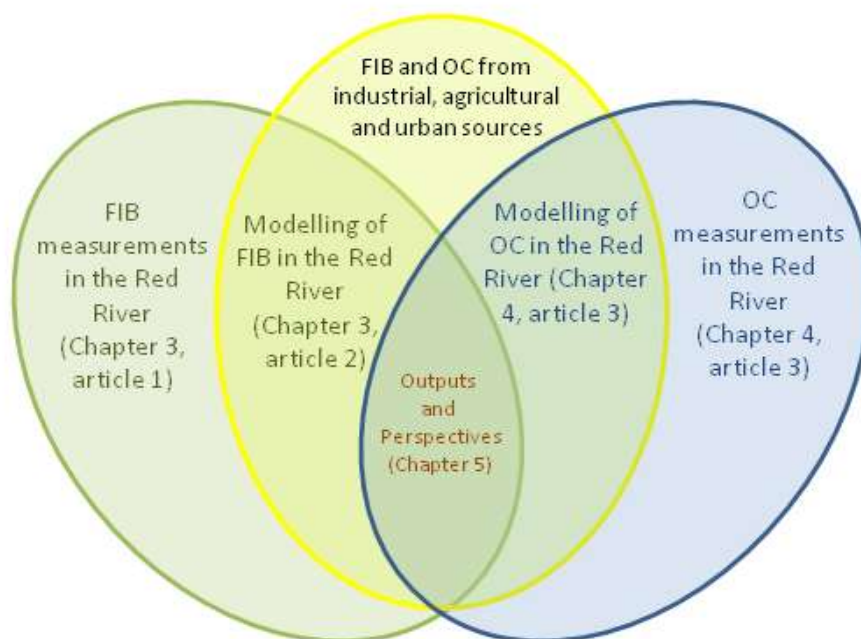


Figure 5.1: Overview of the thesis

Wastewater can contribute large concentrations of OC as well as faecal microorganisms to the aquatic systems. OC concentrations can reach as high as 3000 mgL^{-1} and FIB can be as high as 10^7 - 10^{10} MPN 100ml^{-1} in raw, untreated wastewater (von Sperling, 2007). In this work, the highest values observed were 30.9 mg l^{-1} OC and 1.1×10^9 MPN 100ml^{-1} . Over and above the health aspects associated with the use of water contaminated by wastewater, such high inputs of organic matter and microbes can also have a severe impact on the environment. High wastewater inputs can severely degrade water quality, fundamentally alter the biodiversity of an ecosystem (Dutto *et al.*, 2012; Englert *et al.*, 2013) and have important impacts on the nutrient and carbon cycling of the impacted ecosystem (Brion and Billen, 2000; Garnier *et al.*, 2001; Smith *et al.*, 2011; Trinh *et al.*, 2012; Garnier *et al.*, 2013). Moreover, organic matter is also a controlling factor of microbial activity and high concentrations such as are found in wastewaters may also support the growth of pathogenic

bacteria present in manure and sewage (Byappanahalli and Fujioka, 1998; Byappanahalli *et al.*, 2003; Son *et al.*, 2011; Rochelle-Newall *et al.*, 2015).

In this work, POC and TSS concentrations were significantly correlated (Pearson correlation = 0.519, $p < 0.0001$), reflecting the close association between POC and suspended solids. At the same time, EC_{tot} and EC_{tot} die-off rates was significantly correlated with TSS. It has been proposed that sediments serve as a suitable environment for faecal bacterial survival, perhaps due to the availability of soluble organic matter (Jamieson *et al.*, 2004; Jamieson *et al.*, 2005). However, at Yen Bai, although this station had the highest TSS concentrations and the highest number of attached FIB (EC_{att} and TC_{att}), this was also the station where the highest die-off rates were observed, which seems to contradict this idea. Given the strong relationships between microbial metabolism and organic matter, it may well be that one of the factors controlling the die-off of FIB is the lability of DOC and POC. This might be particularly important during the wet season when the highest die-off rates were observed despite there being high turbidities and high temperatures, both of which are associated with higher bacteria growth rates (Crump and Baross, 1996; Shiah and Ducklow, 1997). Moreover, the highest DOC and POC biodegradation rates were observed during the hotter, rainy season (Chapter 4). This relationship of increased FIB die-off rates at higher temperatures has been observed elsewhere in temperate environments (Ishii *et al.*, 2006; Chahinian *et al.*, 2012) and it was also observed in this work (Chapter 3). Interestingly, at Gian Khau where high percentages of EC_{att} and FC_{att} were observed, die-off rates of EC and FC were much lower. This was despite the fact that this station has lower fraction of labile DOC and POC than at Yen Bai. Of course, it is difficult to draw any firm conclusions without more data nevertheless it would be interesting to verify if the relative importance of organic carbon bioavailability and temperature in determining FIB die-off rates in a controlled laboratory experiments.

The results of the model (Chapters 3 and 4) reflected the importance of land use, discharge and the dominance of diffuse over point sources in this network for both FIB and OC. Indeed, the concentrations observed reflected the large amounts of industrial effluent, agricultural runoff, and domestic sewage that are discharged into the surface water of this river system. Although, domestic and industrial wastewater (point sources) are important sources of FIB and OC in the urban or peri-urban areas such as Ha Noi, Nam Dinh, Truc Phuong, on the scale of the whole river basin, diffuse pathways dominated over point sources even in the populated area of the Delta of Red River. The scenario, based on the predicted changes in future demographics and land use in the Red River system for the 2050 horizon, showed only a limited increase of FIB numbers compared with the present situation at all station. This was particularly the case in Ha Noi even though the population is expected to triple by 2050. This perhaps surprising result further underlines the difficulties that many developing countries are facing in terms of water quality. On one side, such a result can be considered as being relatively positive i.e. no decrease in water quality, at least in terms of FIB loads despite large increases in population. On the other hand, it also means that water quality (FIB and probably OC concentrations) will not improve in the future unless efforts are made to control diffuse sources in the basin. However, the setting up and running of such a program requires extremely large investments, strong legislation and effective education programs.

Moreover, the high inputs of allochthonous OC from both point and diffuse sources mean that the Red River system is a strongly heterotrophic system. The high inputs of OC to the Red River are supporting respiration rates that exceed primary production rates by a factor of 6. This means that the Red River is strong source of CO₂ to the atmosphere. Moreover, considering the results of Chapter 3, if the scenario for 2050 of FIB is also true for OC inputs

(i.e. relatively little change in the future despite large increases in the urban populations), it appears that this system will remain a strong source of CO₂ to the atmosphere in the future.

5.2 Directions for future research

Recognizing and understanding the link between human activities, natural process and microbial functioning and their ultimate impacts on human health are prerequisites for reducing the risks to the exposed populations. The work presented in this thesis provides, to our knowledge, some of the first, internationally published data on FIB numbers in the Red River network. During this thesis, the measured FIB concentrations in the river water were considerably higher than the clean water limits set by the Viet Nameese Government (500 colonies 100 ml⁻¹ for informal domestic water supplies). While it is difficult to estimate the number of people using these informal domestic water supplies in the Red River network, on the scale of the country, WHO estimate that around 97.6% of the population of Viet Nam has access to “improved” drinking water supplies (WHO, 2012). It can therefore be considered that the health risks to the population are low, at least in terms of domestic usage. However, during the hot, rainy season when FIB numbers and OC concentrations are higher, the number of people bathing in the river network may be higher and the associated health risks may be higher too. Nevertheless, it would be interesting to link this data with an epidemiological study to determine the real health risks associated with the use of the river water for leisure or domestic and agricultural activities.

One of the long term objectives of this thesis work is to participate in the development of a tool that can be used to aid the construction of strategies for better water quality management both on the local and, potentially, on the regional scale. A first step towards attaining this objective is to provide a scientific base for discussion with decision makers on the future management of wastewater in the Red River system. Potentially, through the

application of the model to other river systems within the complex drainage network characterizes Northern Viet Nam.

For the implementation of the Seneque-Riverstrhaler model and the simulations, previously published values of OC degradation, FIB die-offs and sedimentation rates were used. For example, we used a OC degradation rate of 0.015 h^{-1} as proposed by Servais et al. (2007), and sedimentation of OC is taken into account in the model through a sedimentation velocity of 0.006 m h^{-1} to model the fluxes of OC and an FIB mortality rate of 1.08 d^{-1} , as proposed by Servais et al. (2007a) was used. The values obtained in this work varied between 0.1 and 1.33 d^{-1} (mean $0.57 \text{ d}^{-1} \pm 0.29$) and so it is probable that improvements could be made using more site specific values. This is something that could be included in a future version of the model.

Moreover, light intensity has been identified as one of the most influential factor causing die-off of FIB (Chan *et al.*, 2015). In this work, die-off rates were conducted in the dark, which are probably appropriate for the Red River conditions where turbidity is high. However, in future work, given the importance of light as a mediator of FIB survival, it will important to take this into account, although, the high turbidities may render the estimation of the water column light field difficult.

Very little work has been published on FIB in the developing countries of Southeast Asia, with the notable exception of some work from a rural catchment in Laos (e.g. Ribolzi *et al.*, 2011; Causse *et al.*, 2015; Ribolzi *et al.*, 2016a). It would therefore be interesting to investigate the dynamics of FIB and OC e.g. in the Mekong River and delta. The large, urbanized delta of this transnational river provides a very interesting opportunity of studying the sources and losses of FIB. Moreover, the presence of a large, shallow, flood plain lake (Ton Le Sap) in Cambodia, upstream of the delta makes this a particularly interesting site to study the dynamics of FIB and OC. Moreover, the lower Mekong remains one of the last

unregulated, given the specific hydrodynamics of the Ton Le Sap – Mekong system, it would be very interesting to study particularly as in many dams are planned in the future on this system (Holtgrieve *et al.*, 2013). Ton Le Sap has many floating villages that have release their wastewater (of both animal and human origin) directly into the lake. Further, by including specific markers of the origins of FIB (stannols or microbial biomarkers), it would be possible to clearly identify the sources of the FIB and, potentially, OC and then further refine the possibilities for management and contamination reduction.

At this stage, besides the acquisition of original data regarding the origins and fate of faecal contamination, one of the major results of this work concerns the carbon metabolism of the Red River. We calculated that the system is highly heterotrophic and so these model results provide an important base for further work. Little data exists on the role of tropical rivers and estuarine systems as a source or sink of CO₂ to the atmosphere and the work presented here shows that the Red River is a large source of CO₂ to the atmosphere. It is therefore important to conduct *in situ* measurements over a period of time to provide some ground-truthing data for these calculations.

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7 Appendices

7.1 Appendix I: List of publications in international journals of Rank A

1- Rochelle-Newall E., Nguyen Thi Mai Huong, Le Thi Phuong Quynh, Sengtaheuanghoung, O, Ribolzi O. A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions, 2015. *Frontiers in Microbiology* section Aquatic Microbiology: 6, 308. doi: 10.3389/fmicb.2015.00308.

2- Nguyen Thi Mai Huong, Le Thi Phuong Quynh, Garnier, J., Janeau, J.-L., Rochelle-Newall, E. 2016. Seasonal variability of faecal indicator bacteria numbers and die-off rates in the Red River basin, North Viet Nam. *Sci. Rep.* 6, 21644; doi: 10.1038/srep21644.

3- Nguyen Thi Mai Huong, Billen, G, Josette Garnier, Emma Rochelle-Newall, Le Thi Phuong Quynh. Modeling of Faecal Indicator Bacteria (FIB) in the Red River basin (Viet Nam). *Submitted to Environmental Monitoring and Assessment (12/01/2016)*.

4- Nguyen Thi Mai Huong, Billen, G, Josette Garnier, Le Thi Phuong Quynh, Emma Rochelle-Newall. Organic carbon transfers in the subtropical Red River system. *In preparation for submission to Biogeochemistry in the spring of 2016*.

7.2 Appendix II: List of oral and poster presentations at conferences and seminars

1-Nguyen Thi Mai Huong, Emma Rochelle-Newall, Le Thi Phuong Quynh, Josette Garnier, Gilles Billen. Preliminary results of DOC and Coliform contents in water environment of the Red river system. UMR iEES, Paris (14th March 2013) *Poster*

2-Nguyen Thi Mai Huong, Emma Rochelle-Newall, Le Thi Phuong Quynh, Josette Garnier, Gilles Billen. Degradation of bacteria and DOC (dissolved organic carbon) in water environment of the Red river system. Paris, UMR METIS (8th January 2014). *Poster*

3-Nguyen Thi Mai Huong, Emma Rochelle-Newall, Le Thi Phuong Quynh, Josette Garnier, Gilles Billen. Water quality from Hanoi to Hung Yen. Hanoi (8th April 2014). *Oral*

4-Nguyen Thi Mai Huong, Emma Rochelle-Newall, Le Thi Phuong Quynh, Josette Garnier, Gilles Billen. Distribution and die-off rates of faecal indicator bacteria in the Red River, Viet Nam. The Asia-Pacific Network for Global Change Research (APN) program. Ha Noi, December, 2014. *Oral*

7.3 Appendix III: List of conference proceedings

1- Carbon Flux and Emissions from the Red River: Human Activities and Climate Change. Thi Phuong Quynh Le, Xi Xi Lu, Josette Garnier, Gilles Billen, Thi Thuy Duong, Cuong Tu Ho, Thi Bich Nga Tran, Thi Mai Huong Nguyen, Thi Bich Ngoc Nguyen and Zhou Yue. APN Science Bulletin Issue 3, March 2013, 92 - 95 ISSN 2185-761x.

2- Carbon Flux and Emissions from the Red River: Human Activities and Climate Change. Thi Phuong Quynh Le, Xi Xi Lu, Josette Garnier, Gilles Billen, Thi Thuy Duong, Cuong Tu Ho, Thi Bich Nga Tran, Thi Mai Huong Nguyen, Thi Bich Ngoc Nguyen and Zhou Yue. APN Science Bulletin Issue 4, March 2014, 68 - 71 ISSN 2185-761x.

3- Carbon Emissions and Fluxes from the Red River (Viet Nam and China): Human Activities and Climate Change (3), 2015. Le Thi Phuong Quynh J. Garnier, G. Billen, XiXi Lu, TT Duong, CT Ho, TBN Tran, Thi Mai Huong Nguyen, TBN Nguyen, DA Vu, BT Nguyen, Quoc Long Pham, et al. APN Science Bulletin (ISSN 2185-761x), 5, 38-39.

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Abstract

In many developing countries, poor water quality poses a major threat to human health and the lack of access to clean drinking water and adequate sanitation continues to be a major brake on development. The Red River is the second largest river in Viet Nam and constitutes the main water source for a large percentage of the population of North Viet Nam. This thesis presents the results from observations and modeling of both faecal indicator bacteria (FIB) and organic carbon (OC) in the Red River system, North Viet Nam. The objective of this work was to measure FIB numbers and OC concentrations in this system and then to model these parameters in order to investigate scenarios for 2050 when population in the area is estimated to have doubled. The dataset was then modeled using the Seneque/Riverstrahler model in order to investigate the dynamics and seasonal distribution of FIB and OC in the Red River and its upstream tributaries. A scenario, based on the predicted changes in future demographics and land use in the Red River system for the 2050 horizon, showed only a limited increase of FIB numbers compared with the present situation. This was particularly the case in Ha Noi even though the population is expected to triple by 2050. The OC inputs and the resulting heterotrophic respiration of this OC resulted in a system that was a strong CO₂ source. The model results also reflected the importance of land use, discharge and the dominance of non-point sources over point sources for FIB and OC in the Red River. This thesis provides some new information on FIB in the Red River as well as providing a base for discussion with decision makers on the future management of wastewater in the Red River.

Keywords: Red River, Faecal Indicator Bacteria, Organic Matter, Seneque/Riverstrahler model, human impacts

Tóm tắt

Ở các nước đang phát triển, ô nhiễm nước đặt ra mối đe dọa lớn đối với sức khỏe con người và thiếu nước sạch và vệ sinh môi trường vẫn tiếp tục là vấn đề chính cho phát triển kinh tế - xã hội. Sông Hồng là con sông lớn thứ hai tại Việt Nam và là nguồn cung cấp nước chính cho bộ phận lớn dân cư ở miền Bắc Việt Nam. Luận án này trình bày các kết quả quan trắc thực tế và kết quả mô hình hóa về vi khuẩn chỉ thị phân (FIB) và cacbon hữu cơ (OC) ở hệ thống sông Hồng, miền Bắc Việt Nam. Mục tiêu của nghiên cứu này nhằm đo đạc thực tế giá trị FIB và OC trên sông Hồng và sau đó sử dụng mô hình mô phỏng các thông số này cho kịch bản năm 2050 khi mà dân số ở khu vực này được ước tính để có tăng gấp đôi. Sử dụng mô hình Seneque/Riverstrahler mô phỏng động học và sự phân bố theo mùa của FIB và OC trong sông Hồng và các sông nhánh thượng lưu. Kết quả mô phỏng từ một kịch bản, dựa trên sự thay đổi trong tương lai về dân số và sử dụng đất trong lưu vực sông Hồng năm 2050, cho thấy chỉ giá trị FIB tăng rất ít so với mô phỏng hiện tại. Điều này là đặc biệt đối với trường hợp tại Hà Nội khi mà dân số dự kiến sẽ tăng gấp ba vào năm 2050. Nguồn cung cấp đầu vào OC và các quá trình hô hấp dị dưỡng OC đã tạo ra trong hệ thống sông một nguồn CO₂ lớn. Các kết quả mô hình cũng phản ánh mức độ quan trọng của việc sử dụng đất, lưu lượng nước và nguồn thải phát tán hơn so với nguồn thải điểm cho FIB và OC ở sông Hồng. Luận án này cung cấp một số thông tin mới về FIB ở sông Hồng cũng như cung cấp cơ sở khoa học cho các nhà hoạch định chính sách về quản lý nước thải của lưu vực sông Hồng trong tương lai.

Từ khóa: Red River, Vi khuẩn chỉ thị phân, Chất hữu cơ, mô hình Seneque/Riverstrahler, tác động của con người.