THE ASSESSMENT OF THE QUALITY OF WATER RESOURCES IN NORTHERN THAILAND: TOWARDS SAFE DRINKING-WATER ACCESS FOR VULNERABLE POPULATIONS

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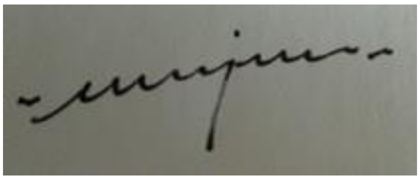
DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.



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SUMMARY

Water quality degradation is a serious environmental problem in Thailand and is inextricably linked to human-related activities. The degradation of water quality not only depletes the supply for beneficial use, but it also poses a threat to human health. Contaminated water supplies may contain hazards and when ingested, health-related problems will ensue. The overarching objective of this study was to assess the quality of water resources in Northern Thailand. Three important local drinking-water quality problems and the associated health hazards were investigated.

Firstly, the extent of faecal contamination of groundwater resources from on-site sanitation systems commonly used in Northern Thailand were assessed. Faecal indicators from wells were monitored for a year to understand the seasonal effects on the level of contamination in local groundwater. All wells showed signs of faecal contamination but generally, shallow wells were more susceptible to contamination. The level of faecal contamination in groundwater generally increased during the wet season. However, concentration of faecal indicators responded differently to rainfall distribution and water table level, implying the importance of the hydroclimatological factors in the transport of faecal contaminants.

Faecally contaminated water sources often contain disease-causing microorganisms. In the following part of this study, the prevalence of *Cryptosporidium* and *Giardia* – two important waterborne pathogens of faecal origin – was investigated. Water bodies from the Mae Kuang Catchment were sampled during the dry and wet seasons. The frequency of detection doubled during the rainy season, reflecting the importance of water in pathogen transport. Alarmingly, both pathogens were detected in 80% of the sampling sites of Lai River which drains into the Mae Kuang Reservoir, an important source of drinking-water to many local towns. Faecal samples from beef and dairy cattle (important hosts of *Cryptosporidium* and *Giardia*) at the study site were screened for the presence of these pathogens. Both pathogens were detected in beef cattle but not in dairy cattle. This discrepancy may be due to the difference in livestock management strategies.

Health hazards in drinking-water are typically associated with contamination from anthropogenic sources. However, naturally occurring health hazards such as fluoride can also occur. Sustained consumption of high-fluoride water can lead to fluorosis, a chronic health condition that is endemic in certain parts of Northern Thailand. In this section, samples from drinking-water wells were tested for fluoride and two high-fluoride zones in the provinces of Chiang Mai and Lamphun were mapped. At the Chiang Mai site, the high-fluoride waters originate from a nearby geothermal field. Fluoride-rich geothermal waters are distributed across the area following natural hydrological pathways of water flow. At the Lamphun site, a well-defined, curvilinear high-fluoride anomalous zone, resembling that of a nearby conspicuous fault, was identified. This similarity provides evidence of the existence of an unmapped, blind fault as well as its likely association to a geogenic source of fluoride related to the faulted zone.

Although Thailand receives abundant rainfall, the growing population and burgeoning economy will further increase water demand beyond the available supply. Additionally, anticipated changes to the climate system will make the management of water more challenging. The quality of drinking-water resources must be continuously monitored and the current wastewater management practices must improve to ensure a sustainable supply of drinking-water for present and future populations.

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CHAPTER 1 – PROLOGUE

1.1 The Global Water Resources

Water is essential for all life on Earth. For human beings, we are aware of its function: water is needed for drinking, producing food, cleaning and washing — in essence, for maintaining our health and dignity. Water is also required for power generation, industrial production and transporting people and goods – all are necessary for allowing societies to function.

Nearly all of the water on the planet – approximately 97.4% – resides in the oceans, and is too saline for consumption without treatment (Jury and Vaux, 2007; Shiklomanov, 1993). A large portion of the rest, about 2.0%, is also unavailable because it is locked in glaciers and ice caps. Humans and all the other terrestrial life must subsist on the remaining 0.6%. The global freshwater resource that is potentially available for human use can be categorised as surface or sub-surface (ground) water, which, collectively amounts to roughly 475 million Gm^3 (Shiklomanov, 1993). This seems like a staggering and sufficient quantity, but much of the deeper sub-surface resources are not typically accessible and oftentimes, not renewable (e.g. fossil water).

Falkenmark and Rockstrom (2004) divided the renewable and readily available freshwater resources into two categories: *green water* and *blue water* (Figure 1.1). Green water is precipitation which subsequently returns to the atmosphere following evaporation or transpiration; blue water is volume that remains. The amount of blue water resources is estimated to be between 33,500 to 47,000 Gm^3, merely a fraction of the total available fresh water in the world (Postel et al., 1996).

Increasingly, water resources are threatened as human populations grow and the ensuing demand for water increases. Today, water scarcity affects 40% of the world's population and this value is expected to increase (UN, 2015). Water scarcity is defined as '*the point at which the aggregate impact of all users impinges on the supply or quality of water under prevailing institutional arrangements to the extent that the demand by all sectors, including the environment, cannot be satisfied fully*' (WWAP, 2012). As such, water scarcity can be physical (lack of water of sufficient quality), economic (lack of adequate infrastructure, due to financial, technical or other constraints) or institutional (lack of institutions for a reliable, secure and equitable supply of water) (UN, 2015).

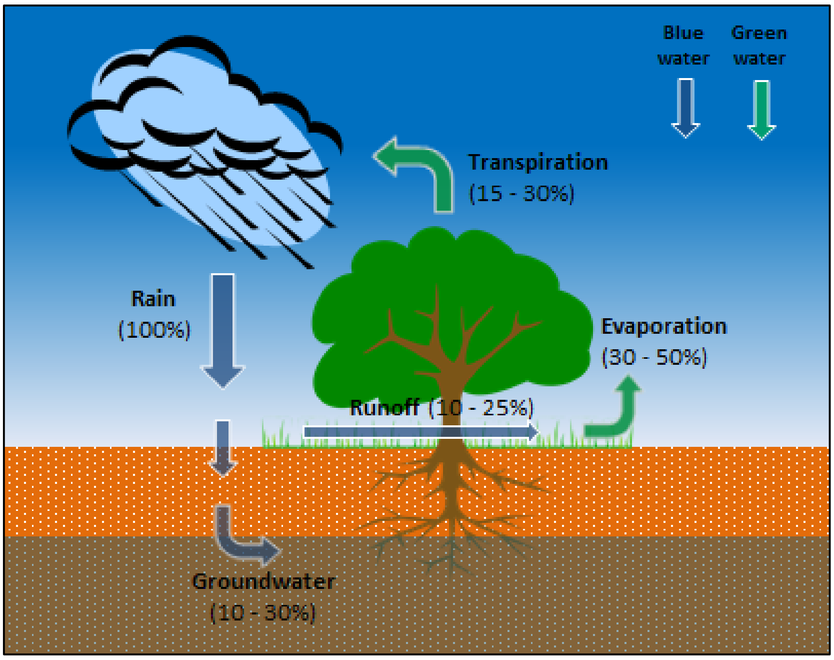

Figure 1.1: Blue and green water balance (after Rockstrom and Falkenmark, 2015)

Currently, one fifth of the global population is living in areas with physical water scarcity (UNEP, 2012). Arid regions (e.g. Northern Africa, Sub-Saharan Africa, Arabian Peninsula, and Central Asia) are most often associated with physical water scarcity. However, water scarcity also occurs in areas where water is apparently abundant but water resources are overcommitted for various usages. At present, municipalities account for 12% of total freshwater withdrawal globally. Industrial sectors account for 19%, while agriculture, the largest consumer of water, takes up the remaining 69%, mostly through irrigation (UN, 2015). Global withdrawals (gross amount of water extracted from any source in the natural environment for human purposes) have tripled over the last fifty years to meet the demands of a growing population with increasing wealth and consumption levels (WWAP, 2009). The demand for water is expected to increase in all sectors; by 2030, the world is projected to face a 40% global water deficit under a 'business-as usual' scenario (UN, 2015; WWAP, 2012).

1.2 Linking Water Quantity and Quality

Compounding water scarcity is water quality degradation. Degradation reduces the amount of water available for drinking in particular. Although inextricably linked, water quantity and quality are not often measured simultaneously (UNEP, 2008). Water quantity is often measured by means of remote hydrological monitoring stations that record water level and velocity. It can therefore be undertaken, to a certain extent, with minimal human involvement. In contrast, water quality (with the exception of a few basic parameters e.g. pH, temperature, turbidity) is more difficult to monitor. It usually is determined by analysing samples of water collected manually from monitoring stations at regular intervals. The costs associated with monitoring all the parameters that influence water quality prevents water quality monitoring from being undertaken as frequently as water quantity monitoring (UNEP, 2008).

The quality of any body of water is a function of either or both natural and human factors. In the absence of anthropogenic influences, water quality is determined by three major natural sources of dissolved and soluble matter: (i) the atmospheric inputs of material; (ii) the degradation of terrestrial organic matter; and (iii) the weathering of geological matter (Meybeck and Helmer, 1989). Notwithstanding cases of naturally-occurring health hazards (e.g. arsenic and fluoride in groundwater; pathogens derived from faecal waste of wild animals), fresh water is generally fit for human consumption and other practical uses.

The degradation of water quality can occur through water *contamination* or *pollution*. Contamination is the presence of elevated concentrations of substances (chemical or biological) in the environment above the natural background level (Chapman, 2007; Sciortino and Ravikumar, 1999). On the other hand, pollution is contamination that results in or can result in adverse biological effects to resident communities (Chapman, 2007; Sciortino and Ravikumar, 1999).

Degradation of water quality at a particular site not only depletes the beneficial supply of water for use, but it also poses a threat to ecosystem and human health. For example, globally, the most prevalent water quality problem is eutrophication – a result of excessive input of nutrients (usually nitrogen and phosphorus) in water bodies (WWAP, 2009). High nutrient loadings may cause blooms of algae which can out-compete other organisms for oxygen leading to the kills of other aquatic lives. While eutrophication is a natural process, increasingly it is associated with human related activities. For example, domestic wastewater and fertiliser from agriculture are major sources of these nutrients.

The improper discharge of inadequately treated domestic wastewater into aquatic environments represents one of the biggest threats to human health. Domestic wastewater contains human excreta that are oftentimes laced with pathogenic microorganisms. More than 80% of wastewater in developing countries worldwide is discharged untreated (WWAP, 2009). The consumption of untreated wastewater can lead to various diseases. Diarrhoea, a common water- and faecal-related disease, is the cause of 1.8 million deaths every year, of which, 88% are water-related (UNEP, 2010). In Southeast Asia alone, diarrhoea is responsible for as much as 8.5% of all deaths. While diarrhoea-related deaths have decreased over the last 50 years, diarrheal morbidity remains high or is increasing (UNEP, 2010). Every year, children in developing countries suffer from 4-5 debilitating episodes of diarrhoea (UNEP, 2010). Recurring bouts of diarrhoea can exacerbate malnutrition, which may result in long-term debilitating effects, including stunting and wasting.

1.3 Water Resources in Thailand

1.3.1 Water Quantity

Thailand, being a tropical country, receives abundant rainfall annually. The rainfall distribution in Thailand varies over space and time. The southern part of the country receives more rain. For example, Songkhla Province (Southern Thailand) receives approximately 2,000 mm rain per year while rainfall in Chiang Mai Province (Northern Thailand) is a little over 1,100 mm annually. The nation’s capital, Bangkok in Central Thailand, receives nearly 1,650 mm every year.

Heavy rainfall events occur during the wet season, between May and October, under the influence of the southwest monsoons and tropical storms hailing from the Bay of Bengal (WEPA, 2012). During the dry season – November to April – the climate is influenced by the northeast monsoon from China and tropical storms from the South China Sea (WEPA, 2012). The total volume of water from rainfall in Thailand is estimated to be approximately 800 Gm^3 (FAO, 2015a), of which about 75% (green water) is lost through evaporation, evapotranspiration and infiltration (WEPA, 2012). The remaining 25% (blue water) constitutes the runoff that flows in the rivers and streams within 25 drainage basins in the country (Figure 1.2).

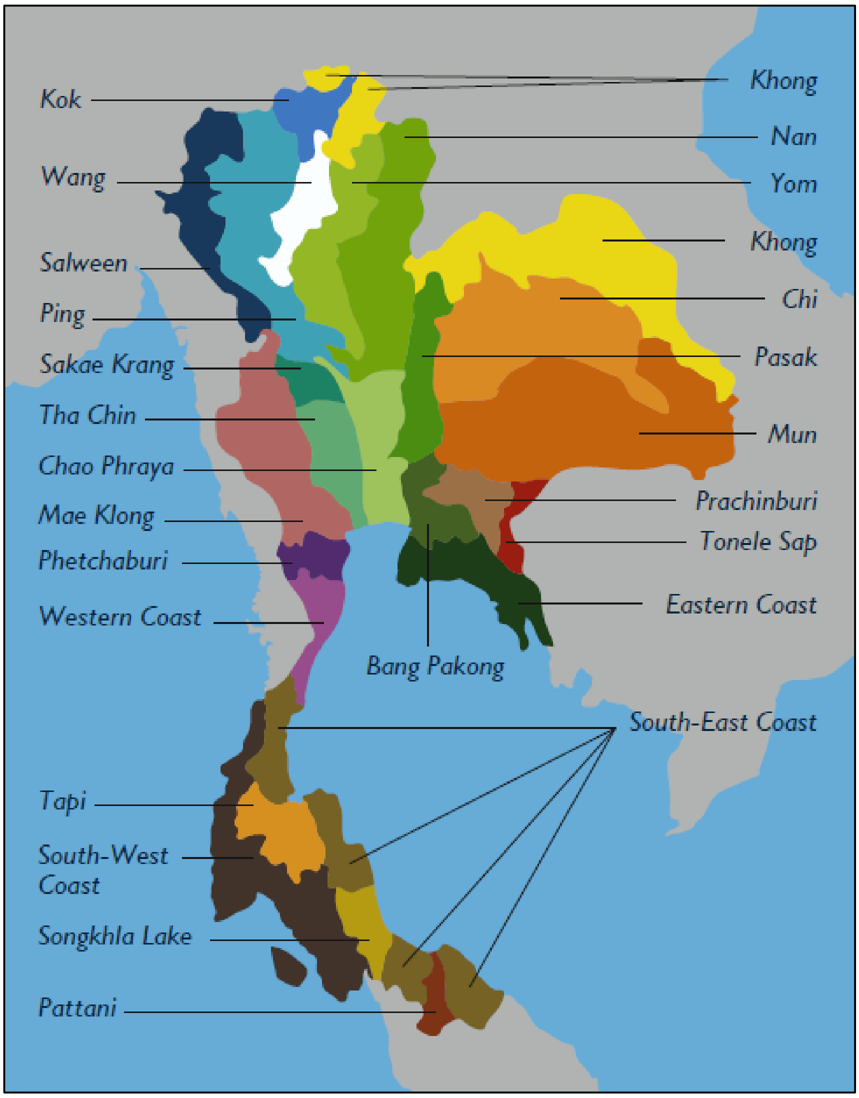

Figure 1.2: The 25 drainage basins in Thailand (Source: WEPA, 2012)

Of the total annual runoff, about 37% of the fresh surface water resources are stored in reservoirs, most of which are managed by the Electricity Generating Authority of Thailand (EGAT) for the production of electricity, in addition to meeting agriculture, industry, and domestic demands (World Bank, 2011). To maintain the minimum water storage requirement for the generation of electricity, only about 60% of the design storage capacity of these reservoirs can be supplied to consumers for use (World Bank, 2011).

With a supply of potentially up to 42 Gm^3 of internal renewable groundwater annually, sub-surface resources represent another important source of water in Thailand (FAO, 2015a). The groundwater system in Thailand is mainly recharged by rainfall and seepage from rivers. Water balance studies show that 5 to 9% of rainfall infiltrating soil would reach and recharge the aquifers (Sethaputra et al., 2001).

In 2007, about 57.3 Gm^3 of fresh water was extracted for agricultural, industrial and domestic use (FAO, 2015a). The agricultural industry is the largest consumer of fresh water in Thailand with 90.4% of the total used in the sector (FAO, 2015a). Fresh water consumption for industrial and domestic uses are approximately 2.8 (4.8%) and 2.7 Gm^3 (4.8%), respectively (Figure 1.3).

Thailand has the second lowest (3,340 m^3/capita/year) renewable water availability per capita in the Southeast Asian region (FAO, 2015a; from a population of 67 million in 2014). Only land-scarce Singapore, with limited catchment area for water storage, ranks lower (Figure 1.4). Despite receiving abundant rainfall yearly, some areas in Thailand are facing severe water scarcity particularly during the dry season. An example is the Chao Phraya River Basin, which covers the northern and central region of Thailand. Hoekstra and Mekonnen (2011) estimated that, between the months of February and April, the demand of water resources within the catchment is nearly seven times more than the available blue (renewable) water (Figure 1.5).

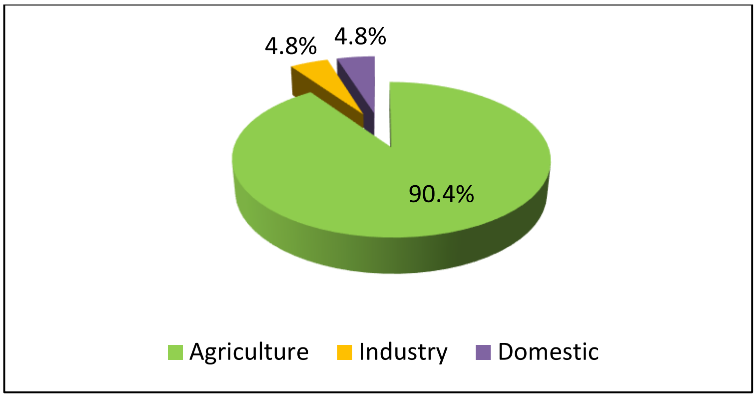

Figure 1.3: Water use by sectors in Thailand (FAO, 2015a)

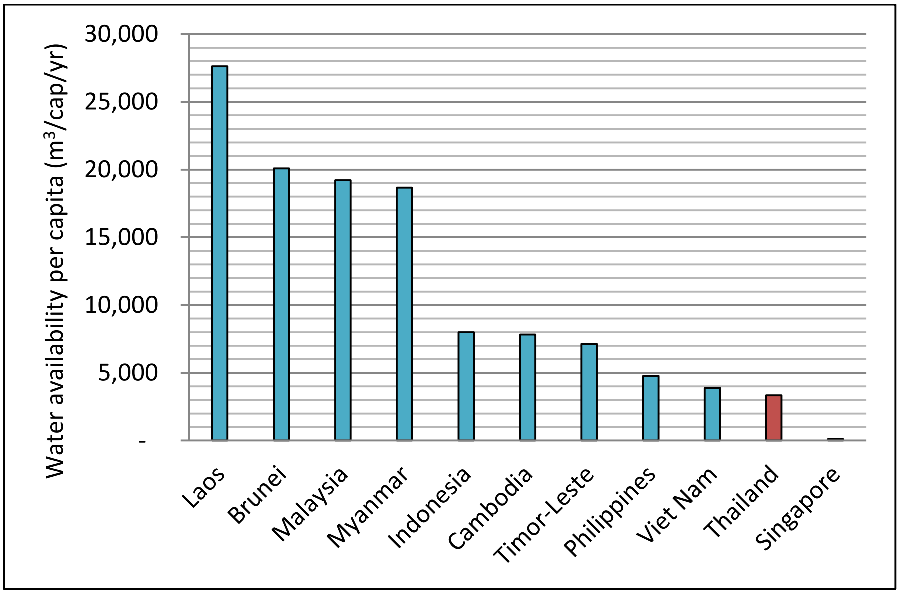

Figure 1.4: Annual renewable water availability per capita of the countries in Southeast Asia (FAO, 2015a)

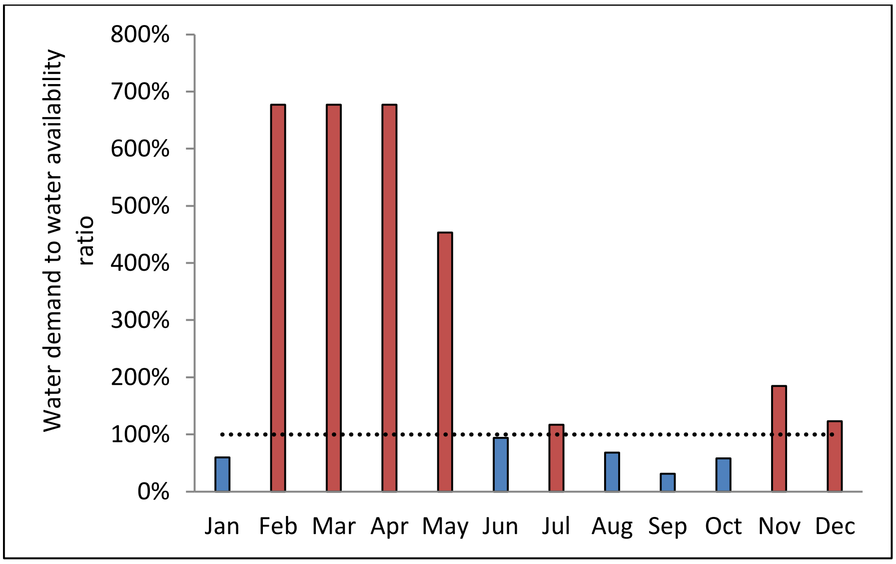

Figure 1.5: Ratio of water demand to blue water availability in the Chao Phraya River Basin (Hoekstra and Mekonnen, 2011). Note: Bars beyond the dotted horizontal line, indicated in red, represent months when water demand is beyond the available blue water supply.

1.3.2 Water Quality

Water quality degradation is one of the most serious environmental problems in Thailand (PCD, 2011; World Bank, 2011). Nearly a quarter of the surface water resources require special, non-conventional treatment processes (e.g. reverse-osmosis membrane filtration) before safe consumption is possible (Bolong et al., 2009; PCD, 2013).

Water quality monitoring is conducted by the Pollution Control Department (PCD) under the Ministry of Natural Resource and Environment (MoNRE) at 366 monitoring stations in 48 major rivers and four standing water bodies: Phayao Lake, Boraphet Lake, Han Lake (*Nong Han*), and Songkhla Lake (PCD, 2013). The overall condition of water quality is indicated by a water quality index (WQI) developed by the PCD. The WQI considers five basic water quality parameters (Table 1.1): (i) Dissolved Oxygen (DO); (ii) Biochemical Oxygen Demand (BOD); (iii) Ammoniacal-Nitrogen (NH_3-N); (iv) Total Coliform Bacteria (TCB); and (v) Faecal Coliform Bacteria (FCB).

Since 2007, none of the monitored water bodies were categorised as '*Very Poor*' (Class 5) indicating some improvements to the quality of water resources (Figure 1.6). However, between 2001 and 2013, none of the monitored water bodies were rated '*Excellent*' (Class 1) implying that all were subjected to some degree of quality degradation (Table 1.2).

Of the five basic water quality parameters used to determine the WQI, concentrations of Total Coliform Bacteria and Faecal Coliform Bacteria are likeliest to be above water quality standard values (20,000 MPN/100 mL and 4,000 MPN/100 mL, respectively), implying that faecal contamination is the most significant cause of water impairment nationwide (Figure 1.7).

The types of contaminants in the water bodies also vary spatially across the regions in Thailand. The source of contamination is contingent upon the types of land use and socio-economic activities of the specific area. For example, in terms of organic matter contamination, the BOD of the water bodies in the highly urbanised city of Bangkok is only affected by the domestic (81%) and industrial (19%) sectors (Figure 1.8). In Northeast Thailand, where farming is an integral part of the local community's livelihood, the agriculture sector also contributes significantly (41%) to the BOD of the water bodies of the region (Figure 1.8).

Table 1.1: Brief description of the five basic water quality parameters used for the determination of the Thai Water Quality Index

Parameter	Description
DO	▪ Oxygen dissolved in a water column is one of the most important components of an aquatic system. ▪ Oxygen is required for the metabolism of aerobic organisms and it also influences inorganic chemical reactions. ▪ Oxygen enters water through diffusion across the water's surface or as a by-product of photosynthesis. ▪ High concentration of DO usually represents good water quality.
BOD	▪ The Biochemical Oxygen Demand of water body reflects the degree of organic matter contamination. ▪ Specifically, BOD is a measure of the amount of oxygen removed from aquatic environments by aerobic microorganisms for their metabolic requirements during the breakdown of organic matter. ▪ Systems with high BOD tend to have low DO concentrations.
NH_3	▪ Ammonia is a chemical species of inorganic nitrogen and its presence in water reflects the degree of nutrient contamination. ▪ NH_3, along with other species of inorganic nitrogen (nitrate, nitrite), are usually present in natural waters in low concentrations and are essential for the survival and growth of aquatic organism. ▪ However, excessive loadings of nutrients entering a body of water – in a process called '*eutrophication*' – may result in algal bloom, which in turn may result in the depletion of oxygen in water. ▪ Fertiliser and domestic wastewater are important sources of nutrients.
TCB	▪ Total coliform bacteria include a wide range of aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacilli capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 hours at 35 – 37 °C. ▪ Coliform bacteria may exist in natural waters but some (e.g. *E. coli*) are excreted with the faeces of humans and animals. ▪ As such, TCB is commonly used as indicators of faecal contamination.
FCB	▪ Coliform bacteria that are able to ferment lactose at 44 – 45 °C are known as thermotolerant or faecal coliform bacteria. ▪ In most waters, the predominant genus is *Escherichia*, but some types of *Citrobacter*, *Klebsiella* and *Enterobacter* are also thermotolerant. ▪ *Escherichia coli* occurs in high numbers in human and animal faeces, sewage and water subject to recent faecal contamination and as such FCB is also used as indicators of faecal contamination.

Table 1.2: PCD's water quality classification

Category	Description and Use
Class 1 (Excellent)	▪ Pristine and extra clean water resources
Class 2 (Good)	▪ Very clean water resources ▪ Ordinary water treatment processes required for consumption ▪ Suitable for aquaculture, recreation
Class 3 (Fair)	▪ Fairly clean water resources ▪ Ordinary water treatment processes required for consumption ▪ Suitable for agricultural uses
Class 4 (Poor)	▪ Deteriorated water resources ▪ Special water treatment processes required for consumption ▪ Suitable for industrial uses
Class 5 (Very Poor)	▪ Highly deteriorated water resources ▪ Suitable for navigational uses only

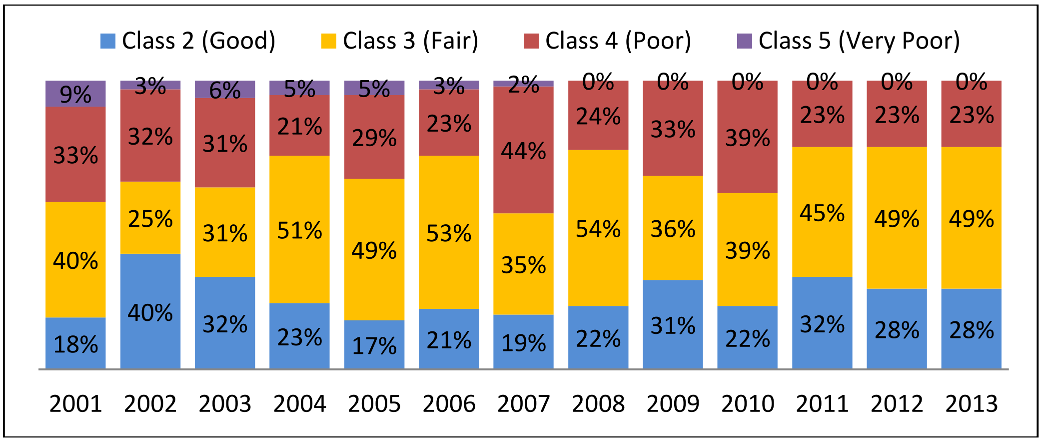

Figure 1.6: Status of the inland water resources based on the Thai Water Quality Index between 2001 and 2013 (PCD, 2005; PCD, 2008; PCD, 2013)

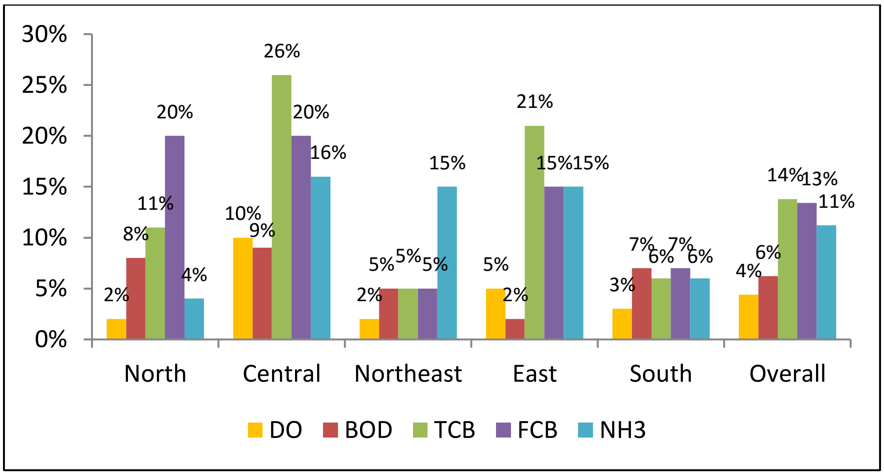

Figure 1.7: Water quality parameters of water bodies not in compliance with water quality standards by region in 2010 (PCD, 2010)

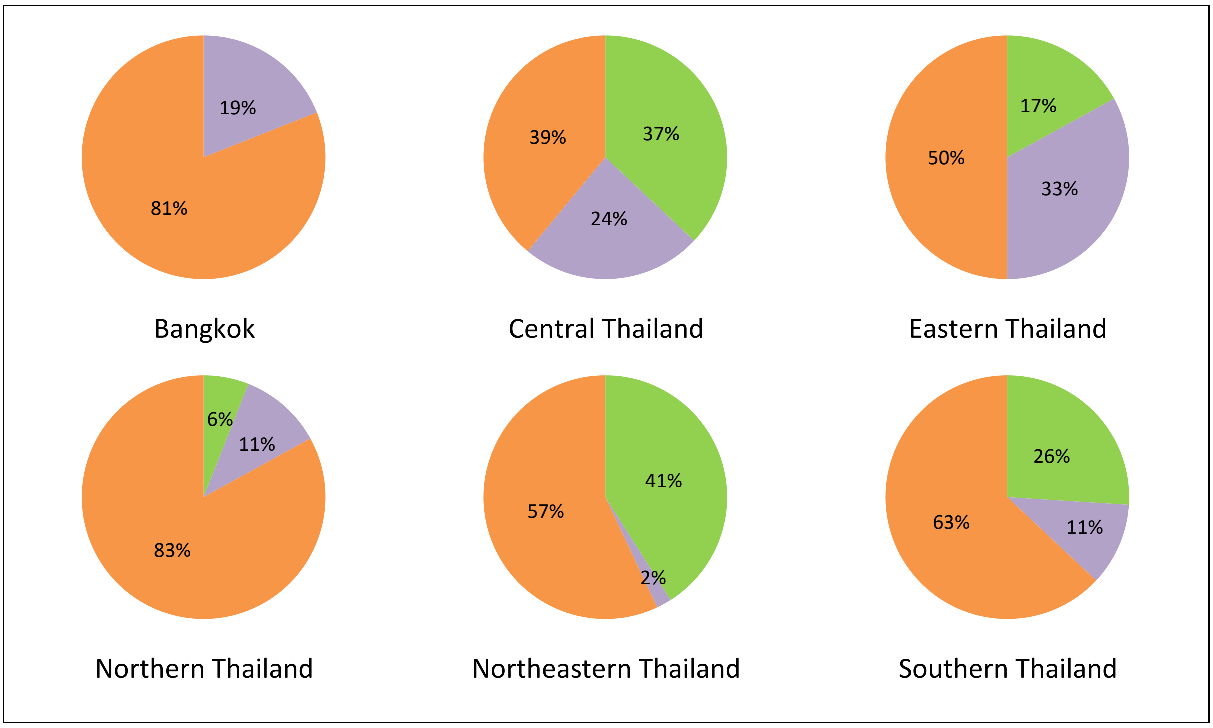

Figure 1.8: Sector contribution of BOD in water bodies of the regions of Thailand (World Bank, 2001). Note: Orange – Domestic source; Green – Agricultural source; Purple – Industrial source.

1.3.3 Drinking-Water Management

The Metropolitan Waterworks Authority (MWA) is responsible for the management of the domestic and drinking-water resources in Bangkok and its vicinity. In 2006, MWA's water production capacity was approximately 5.5 million m^3 daily, servicing nearly 7.8 million people (World Bank, 2008). The management of the drinking-water resources for the rest of the other urban areas (e.g. district and provincial capitals) is under the responsibility of the Provincial Waterworks Authority (PWA). In 2006, the production capacity of PWA's water treatment plants was over 980 million m^3 of water, serving almost 2.5 million domestic and commercial subscribers covering around 10.5 million people in the country (World Bank, 2008). Sources of water are typically large reservoirs (surface water). In the dry season, some areas are also supplemented with groundwater. The conventional coagulation-flocculation-sedimentation-filtration-disinfection (chlorination) system is used for the treatment of raw water before distribution by both MWA and PWA (Figure 1.9). Water is also routinely treated by both MWA and PWA prior to the distribution to consumers. Up to thirty parameters are tested, including both chemical and biological water quality parameters.

In areas not served by either MWA or PWA – mostly rural areas – the management of the drinking-water resources are handled by the village communities themselves. According to a survey by the Ministry of Interior in 2007, there were almost 70,000 villages in the rural areas of Thailand (World Bank, 2008). Approximately 78% of the rural communities have access to community-managed drinking-water supply piped to their residences (World Bank, 2008). The remaining ~22% rely on drinking-water resources managed at the household level. Sources of drinking-water supplies may be of surface or sub-surface origin for both community- and household-managed water resources. For surface sources, water is siphoned from rivers while sub-surface sources are accessed with wells. Treatment of water resources is often minimal and wherever available, raw water sources are usually treated by sand-filtration only. In the rural areas, the drinking-water resources are not subjected to any routine tests to ensure the suitability for consumption.

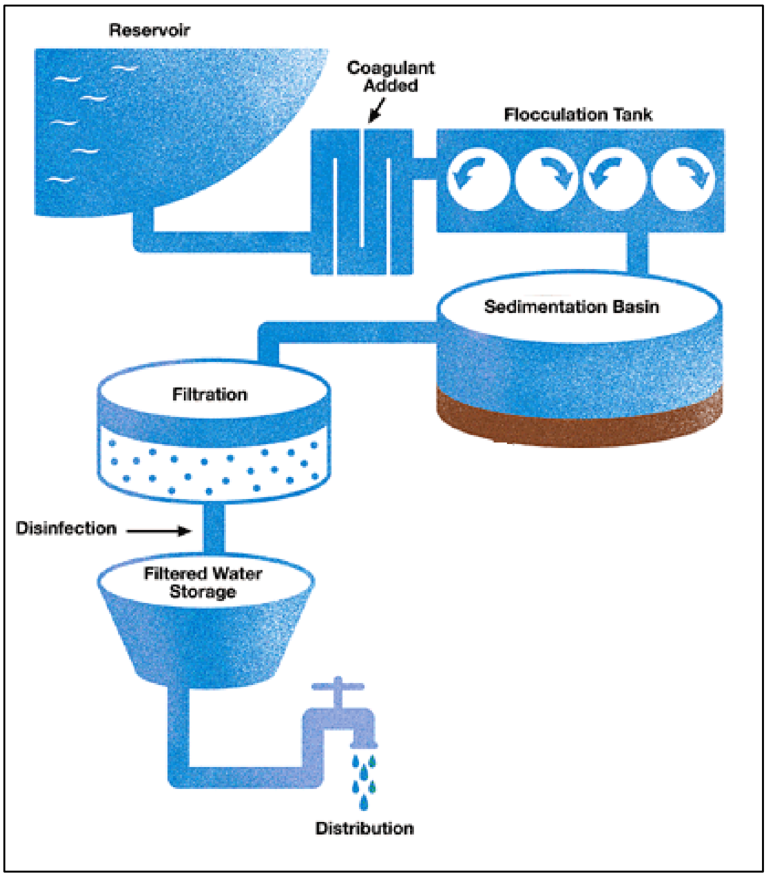

Figure 1.9: The water treatment processes by the Metropolitan and Provincial Waterworks Authority of Thailand

1.3.4 Drinking-Water Quality Issues and Associated Health Hazards

Studies pertaining to the quality of drinking-water resources in Thailand are very limited. While the PCD carries out routine water quality surveys, only five basic water quality parameters (i.e. DO, BOD, NH3-N, TCB and FCB; Section 1.3.2) are tested. These parameters are merely indicators used to assess the general status of the monitored water bodies (e.g. TCB and FCB are indicators of faecal contamination; DO and BOD for organic matter contamination; NH_3-N for agricultural for domestic wastewater contamination).

Where public health is concerned, very little is known of the presence of actual hazards in drinking-water sources. These drinking-water hazards may occur as various biological and chemical constituents, usually resulting from human-related activities. As an agrarian country and one of the world's major food exporters, Thailand relies heavily on the use of pesticides to protect crops and increase yields (Panuwet et al., 2012). The usage of these agrochemicals may contaminate drinking-water sources (Panuwet et al., 2012; Poolpak et al., 2008; Sangchan et al., 2014) where some of these pesticides are recognised as probable human carcinogens (e.g. DDT, heptachlor, chlordane, toxaphene) and are highly persistent (resistant to degradation) in the environment.

The flourishing industrial sector – which contributes to over 40% of the Thailand's Gross Domestic Product (GDP) in 2014 – may also contribute to the degradation of drinking-water resources. Industrial effluents too may contain highly toxic and persistent chemicals. For example, perfluorinated compounds that are used in protective coatings for fabrics, firefighting foam, hydraulic fluids, paints etc. have been detected not only in environmental waters of Thailand but also in treated wastewater and drinking-water, implying that these chemicals cannot be effectively removed by conventional treatment systems (Boontanon et al., 2013; Kunacheva et al., 2011). Some perfluorinated compounds such as perfluorooctanoic acid are carcinogens; liver, developmental and immune system toxicants, and can also exert hormonal effects including alteration of thyroid hormone levels (Lau et al., 2007).

The population boom in Thailand – from 21 million in 1950 to 68 million today (UN-DESA, 2015) – have resulted in a corresponding increase in domestic wastewater generation. Approximately 14 million m^3 of domestic wastewater is generated every day but almost 80% of this total is discharged directly without any treatment (World Bank, 2008). Domestic wastewater may also contain chemical and biological drinking-water hazards. Pharmaceuticals and personal care products (PPCPs) belong to a new class of emerging water contaminants that are often found in domestic wastewater worldwide. While studies have thus far been limited, PPCPs have also been detected in the water bodies of Thailand (Tewari et al., 2013). Many PPCPs have been identified as endocrine-disrupting compounds (Daughton and Ternes, 1999). Endocrine disruptors are synthetic chemicals that block or mimic natural hormones in the body, disrupting normal organ function even at extremely low concentrations.

Improper disposal of domestic wastewater may also lead to faecal contamination in drinking-water resources. Water contaminated with faecal matter oftentimes contain pathogens (disease-causing microorganisms) and when consumed without adequate treatment, can lead to various waterborne diseases. Diarrheal diseases are the most common. The risk of infection from waterborne diseases in Thailand is alarmingly high. Between 2005 and 2006, at least 46% of children under the age of five were treated for diarrhoea (UNICEF and WHO, 2009). Various waterborne pathogens such has *Vibrio cholerae*, *Shigella* spp., *Leptospira* spp., *Giardia* spp., *Cryptosporidium* spp. etc. have been detected in water resources in Thailand (Chaturongkasumrit et al., 2013; Diallo et al., 2008).

Drinking-water hazards may also occur naturally. One such example is fluoride. In small amounts, fluoride is beneficial for oral health. However, prolonged exposure to high doses of fluoride may lead to fluorosis, a disease that can lead to the destruction of teeth (dental fluorosis) for mild cases or bones (skeletal fluorosis) for severe cases. Cases of fluorosis have been recorded in Thailand as early as the 1960's (Leatherwood et al., 1965). High-fluoride water is typically found in sub-surface resources and is most prevalent in the northern region of Thailand (Leatherwood et al., 1965; Namkaew and Wiwatanadate, 2012; Ratanasthien and Ramingwon, 1982).

1.4 Objective

In view of the pressing drinking-water quality problems as presented in the preceding sections, as well as the apparent vulnerability of the rural communities to drinking-water hazards, the overarching objective of this study was to assess the quality of drinking-water resources in Thailand. As there are numerous water quality parameters that could potentially be assessed, it is not feasible to evaluate the entire spectrum within the timeframe allocated in this study. Only a selected few which were identified as health hazards and are of direct pertinence to the public health of the vulnerable rural populations were investigated. Drinking-water resources from both surface and sub-surface supplies were investigated. Health hazards in drinking-water of anthropogenic origin as well as those that occur naturally were also studied. For the selected water quality parameters, the following research questions were asked and then discussed:

- What is the source of the selected drinking-water health hazard?
- What are the important processes and factors that govern the transport of the drinking-water health hazard from source to sink?
- What are the implications of the presence of the selected drinking-water hazard to the management of the local drinking-water resources?

1.5 Layout of Thesis

Chapter 1 – Prologue

The introductory chapter of this research endeavour exposes the readers to water quantity- and quality-related problems in the world and Thailand. The impact of the degradation of water quality, particularly to the public health, is emphasised. The aim of this research is also highlighted. Finally, the layout and a brief overview of all the chapters in this dissertation are presented.

Chapter 2 – Faecal Contamination of Groundwater from On-Site Sanitation Systems

Faecal contamination represents one of the biggest environmental and health-related problems in Thailand. Inadequately domestic wastewater is a major source of faecal matter in water resources. A longitudinal study which describes the impact of the prevalent on-site sanitation systems used in rural Thai villages to the quality of local groundwater is presented in this chapter. This study also considered the hydroclimatological factors which affect the level of faecal contamination in the local groundwater resources.

Chapter 3 – Cryptosporidium and Giardia: Waterborne Pathogens in Surface Waters

Water resources contaminated with domestic wastewater and faecal matter may contain harmful microorganisms including viruses, bacteria, protozoa and helminths. The ingestion of these pathogens can lead to various diseases. *Cryptosporidium and Giardia* are two of the most significant and deadly etiological agents of diarrhoeal diseases worldwide. In this chapter, the prevalence of these waterborne pathogens in the surface waters of an important drinking-water catchment in Northern Thailand is described. The prevalence of both *Cryptosporidium and Giardia* in their hosts – meat and dairy cattle – was also explored in this study.

Chapter 4 – Fluoride: A Naturally-Occurring Health Hazard in Drinking-Water Resources

While health hazards in drinking-water are typically associated to pollution from anthropogenic sources, naturally occurring health hazards can also occur. One such example is fluoride. Cases of mild to severe fluorosis (fluoride poisoning) have been documented in Thailand. This chapter describes a study which aimed to map the extent of high-fluoride zones in two fluorosis endemic provinces in Northern Thailand. The genesis of fluoride and its transport from the source to the drinking-water supplies of the affects areas were also discussed in this study.

Chapter 5 – Epilogue

In the concluding chapter, the on-going and future challenges in the management of drinking-water resources in Thailand are discussed. Finally, recommendations towards the provision of safe drinking-water supplies are put forth for consideration.

CHAPTER 2 – FAECAL CONTAMINATION OF GROUNDWATER FROM ON-SITE SANITATION SYSTEMS

2.1 Introduction

Worldwide, water resources contaminated with excreta from inadequate treatment and improper disposal are correlated with the presence of disease-causing pathogens that are predominantly of faecal origin (Ashbolt et al., 2001; Corcoran et al., 2010; Hunter et al., 2003; IOM, 2009; Leclerc et al., 2001). According to the World Health Organisation (WHO), poor management of water resources and sanitation is responsible for nearly 10% of the total burden of disease (disability adjusted life years), contributing to more than three million deaths worldwide annually (Pruss-Ustun et al., 2008).

Water plays a vital role in the transmission of the diseases associated with enteric pathogens (Mor and Griffiths, 2011; White et al., 1972). For waterborne diseases, water is the vehicle of transport for the etiological agents. The distribution of waterborne organisms is purely mechanical, as mobilisation occurs with the flow of either surface or subsurface water. Transmission and infection occur following the ingestion of water contaminated with pathogens. Diarrhoea is one of the most common symptoms of waterborne diseases, including cholera, shigellosis, cryptosporidiosis and giardiasis, which are caused by a myriad of waterborne pathogens such as viruses, bacteria and protozoa (e.g. noroviruses, *Vibrio cholerae*, *Shigella dysentariae*, *Cryptosporidium parvum*, *Giardia lamblia*). WHO (2008) ranked diarrhoeal diseases within the top-five leading causes of mortalities worldwide. In 2004, diarrhoeal illnesses claimed more than two million lives globally (WHO, 2008a).

In Thailand, approximately 14 million m^3 of wastewater are generated daily. However, only a little over 20% from this total volume is channelled to centralised wastewater treatment facilities, which are typically located only in major urban areas and tourist attractions; the rest is discharged into the environment untreated (Simachaya, 2009; World Bank, 2008). In unsewered areas of Thailand, on-site sanitation systems are typically implemented to treat household waste (Giri et al., 2006; Tsuzuki et al., 2010).

A survey of the management of on-site sanitation systems in Thailand by AECOM and EAWAG (2010) revealed that the approved on-site sanitation system design generally includes the building of non-watertight systems. Thus, many of these sanctioned systems allow the discharge of wastewater into the surrounding soil matrix, where it may then enter the local groundwater. Numerous studies have demonstrated the susceptibility of groundwater to contamination from on-site sanitation systems (Douagui et al., 2012; Dzwairo et al., 2006; Howard et al., 2003; Pant, 2011; Pujari et al., 2007; Scandura and Sobsey, 1997; Wright et al., 2012). Furthermore, although sample construction designs and guidelines for inspection are available to builders and local governing authorities (e.g. via the Pollution Control Department), many on-site sanitation systems are poorly built, unregulated, and improperly maintained (during and post-construction), creating a higher risk for pathogens to enter water bodies unabated. The AECOM and EAWAG survey showed that while guidelines may establish the physical standards of a system, there is no enforcement body to ensure owners maintain their proper functioning. Permitting has not been effective, especially in rural areas, leading the widespread construction of systems that do not meet the standards and cannot be maintained easily (AECOM and EAWAG, 2010).

The promotion of sanitation in Thailand can be dated back to 1897 with the implementation of the first sanitation law was directed at curbing communicable diseases in Bangkok through the building of public latrines, in addition to promoting proper management of solid waste in general (Luong et al., 2000). In 1926, open defecation in rivers and canals was banned by the Ministry of Interior (Luong et al., 2000). The first momentous step towards universal improvement of sanitation came with the initiation of the Village Health and Sanitation Project in 1960 (Graham, 2011). At the time, basic sanitation coverage in rural areas was less than one percent. Directed by the newly-formed Ministry of Public Health, the project endeavoured to reduce the incidence of prevalent waterborne diseases, particularly those associated with faecal matter. The Village Health and Sanitation Project was the impetus for the expansion of a sanitation improvement programme nationwide the following year, when it was integrated into the National Economic and Social Development Plan and subsequently renamed the Rural Environmental Sanitation Program (Graham, 2011). Today, nearly all urban (90%) and rural (96%) areas of Thailand have access to basic sanitation facilities based on the Joint Monitoring Programme for Sanitation by UNICEF and WHO (2015). Consequently, between 1960 and 2000, deaths associated with diarrhoeal diseases decreased more than 90% (Graham, 2011).

However, in spite of the reduction in mortality, the risk of infection from waterborne diseases remains alarmingly high. Between 2005 and 2006, at least 46% of children under the age of five were treated for diarrhoea (UNICEF and WHO, 2009). According to the Thai Ministry of Public Health, the incidence of acute diarrhoea increased from 1 to 16 cases per population of 1,000 between 1973 and 2001 (Boonyakarnkul, 2003). In 1999, more than one million cases of diarrhoea were registered. Of these, 323 cases resulted in premature death. Also reported in the same year were more than 7,000 cases of typhoid fever and nearly 60,000 cases of dysentery. In total, more than one hundred thousand people needed medical attention and the estimated total costs of treatment and hospitalisation amounted to approximately 7.5 million US dollars (World Bank, 2001).

The Thai Pollution Control Department (PCD) has identified faecal contamination as an important water quality concern in Thailand (Simachaya, 2002). Predictably, the effects of faecal contamination are especially pronounced in surface waters draining densely populated areas. Independent studies generally concur with the PCD’s findings and highlight the severity of the faecal and microbial contamination of surface waters (Chaturongkasumrit et al., 2013; Diallo et al., 2008; Ferrer et al., 2012; Kittigul et al., 2006; Koompapong and Sukthana, 2012; Widmer et al., 2013). More importantly, these findings also report the presence of various kinds of waterborne pathogens in these faecal-contaminated water sources including Hepatitis A virus, pathogenic strains of *E. coli* (ETEC, EPEC, STEC), *Vibrio cholerae*, *Shigella* sp., *Entamoeba histolytica*, *Cryptosporidium parvum* and *Giardia lamblia*.

In general, groundwater quality monitoring studies addressing wastewater contamination in Thailand are rare (Karnchanawong et al., 1993; Lawrence et al., 2000; Vaccari et al., 2010). This paucity of information is of concern because an estimated 75% of the national domestic water supply – up to

2.7 billion m^3 annually – is derived from sub-surface water sources (Sethaputra et al., 2001). New research is therefore needed to help develop sound policies to protect both urban and rural populations from acquiring waterborne or water-related diseases.

The objective of this study was to assess the extent that household on-site sanitation systems contribute to the groundwater contamination. Other objectives were to evaluate the risk of faecal-associated waterborne diseases and explore the implications of current sanitation practices to management of drinking-water and wastewater in Thailand.

2.2 Site Description

The study was conducted in Bo Hin Village (population: 984) and Pa Kang Village (756) of San Sai District, Chiang Mai Province, Northern Thailand (Figure 2.1). Approximately 10 km to the west of these villages is the Ping River, one of the most important sources of water supply in northern Thailand. The villages are located approximately 12 km northeast of Chiang Mai city (18^o 51’ 00” – 18^o 51’ 30” N, 99^o 04’ 30” – 99^o 05’ 00” E). Chiang Mai is one of the most populous, economically important and culturally significant cities in the country (Tubtim, 2012).

The region has a tropical savannah (wet and dry) climate (Köppen *Aw*). Annual rainfall ranges from 800 mm in the lowlands to 1,500 mm in the highlands with seasonal rainfall between May and October accounting for over 90% of the annual total (Lim et al., 2012; Margane and Tatong, 1999; Wood and Ziegler, 2008). Natural and anthropogenic factors including climate (wet/dry spell) and groundwater abstraction affect the fluctuation of the water table in the Ping River Basin (Uppasit, 2004). Historical records of groundwater levels from the Department of Groundwater Resources showed that water table in Chiang Mai Province were typically lowest at the end of the dry season between March and May (Uppasit, 2004). Groundwater recharge occurs during the wet season. The water table was observed to be highest between August and November (Uppasit, 2004). The water table in the vicinity of San Sai District is generally only an average of 1.25 m below ground level (Uppasit, 2004).

Figure 2.1: Location of San Sai District in the Chiang Mai Province, Northern Thailand

Like most parts of Thailand, the villages of Bo Hin and Pa Kang are not served by centralised wastewater treatment systems. Instead, individuals in these villages use non-watertight, open-bottomed on-site sanitation systems. These systems typically consist of single-compartment cess pits with reinforced walls constructed by stacking pre-cast concrete rings in holes excavated adjacent to houses or in free-standing toilet facilities. The bottoms of these systems are commonly unlined, allowing for the movement of wastewater into the underlying soil strata. Pit depths vary from 1.5 and

2.0 m. The removal of sludge from the cess pits typically occurs when the systems are blocked or full — on the order of once a year or less frequently.

A small number of the households south of Bo Hin Village have access to treated water that is piped from a local reservoir and treatment facility. These houses are typically located near water mains that run along a major road. Most houses in the two study villages, however, rely on local groundwater wells as the principle source of domestic water for household activities. While treated bottled drinking-water is available, some villagers – especially those from lower income households – still drink water from these wells. In both villages, both hand-dug wells and bored wells are used access ground water.

2.3 Materials and Methods

2.3.1 Sample Collection

From the two study villages, 13 pairs of wells were selected for observation (Figure 2.2). Each pair consisted of one hand-dug well and one bored well. The hand-dug wells had depths ranging from approximately 3 to 5 m. The bored wells were deeper, with depths ranging between 10 and 12 m. The hand-dug wells are referred to herein as 'shallow wells' while the bored wells are termed 'deep wells'.

Selection of the sampled well pairs was based on three criteria. First was the availability of shallow-deep pairs in close proximity such that water quality could be compared. Paired wells were typically found within the compound of one or two adjacent households. Secondly, all selected pairs of wells were spatially distributed across the extent of the village areas to allow a village-wide assessment of the groundwater quality. Lastly, permission from house owners to collect water samples throughout the duration of the study was needed. In total, six pairs of wells in Bo Hin Village (BH1, BH2, BH3, BH4, BH5 and BH6) and seven pairs of wells in Pa Kang Village were sampled (PK1, PK2, PK3, PK4, PK5, PK6 and PK7).

Prior to collection, the hand-dug wells were cleaned and inspected for potential points of surface water intrusion at the base of elevated concrete well walls. All detected cracks and cavities were grout-sealed to prevent the influx of surface water. Throughout the entire monitoring period, the wells were covered to prevent the entry of above-ground debris.

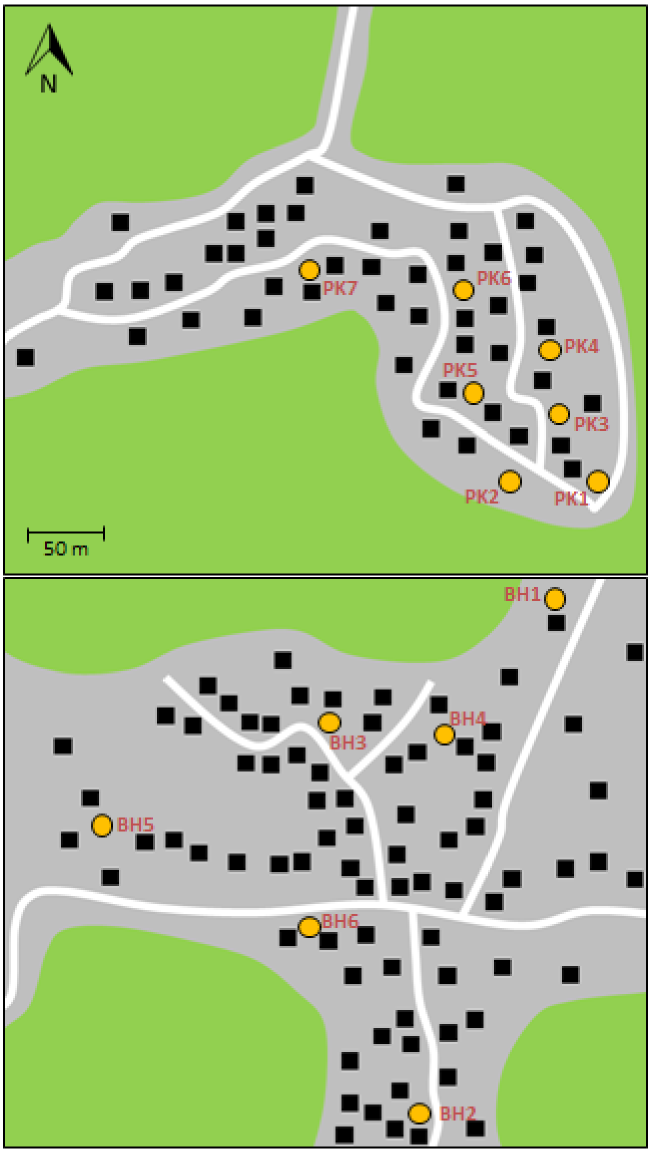

Figure 2.2: The villages of Pa Kang (top) and Bo Hin (bottom) as well as the locations of the wells. PK7 is approximately 400 m northeast of BH1. Green areas denote agricultural lands/rice fields.

Water sampling times were restricted to 9:00 to 11:00 a.m. to ensure consistency throughout the study. Prior work has shown the level to faecal contamination in groundwater from sanitation systems may vary diurnally. A study by Ekklesia et al. (2015a) revealed that faecal contamination originating from sanitation systems peaked twice a day (in the day time from 10:00 a.m. to 2:00 p.m. time and in the night time around 8:00 p.m.), presumably in response to periods of highest use.

Water samples from hand-dug wells were collected using a bucket lowered from above. Water table depth was measured with a weighted graduated scale. Water samples from bored wells were collected directly from taps after allowing the water to run for 30s. Approximately 500 mL of water were collected from both sources during each sampling session. Samples were stored in polyethylene bottles and refrigerated (approximately 4 ^{o}C) in the dark prior to the subsequent processing and analysis, which typically was performed within 12 hours following collection.

2.3.2 Faecal Indicator Organisms

Drinking-water resources should ideally be tested for the presence of all known pathogens, particularly those of enteric origin. However, given that water potentially contains many different pathogens, and that the types of pathogens vary over time, it is not practical to test for all. Moreover, methods for the direct detection of many pathogens in water are still in the developmental stage (Medema et al., 2003). Hence, only a few pathogens are currently detectable. Thus, for this study, we tested for two faecal indicator organisms, *Escherichia coli* (*E. coli*) and *Enterococcus* spp., to assess the potential presence of faecal-derived pathogens in general.

Both *Escherichia coli* (*E. coli*) and *Enterococcus* spp. are natural inhabitants in the gastrointestinal tract of humans and are released in large quantities along with faeces during defecation, making these bacteria ideal indicators of faecal contamination (Fisher and Philips, 2009; Leclerc et al., 2001; WHO, 2011). The use of faecal contamination indicators is based upon the principle that the detection of these bacteria implies faecal contamination, and therefore, the possible presence of faeces-derived pathogens (Ekklesia et al., 2015a; Ekklesia et al., 2015b; Pitkanen et al., 2011; Rochelle-Newall et al., 2015; Soller et al., 2010).

E. coli and *Enterococcus* were cultured using Colilert® and Enterolert® (IDEXX Laboratories, Westbrook, ME, USA), which are commercially-available, enzyme-substrate media. Both of the faecal indicator organisms were enumerated using the Most-Probable Number (MPN) method, in accordance with the Quanti-Tray/2000® enumeration procedure (IDEXX Laboratories, Westbrook, ME, USA). These methods are approved by the United States Environmental Protection Agency (USEPA) and have been included in the *Standard Methods for Examination of Water and Wastewater* by the American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF).

Briefly, for each sample, 100 mL of sample water were first aliquoted from the sampling bottles into sterile 100 mL-volumetric flasks. Colilert® or Enterolert® media were mixed until dissolved by gently inverting the flasks repeatedly. The reagent-sample mixtures were then incubated in sealed trays (Quanti-Tray/2000®) at 35 ^{o}C and 41 ^{o}C for *E. coli* and *Enterococcus*, respectively. The results were registered after an incubation period of 24 – 28 hours according to manufacturer instructions.

2.3.3 Nitrate

Nitrate (NO_3^-) can be found in inorganic fertilisers and used as oxidising agents (WHO, 2011). Additionally, nitrate may also be derived from human and animal excreta. Nitrate is formed as a result of the oxidation of nitrogenous matter that is found in abundance in faeces (Wakida and Lerner, 2005). Numerous studies have revealed that water resources subjected to wastewater contamination often contain high levels of nitrogen (Gill et al., 2009; Nyenje et al., 2013; Wakida and Lerner, 2005; Withers et al., 2011).

As such, nitrate concentrations in the selected wells were also measured as a secondary indicator of faecal contamination. Collected water samples were stored (refrigerated at approximately 4 ^{o}C in the dark) between four and six weeks before delivering to a laboratory at the Department of Geography, National University of Singapore (Singapore) for chemical analyses. Concentrations of nitrate in water samples were measured using a high-pressure ion chromatography system (Dionex™ ICS-5000, Thermo Scientific™, Sunnyvale, CA, USA).

2.4 Results

2.4.1 Rainfall

The one-year monitoring period for this study extended from January 2014 to January 2015. Rainfall was first detected in early April. Monthly rainfall then gradually increased, peaking in September before ceasing completely by early November. A total of 809 mm of rain from 90 rainfall days was recorded during the monitoring period with a tipping bucket rain gauge. Approximately 90% of the total recorded rainfall recorded occurred between mid-April and mid-October (~6 months). Herein, this period is referred to as the 'wet season' (Figure 2.3).

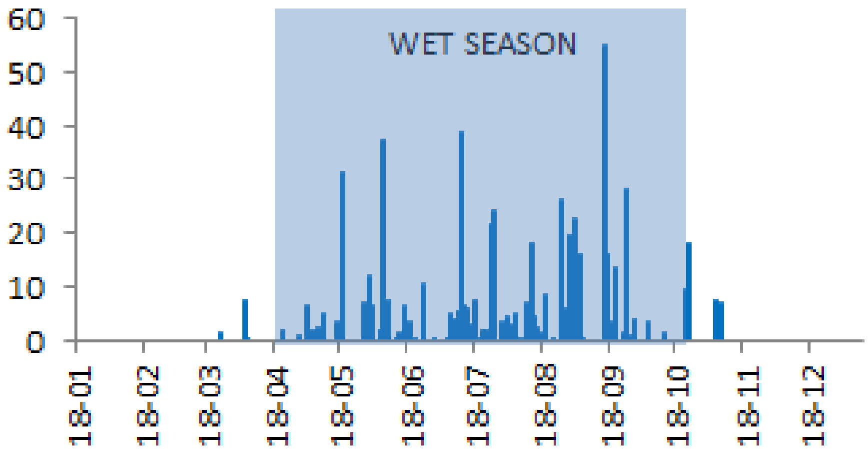

Figure 2.3: Total daily rainfall (mm) for the entire study period

2.4.2 Shallow Wells

A total of 382 water samples from (shallow) hand-dug wells were collected. Water samples from several dry wells towards the end of the dry season were not collected. These wells are BH4 (dry from 27 April to 11 May 2014), PK1 (7^{th} February to 11^{th} May 2014), PK3 (7^{th} February to 11^{th} May 2014), and PK6 (11^{th} May 2015).

All samples collected throughout the duration of the study contained either *E. coli* or *Enterococcus* with concentrations greater than 1 MPN/100 mL (Table 2.1). All samples contained at least 1 MPN/100 mL *Enterococcus*; approximately 96% contained at least 1 MPN/100 mL *E. coli*. All samples were therefore considered unsuitable for drinking without treatment in accordance to the WHO guidelines for drinking-water quality (WHO, 2011).

The levels of faecal contamination, based on the concentrations of the faecal indicator organisms, vary markedly from one well to another. For example, almost 70% of the water samples in BH3 contained high levels of *E. coli* (from 1,300 – 24,196 MPN/100 mL). At the other extreme, approximately 85% of the water samples in BH6, approximately 150 m away, contained *E. coli* concentrations of less than 100 MPN/100 mL. Wells sampled in the dry and wet seasons exhibited different levels of faecal contamination. The concentrations of the faecal indicator organisms were generally elevated during the wet season. However, the seasonal fluctuation trends of *E. coli* and *Enterococcus* concentrations showed variability between the monitored shallow wells (Figure 2.4).

From the 13 shallow wells monitored in this study, five recorded incidences of nitrate levels exceeding 50 mg/L, the concentration that is considered safe for drinking water by the World Health Organization (WHO, 2011). These wells include BH4 (maximum = 82 mg/L), BH5 (119 mg/L), PK4 (79 mg/L), PK5 (55 mg/L) and PK7 (72 mg/L). Water samples from PK1 and PK2 also contain levels of nitrate that fall just short of the accepted water quality guideline values, with maximums of approximately 45 and 38 mg/L recorded, respectively. The hydroclimatic effects of the study site appeared to influence the concentration of nitrates. With the exception of PK1 and PK4, concentrations of nitrate in shallow wells were generally higher during the wet season. However, like the *E. coli* and *Enterococcus* counts, the temporal trends of nitrate concentrations varied among the monitored wells (Figure 2.4).

Table 2.1: Summary of results showing concentration ranges of faecal contamination indicators and associated archetypal response to hydroclimatic factors in shallow and deep wells.

Site	Well	*E. coli* (MPN/100 ML)				*Enterococcus* (MPN/100 ML)				Nitrate (mg/L)			
		Min	–	Max	Response	Min	–	Max	Response	Min	–	Max	Response
BH1	Shallow	< 1	–	11,199	**SYN**	16	–	6,499	**SYN**	0.68	–	14.53	**CON, SYN**
	Deep	< 1	–	2	n/a	< 1	–	21	n/a	0.01	–	0.79	n/a
BH2	Shallow	< 1	–	816	**CON**	3	–	3,973	**DIL**	0.48	–	12.01	**SYN**
	Deep	< 1	–	< 1	n/a	< 1	–	11	n/a	0.01	–	0.42	n/a
BH3	Shallow	8.5	–	24,196	**FLU, CON**	13	–	24,196	**FLU, CON**	0.05	–	8.79	**SYN**
	Deep	< 1	–	4	n/a	< 1	–	15	n/a	0.01	–	22.15	**CON, SYN**
BH4	Shallow	< 1	–	9,678	**FLU, SYN**	3	–	7,765	**FLU, DIL**	22.12	–	81.62	**CON, SYN**
	Deep	< 1	–	< 1	n/a	< 1	–	5	n/a	0.01	–	25.96	**DIL**
BH5	Shallow	< 1	–	12,.98	**SYN**	40	–	12,098	**FLU, DIL**	0.01	–	119.26	**DIL**
	Deep	< 1	–	< 1	n/a	< 1	–	11	n/a	0.01	–	0.93	n/a
BH6	Shallow	< 1	–	9,678	n/a	11	–	2,420	n/a	0.01	–	6.78	n/a
	Deep	< 1	–	< 1	n/a	< 1	–	4	n/a	0.01	–	1.01	n/a
PK1	Shallow	< 1	–	7,945	**FLU**	23	–	12,098	**FLU, SYN**	0.37	–	44.83	**DIL**
	Deep	< 1	–	< 1	n/a	< 1	–	8	n/a	0.01	–	0.49	n/a
PK2	Shallow	< 1	–	2,827	n/a	60	–	9,932	**FLU**	0.5	–	37.53	**SYN**
	Deep	< 1	–	3	n/a	< 1	–	16	n/a	0.01	–	1.21	n/a
PK3	Shallow	< 1	–	14,136	**CON**	345	–	10,390	**CON**	1.41	–	18.74	**SYN**
	Deep	< 1	–	< 1	n/a	< 1	–	10	n/a	0.01	–	0.57	n/a
PK4	Shallow	< 1	–	12,997	n/a	28	–	14,136	**FLU, CON**	0.01	–	79.33	**DIL**
	Deep	< 1	–	3	n/a	< 1	–	28	n/a	0.01	–	1.19	n/a
PK5	Shallow	1	–	13,136	n/a	7	–	12,098	**FLU**	0.21	–	54.95	**CON**
	Deep	< 1	–	< 1	n/a	< 1	–	17	n/a	0.01	–	0.8	n/a
PK6	Shallow	2	–	9,678	**FLU, CON**	11	–	19,863	**FLU**	0.35	–	19.74	**CON**
	Deep	< 1	–	2	n/a	< 1	–	22	n/a	0.01	–	11.67	**DIL**
PK7	Shallow	< 1	–	3,973	**SYN**	2	–	1,553	**SYN**	0.72	–	72.44	n/a
	Deep	< 1	–	2	n/a	< 1	–	11	n/a	0.01	–	0.65	n/a

FLU – Seasonal Flush response; **DIL** – Dilution response; **CON** – Concentrating response; **SYN** – Synoptic response; n/a – not applicable (no matching archetypal response observed)

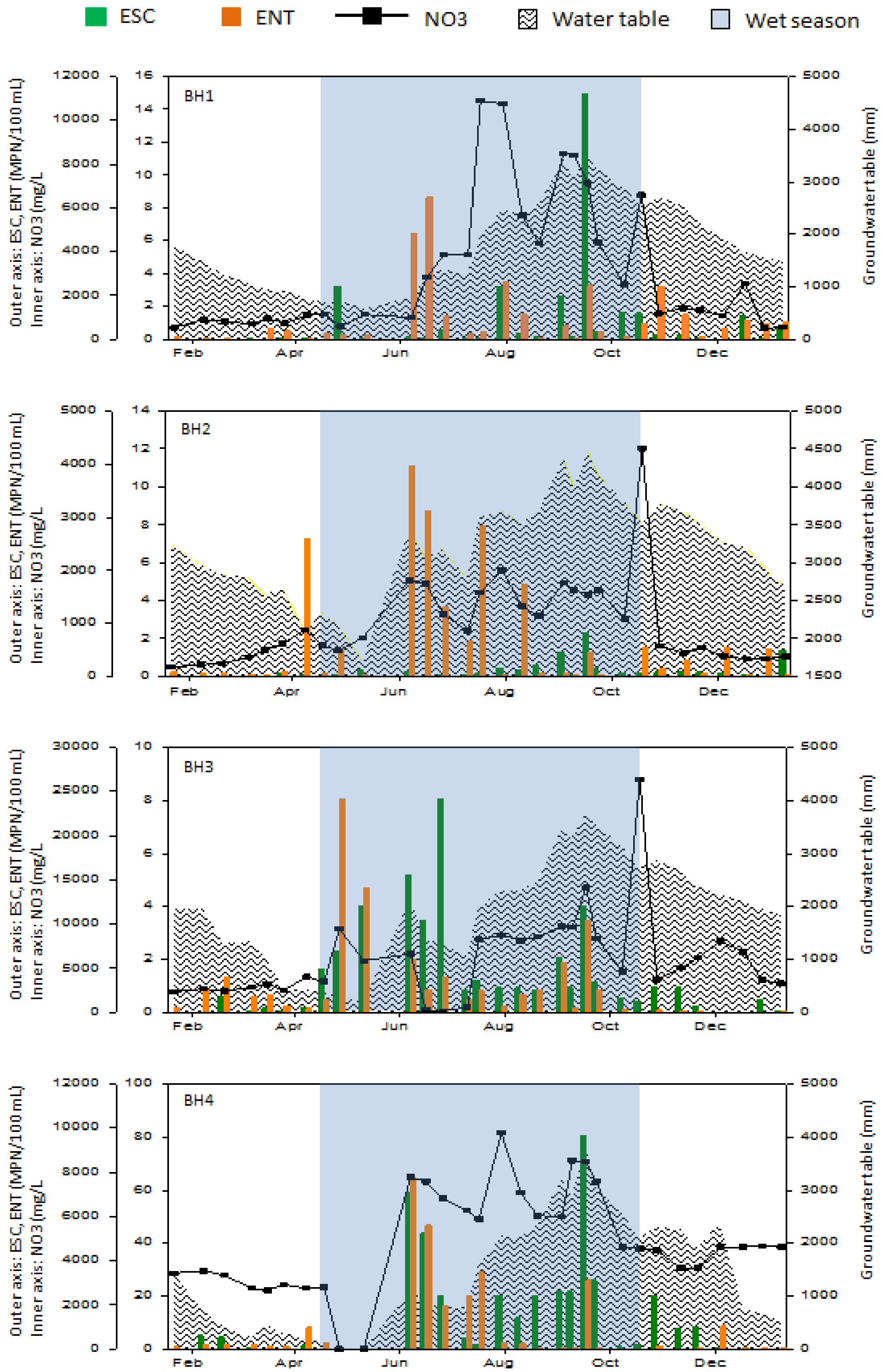

Figure 2.4: Temporal variation of *E. coli* (ESC), *Enterococcus* (ENT) and nitrate (NO3) concentration in shallow wells in response to water table fluctuation.

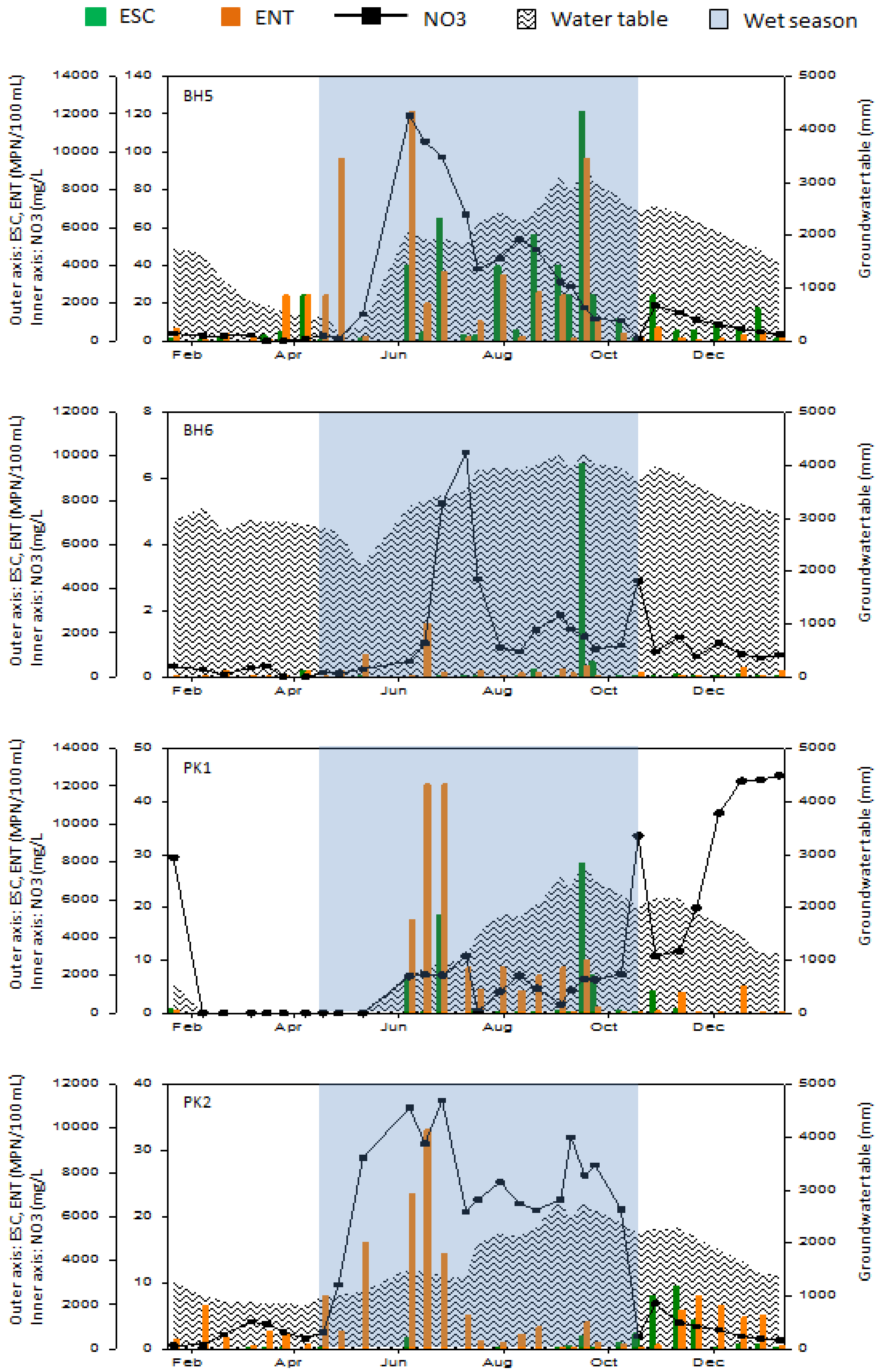

Figure 2.4 (*continued*): Temporal variation of *E. coli* (ESC), *Enterococcus* (ENT) and nitrate (NO3) concentration in shallow wells in response to water table fluctuation.

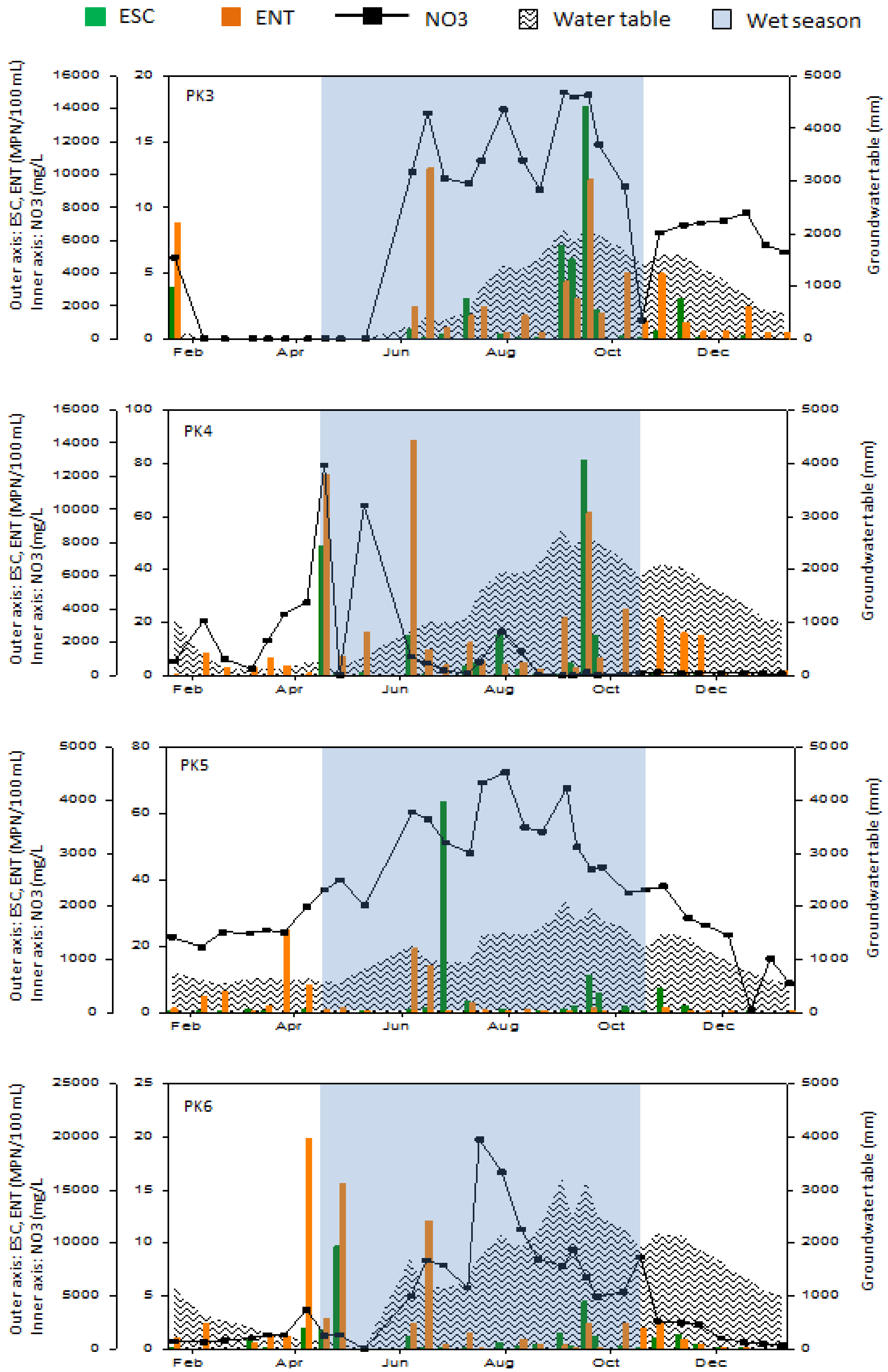

Figure 2.4 (*continued*): Temporal variation of *E. coli* (ESC), *Enterococcus* (ENT) and nitrate (NO3) concentration in shallow wells in response to water table fluctuation.

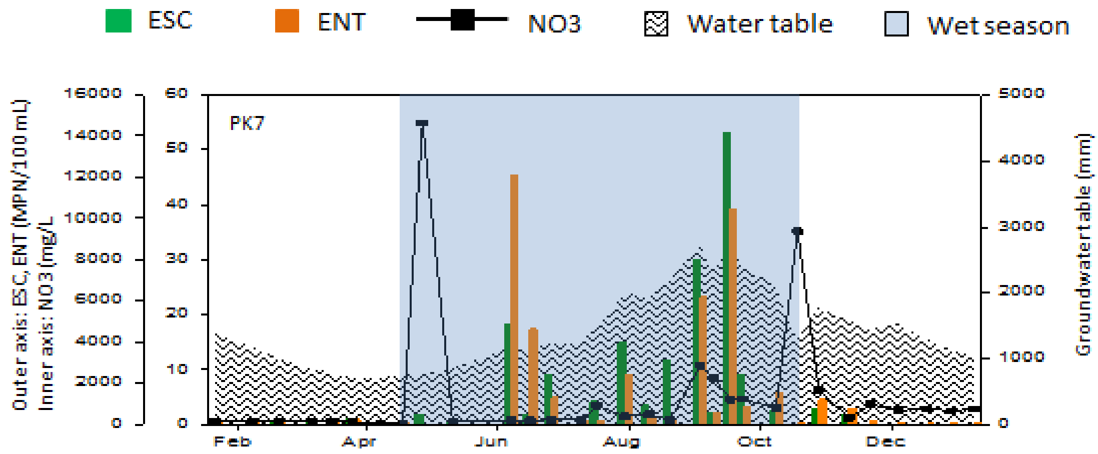

Figure 2.4 (*continued*): Temporal variation of *E. coli* (ESC), *Enterococcus* (ENT) and nitrate (NO3) concentration in shallow wells in response to water table fluctuation.

2.4.3 Deep Wells

A total of 169 samples were collected from 13 (deep) bored wells. Water samples collected from these deeper wells had lower levels of faecal contamination than shallow wells (Table 2.1). *E. coli* concentrations beyond the threshold of the WHO guidelines for drinking-water quality were recorded in only 4% of the total samples collected. All samples had *E. coli* concentrations of less than 5 MPN/100 mL. The incidence of *Enterococcus* detection with concentrations of at least 1 MPN/100 mL was higher. Approximately 23% of the water samples recorded *Enterococcus* concentrations between 2 to 28 MPN/100 mL.

Seasonality appeared to influence the level of faecal contamination in the deep wells. During the dry season, 4% of the collected water samples contained *E. coli* or *Enterococcus* concentrations of at least 1 MPN/100 mL. In comparison, approximately 24% of the water samples collected during the wet season contained unsafe levels of faecal indicator organisms.

Water supplies from the deep wells were also less prone to nitrate enrichment compared with shallow wells (Table 2.1). Nitrate concentrations were generally constantly less than 1 mg/L throughout the year. Only the water samples of the deep wells at BH3, BH4 and PK6 recorded higher concentrations of nitrate and showed seasonal variability. None of the water samples from the deep wells contained nitrate level beyond the WHO's 50 mg/L threshold for safe drinking-water. Nitrate concentrations in the water samples from the deep well at BH3 were consistently higher than those from the shallow well, with the higher concentrations recorded mainly in the wet season (Figure 2.5). For the deep well at BH4, water samples with higher concentrations of nitrate were detected during the dry season (Figure 2.5). Nitrate concentrations of water samples from the deep well at BH4 were generally lower than those sampled from the shallow well. Nitrate concentrations of water samples from the deep well at PK6 displayed a similar seasonal response as BH4, whereby elevated levels were also observed during the dry season (Figure 2.5). During the dry season, nitrate concentrations from deep well samples were higher than those from the shallow well at PK6.

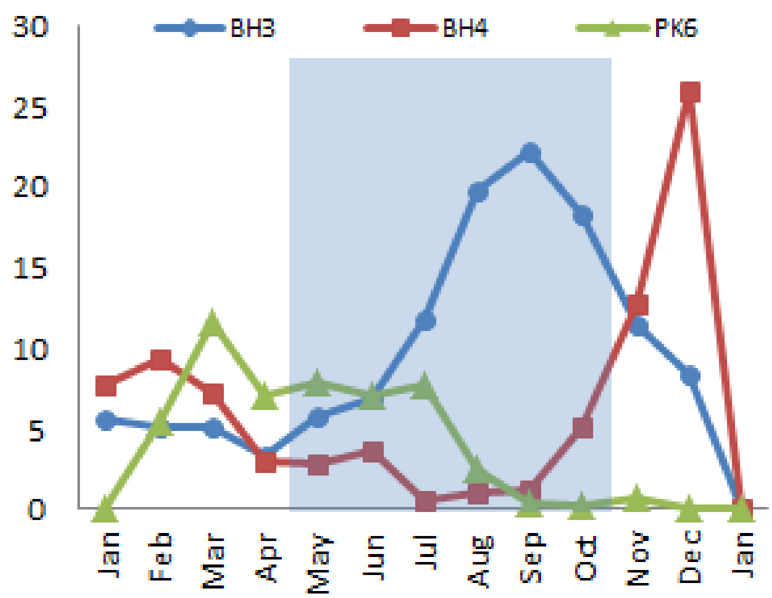

Figure 2.5: Temporal variation of nitrate concentration (mg/L) in deep wells of BH3, BH4 and PK6. Nitrate concentration in other wells are not presented as they are generally low (< 1 mg/L) and remain relatively constant throughout the monitoring period.

2.5 Discussion

2.5.1 Pollutant Transport to Shallow Wells

The shallow well data demonstrated that the observed concentrations of pollutants are affected by underlying flow transport mechanisms linking the sampled wells and pollution sources, which in this case are household on-site sanitation systems. We observed four archetypal responses that are inherently influenced by rainfall distribution and water table fluctuations: (1) Seasonal flush response;

(2) Dilution response; (3) Concentrating response; and (4) Synoptic response. In some cases, these responses were not mutually exclusive.

A *seasonal flush response* was characterised by disproportionately high initial concentrations of pollutants in sampled well water early in the wet season, followed by a rapid decline thereafter (Figure 2.6a). *E. coli* and *Enterococcus* have a high likelihood to be affected by the seasonal flush effect as this response can be observed in eight shallow wells: BH3, BH4, BH5, PK1, PK2, PK4, PK5 and PK6 (Table 2.1, Figure 2.4). During the dry season, a low groundwater table limits the transfer of waste from on-site sanitation systems to the groundwater. Faecal matter in wastewater draining from the cess pits is filtered in the soil matrix, where it is retained until a rising water table or downward percolation of rain water at the commencement of the wet season restores the connection (Figure 2.6b). During the initial period, the stored faecal matter in soil is flushed in relatively high concentrations into the groundwater, where it is transferred laterally to nearby water wells (Figure 2.6c).

Some shallow wells exhibited a *dilution response*, whereby concentrations of the faecal pollutants had an inverse relationship with the groundwater level (Figure 2.7a). This archetypal response was observed in the shallow wells of BH2, BH4, BH5, PK1, PK4 and PK6 (Table 2.1, Figure 2.4). As the water table rose during the wet season, the increasing volume of the groundwater diluted the contaminants' concentrations of the sewage water coming from the on-site sanitation systems (Figures 2.7b and 2.7c). The reverse effect occurred in the dry season. The lowering of the water table, and thus the decrease of groundwater volume in the well, amplified the concentrations of faecal contaminants.

A *concentrating response* archetype was also produced in association with a rise of the water table and contact with an on-site sanitation system. In contrast to the dilution response, the concentration of pollutants in shallow wells represented by this archetype has a direct relationship with the level of water table. Concentrations increase/decrease with the rise/fall of the groundwater table (Figure 2.8a). Shallow wells at BH1, BH2, BH3, BH4, PK3, PK4, PK5 and PK6 exhibited this response (Table 2.1, Figure 2.4). In this case, the distance of on-site sanitation systems to the water table plays an important role in connecting the pollution source and groundwater (Figures 2.8b and 2.8c). In the wet season, the water table rises near the ground surface, flooding cess pits almost entirely, allowing faecal material to potentially bypass any filtration afforded by the soil layers. In most cases, once the connection between the groundwater and a cess pit is established, the movement of contaminants to the water well should remain high during the remainder of the wet period.

A *synoptic response* archetype is typified by consistently elevated concentrations of faecal contamination indicators throughout the wet season in response to individual rainfall events (Figure 2.9a). This response was observed in the all the shallow wells except the ones at BH6, PK4, PK5 and PK6 (Table 2.1, Figure 2.4). This response may be related to a reconnection of a rising groundwater and the on-site sanitation system and/or by percolating rainwater through the soil layer thereby mobilising stored contaminants into the ground water (Figures 2.9b and 2.9c). Given the infrequent sampling, it is difficult to distinguish the synoptic response from a concentrating response. Daily testing would be required to accomplish this.

Despite aligning with four basic archetypical responses, the observed temporal patterns of all faecal indicator (*E. coli*, *Enterococcus* and nitrate) concentrations in the surveyed wells were variable. The concentration changes in response to the hydroclimatic factors need not be similar, even if the pollutants originate from the same source because these indicators have inherent qualities that affect their transport and concentrations in groundwater. For example, *E. coli* and *Enterococcus* likely undergo some degree of filtering depending on the properties of the soil (Foppen and Schijven, 2006; O'Luanaigh et al., 2012; Stevik et al., 2004). In contrast, dissolved nitrate would not be filtered by the soil in between a cess pit and the water table.

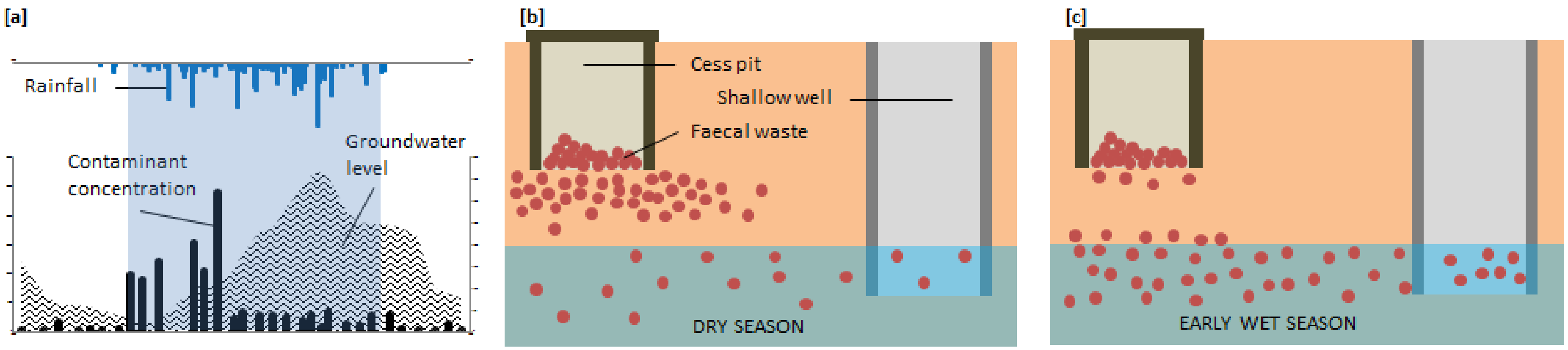

Figure 2.6: Seasonal flush response of faecal indicators in shallow wells. [a] Generic faecal indicator concentration response to rainfall and groundwater level for the seasonal flush response archetype where the initial contaminant concentration is disproportionately high followed by a rapid decline after the early rainfall events of the wet season. [b] Contaminants are accumulated in the soil strata underneath the on-site sanitation system during the wet season. [c] Early rainfall at the beginning of the wet season flushes the accumulated contaminants into the groundwater.

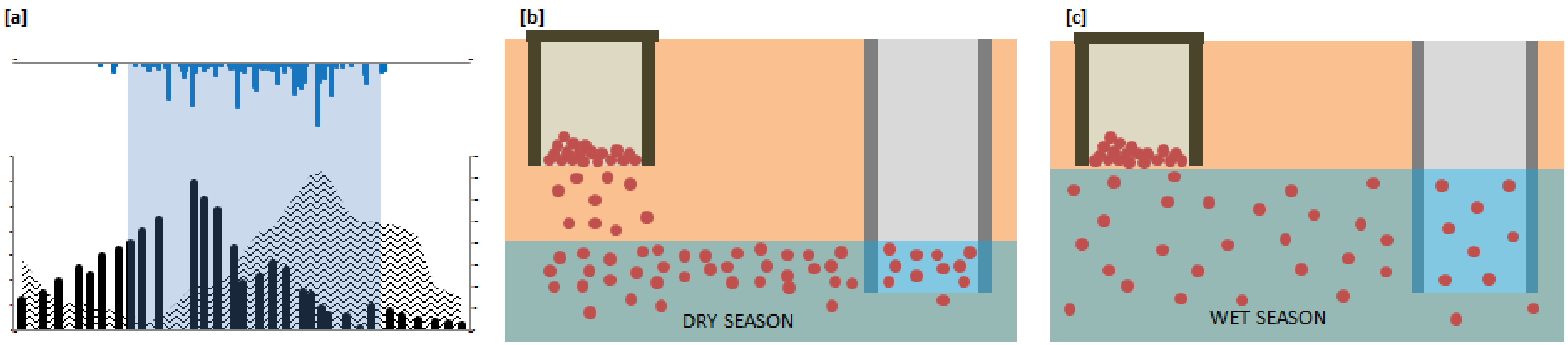

Figure 2.7: Dilution response of faecal indicators in shallow wells. [a] Generic faecal indicator concentration response to rainfall and groundwater level for the dilution response archetype where concentrations of contaminants have an inverse relationship with groundwater level . [b] Constant stream of wastewater leaks into low groundwater during the dry season resulting in high concentrations of faecal contaminants. [c] The rise of the water table during the wet season increases the volume of groundwater which in turn dilutes the concentration of contaminants.

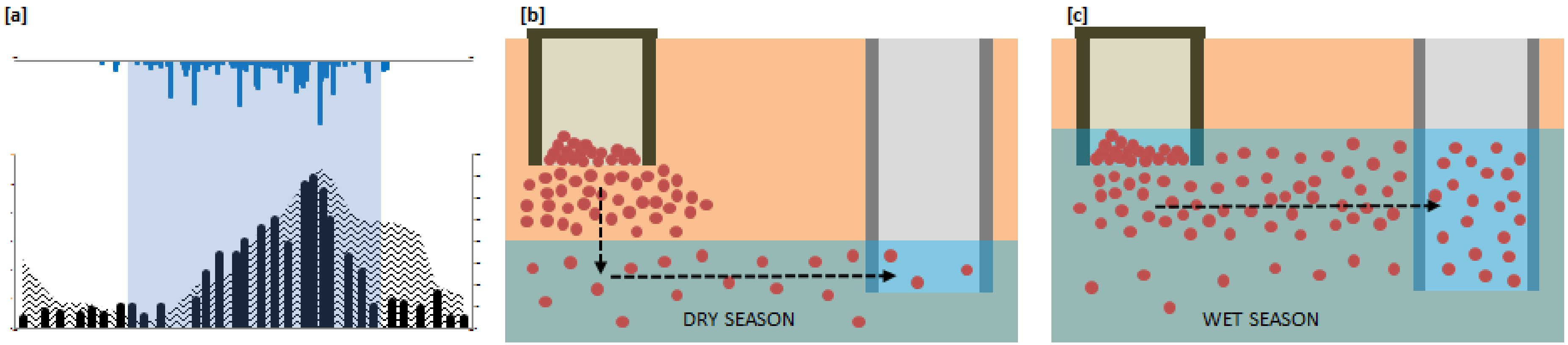

Figure 2.8: Concentrating response of faecal indicators in shallow wells. [a] Generic faecal indicator concentration response to rainfall and groundwater level for the concentrating response archetype where concentration of contaminants has a direct relationship with the water table. [b] During the dry season, contaminants percolate through greater distance through the soil strata before reaching low groundwater and consequently transported laterally to wells. [c] The rise of water table during the wet season decreases the distance between the base of on-site sanitation systems and groundwater (sometimes, flooding the cess pits) which facilitates the lateral transport of contaminants to wells.

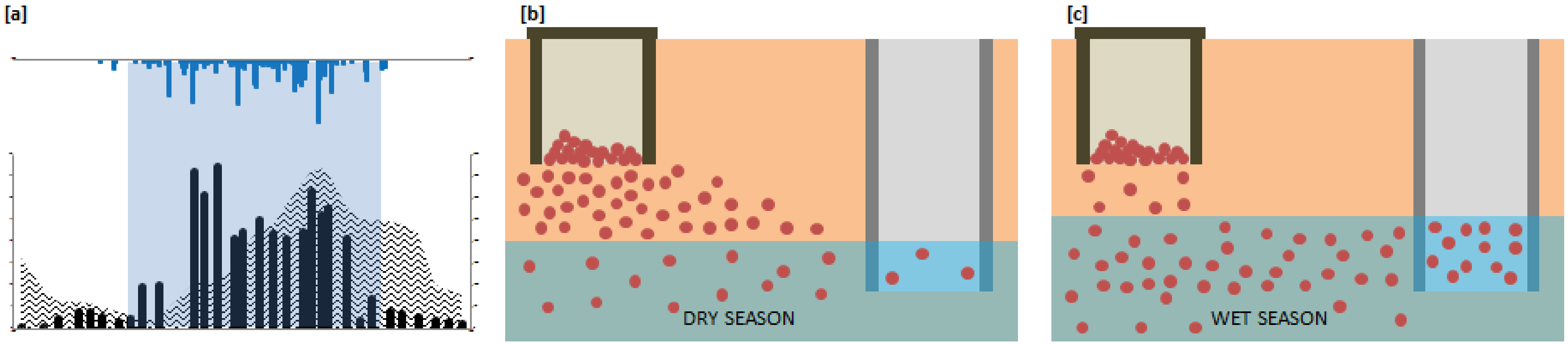

Figure 2.9: Synoptic response of faecal indicators in shallow wells. [a] Generic faecal indicator concentration response to rainfall and groundwater level for the synoptic response archetype where consistently elevated concentrations of contaminants are monitored throughout the entire wet season. [b] During the dry season, contaminants from on-site sanitation systems leak into groundwater at a constant rate. [c] During the wet season, percolating rain facilitates the mobilisation of the vertical transport of contaminants into groundwater system. The rising of the water table may also contribute to the increase of contaminant concentration in wells as described in the concentrating response archetype above. Due to the infrequent sampling, it is difficult to distinguish the synoptic response from a concentrating response.

The two faecal indicator bacteria used in this study also possess some differing intrinsic qualities that may affect their measured concentrations in groundwater. *E. coli* bacteria are usually found in greater numbers in human faeces, generally about an order of magnitude higher, compared to intestinal enterococci (WHO, 2011). In comparison, *Enterococcus* bacteria are less prone to die-off and decay as they are able to endure a bigger range of stresses (e.g. temperature, pH, salinity) that allow them to survive longer in environments outside their natural habitat (Fisher and Phillips, 2009; WHO, 2011).

The higher concentrations of faecal contaminants found in the shallow wells, compared with the deep wells, suggest a dominant influence of shallow lateral transport of wastewater from on-site sanitation systems rather than deep vertical transport to underlying aquifers. This mechanism facilitates the occurrence of all the archetypical responses that were observed in the wells. Further, the data suggest that the unregulated use of unlined on-site sanitation systems has resulted in elevated faecal, microbial and nitrate concentrations in groundwater at the study site. As all the sampling wells were sealed to prevent intrusion of surface water, the sources of these faecal contaminants are unlikely to originate from rainfall runoff.

2.5.2 Pollutant Transport to Deep Wells

The results from this study revealed that the deep wells in both villages were generally less susceptible to microbial contamination from on-site sanitation systems. Concentrations of both faecal indicator organisms – *E. coli* and *Enterococcus* – in the deep wells were lower than those recorded in the shallow wells throughout the sampling period. The soil strata underneath the on-site sanitation systems therefore have played a role restricting the movement of faecal matter to deeper aquifers by filtering through straining or adsorption (Ginn et al., 2002; Stevik et al., 2004). In addition, Margane and Tatong (1999) noted the existence of a layer of low-permeability silt and clay within the soil profile of San Sai District. This layer could exist between the shallow and deep wells and hence, functioned as an aquitard that disconnect shallow and deep aquifers.

Nitrate levels in water samples from the deep wells were also generally lower than water samples from shallow wells. Again, the presence of a low-permeability silt/clay layer would likely limited the vertical transport of nitrate into the deeper aquifers. Only deep well samples from BH3 and BH4 had higher nitrate concentrations than their corresponding shallow well samples. In these locations, the proximity of nitrate sources to these wells may have influenced the observed concentration differences. For example, the BH3 deep well was constructed only 5 m from the nearest on-site sanitation system, while the shallow well was located approximately 20 m away. Similarly, for BH4, the bored well was constructed at the back of the house, in closer proximity to the toilet compared to the hand-dug well which was located in front of the house.

2.5.3 Risks of Infections and Diseases

The observed high concentrations of faecal indicators (*E. coli*, *Enterococcus* and nitrate) show that shallow aquifers in the studied villages are subjected to contamination from on-site sanitation systems. Ingestion of water from these shallow wells without sufficient treatment (e.g. filtration, chlorination, boiling) presents a risk of contracting waterborne diseases. Elsewhere, overwhelming evidence has shown that wastewater originating from sanitary sewage systems often contain highly infectious pathogens including viruses, bacteria, protozoa and helminths (e.g. Cheng et al., 2009; Gallas-Lindemann et al., 2013; Grondahl-Rosado et al., 2014; Hellmer et al., 2014; Kitajima et al., 2014; Steyer et al., 2015).

In neighbouring Vietnam, for example, Yen-Phi et al. (2010) discovered that between 60 and 70% of the samples collected from untreated and partially treated sludge in household septic tanks contained *Salmonella* spp., the bacterial agent that causes typhoid fever. In addition, 95% of the collected samples tested positive for 12 varieties of helminth ova. The dominant variety was *Ascaris lumbricoides*, the etiological agent for ascariasis, a neglected tropical disease, which is also transmitted via the faecal-oral route (Dold and Holland, 2011; WHO, 2010). The eggs of this nematode (roundworm) were detected in nearly 50% of the samples and had a mean concentration of almost 8,000 viable ova per litre of septage sludge.

Despite lower levels of *E. coli* and *Enterococcus* detected in deep wells, the threat of waterborne diseases still persists if the water is consumed untreated. As described above, the layers of soil beneath the on-site sanitations may remove some bacteria and protozoa by filtration, resulting in decreased concentrations in the deeper aquifers. However, some smaller-sized pathogens, specifically viruses, may escape filtration. Enteric viruses, for example, are much smaller (20 – 80 nm) than both faecal indicator organisms used in this study, *E. coli* (rod shaped: 2.0 – 6.0 µm x 1.1 – 1.5 µm) and *Enterococcus* (coccoid, diameter: 0.5 – 1.5 µm) (Kokkinos et al., 1998; Percival et al., 2004; Tufenkji and Emelko, 2011).

Borchardt et al. (2007) found this phenomenon in their study of the occurrence of human enteric viruses in deep municipal wells (depth > 200 m) in Madison, Wisconsin (U.S.A.). These wells drew water from an aquifer confined by an aquitard of clayey-sandy siltstone with thin laminae of fine-grained siltstone and shale units. In spite of low-permeability, which is commonly assumed to protect underlying aquifers from contamination from the shallow aquifers, approximately 23% of the samples tested positive for human enteric viruses, including the infectious, pathogenic echovirus 18, the etiological agent for aseptic meningitis.

Again, the soil strata can protect underlying groundwater via filtration of the larger-sized pathogens. However, very small contaminants and those in dissolved forms will not typically be removed, as was observed at deep wells BH3, BH4 and PK6 where elevated levels of nitrate were found (Figure 2.5). Although the recorded values are still within safe limits for drinking, an increase in population and the corresponding increase in sewage would likely increase the concentrations of nitrate, potentially rendering the groundwater unsuitable for consumption.

Many studies have established a link between precipitation and the incidence of waterborne diseases. Most studies showed strong, positive correlations between rainfall and occurrence of waterborne diseases (Akanda et al., 1999; Curreiro et al., 2001; Drayna et al., 2010; Hashizume et al., 2007; Singh et al., 2001; Thomas et al., 2006). At our study site, most wells showed substantial increase in faecal contamination during the wet season, implying a greater risk of the transmission of enteric pathogens during this part of the year. Kaewkes et al., (2012) also determined that the highest levels of faecal contamination were recorded during the rainy months in Northeast Thailand. Curreiro et al. (2001) reported that waterborne disease outbreaks were commonly preceded by precipitation events in the United States between 1948 and 1994. This finding is in line with the *flush response* we found at the study villages. Carlton et al. (2013) also made similar observations where they noted increased diarrhoea incidence was associated with heavy rainfall events that occurred following relatively dry periods in Ecuador. In addition, Carlton et al. (2013) also reported that the number of diarrhoea cases decreased following relatively wet periods. This finding is consistent with the *dilution response* we observed from this study. Pinfold et al. (1991) also made a similar observation in Northeast Thailand where they found that incidences of diarrhoeal diseases were consistently reduced during the peak of the rainy season during their entire study period between 1982 and 1987.

The excessively high levels of faecal contamination observed in shallow versus deep wells at the study site indicate the dominance of the lateral transport processes of contaminants in the aquifer. Human excreta and associated contaminants move from unlined on-site sanitation systems to these wells following the flow of groundwater. As surface and sub-surface water systems are linked components of a hydrologic continuum (Sophocleous, 2002), it is therefore also possible for faecal contaminants from on-site sanitation systems to be transported to nearby surface water bodies. The extensive networks of irrigation canals in rural agricultural landscapes of Thailand can facilitate the movement of these contaminants including pathogens across the landscape via surface flow (Cohen and Pearson, 1989). In addition, local streams and storm drains are also recipients of these faecal-contaminated sub-surface water resources.

The transport of the wastewater from on-site sanitation systems to surface water conveyance systems is a particular concern as these water resources eventually drain into the Ping River, an important source of drinking-water supply in many areas of northern Thailand. At present there are several water treatment plants in Chiang Mai that draw water from the Ping River. These plants are managed by the Provincial Waterworks Authorities (PWA) and supply water to consumers in high density urban areas like Chiang Mai City and district centres. Many villages also depend on river water for domestic use. Unlike the water supplied by the PWA, many of these village-managed water supplies rely solely on filtration for treatment and do use any form of disinfection (e.g. chlorination). Even so, chlorinated water supplies may not necessarily be disinfected sufficiently. Some enteric pathogens, for example, *Cryptosporidium* spp., have high resistance to chlorination and are small enough (diameter: 4 – 6 µm) to bypass the filtration units in water treatment plants (Fayer et al., 2000; King and Monis, 2007). As such, an increase in the level of faecal contamination in these surface water resources will therefore increase the risk of exposure to waterborne diseases for local communities utilising river water as a source of drinking-water supplies.

2.5.4 Management Implications

As of 2009, there were only 95 municipal wastewater treatment plants in Thailand. This small number of treatment facilities could only treat approximately 20% of the wastewater generated by a population of 66 million. Almost all of these wastewater treatment plants were constructed after the turn of the new millennium. Simachaya (2009) reported that virtually all of these systems experience some type of operating problems that adversely affects the treatment process: e.g. damaged or non-functioning equipment or high rates of surface and groundwater infiltration into the collection system. As a result, a significant portion of the wastewater entering these systems is discharged into the environment without sufficient treatment.

The major reasons cited for the poor performance of wastewater systems are lack of funding for maintenance and unavailability of reliable personnel to operate the equipment (Simachaya, 2009). In Thailand, the central government agencies that are in charge of planning and implementing waste treatment facilities have thus far been unable to ensure that the systems can be operated sustainably (Simachaya, 2009). The local government authorities who are responsible for the operations and maintenance of these wastewater treatment facilities often receive inadequate training to prepare them to manage these systems effectively (Simachaya, 2009).

It is unclear when the complete centralisation of wastewater treatment in Thailand will be accomplished (Simachaya, 2009). Even in the United States, many areas are still served only by decentralised systems, despite the passing of the Clean Water Act in the early 1970s (Stevik et al., 2004; Tchobanoglous et al., 2004). As such, especially in the peri-urban and rural areas, on-site sanitation systems may be the ideal long-term option for wastewater management in Thailand. The many advantages of decentralised wastewater management systems, including cost effectiveness and flexibility in management, are well established (Libralato et al., 2012; Massoud et al., 2009; Tchobanoglous et al., 2004).

Tchobanoglous et al., (2004) noted that the proper implementation of on-site sanitation systems is a good opportunity to catalyse the paradigm shift from the traditional wastewater *disposal* to the more sustainable waste *reuse*. Nutrient-rich wastewater and sludge from on-site sanitation systems are viable irrigation sources. In a case study in Thailand, Schouw et al., (2003) analysed the composition of human excreta and concluded that human faecal matter constitute a large fertiliser resource that was not being utilised fully. Wastes from the on-site sanitation systems, when adequately sterilised and treated, can be used as organic fertilisers by the predominantly agricultural-based societies in Northern Thailand.

Finally, the 2015 drought in Thailand was a harsh reminder of the unpredictability of the climate (Tang, 2015). This environmental hazard depleted many surface water resources, with the main reservoirs falling to the lowest levels in two decades (Suwannakij, 2015). Mandated rationing was implemented in almost one third of the country (Regan, 2015). In times of drought, local groundwater resources can be potential supplemental sources of water for areas reliant on reservoirs. However, the findings of this study suggest that hand-dug (depth: 3 – 5 m) and bored wells (10 – 12 m) are vulnerable to contamination by wastewater from-site sanitation household on-site sanitation systems.

Nationwide, many areas now extract groundwater from deep sources (e.g. > 50 m), usually from protected confined aquifers – many of which have very low recharge rate (Phien-wej et al., 2006; Subtavewung, 2006). While the extraction of deep groundwater may be an alternative source of safe drinking-water supply in some areas, it is not a viable option in other parts of the country where the occurrence of naturally occurring water constituents originating from these deeper aquifers represent a health hazard. For example, at the city of Hat Yai in Southern Thailand, arsenic concentrations (up to 1,000 µg/L) exceeding the WHO recommended guideline values for drinking-water of 10 µg/L have been measured in groundwater from deep (30 – 50 m) wells (Lawrence et al., 2000). In Chiang Mai and the neighbouring province of Lamphun, hazardous levels of fluoride exceeding the WHO recommended guideline values for drinking-water of 1.5 mg/L have also been recorded in deep well water (Namkaew and Wiwatanadate, 2012; Takizawa et al., 2010). Consequently, the consumption of the high-fluoride water had resulted in the increased incidences of fluorosis in these areas (McGrady et al., 2012; Namkaew and Wiwatanadate, 2012; Takizawa et al., 2010). With the presence of these naturally-occurring drinking-water hazards in the deeper groundwater sources, affected areas, particularly villages without municipal water supply, are therefore highly reliant on the shallow aquifers as their primary source drinking- and domestic water resources. However, as we have presented in this study, without a proper wastewater management system in place, these shallow groundwater resources are susceptible to faecal contamination.

2.6 Conclusion

Target 7C of the United Nations' Millennium Development Goals established in the year 2000 was to "*halve the proportion of people without sustainable access to basic sanitation and safe drinking water by 2015*". Thailand appears to have achieved this goal with nearly all of the population having access to basic sanitation facilities and drinking-water sources today (UNICEF and WHO, 2015). This is a laudable feat that has contributed to a decrease in morbidity and mortality stemming from faecal- and water-related diseases in the country.

Yet, overlooked is the implication of the unregulated installation of the non-watertight on-site sanitation systems that provide minimal protection to local groundwater resources against faecal contamination. Our study demonstrates that the implementation of these on-site sanitation systems have resulted in water quality degradation of local groundwater resources. All of the sampled wells showed signs of faecal contamination and therefore, water from these wells are unsuitable for drinking without treatment. Consequently, many of these wells have been abandoned leading to the neglect of a viable source of water for drinking and other domestic purposes. These local groundwater resources, when adequately protected and managed, can represent viable, renewable and hence, sustainable sources of drinking-water especially for the rural populations without access to treated municipal water supplies.

Finally, to ensure the successful implementation of these on-site sanitation systems, the management strategies must be site-specific, accounting for environmental conditions in the target area. The understanding of the receiving environment and the hydroclimatological factors which affect the transport of faecal wastes are particularly crucial for the selection of the most appropriate technology to collect, store and treat wastewater on-site. For example, the suitability of open-bottomed on-site sanitation systems in areas with high water table — akin to those investigated in this study site, should be re-evaluated to ensure that the underlying aquifers are sufficiently protected against contamination.

CHAPTER 3 – *CRYPTOSPORIDIUM* AND *GIARDIA*: WATERBORNE PARASITES IN SURFACE WATERS

3.1 Introduction

Diarrhoea claims more lives of children than AIDS, malaria and measles combined and is ranked as the second most common cause of death for children under five years of age worldwide after pneumonia (UNICEF and WHO, 2009). Nearly one in five children die each year from diarrhoea (UNICEF and WHO, 2009). Diarrhoea is caused by a wide range of pathogens including viruses, bacteria and protozoa. Of these, *Cryptosporidium* and *Giardia* are two of the most globally dominant and dangerous parasitic protozoa that infect not only humans, but also domestic animals and wildlife, (Caccio et al., 2005; Haque, 2007; Hunter and Thompson, 2005).

Cryptosporidium and *Giardia* are monoxenous: they complete their life-cycles within a single host, which excretes large numbers of infective stages (*Cryptosporidium* oocysts and *Giardia* cysts) in faeces. A gram of faeces from an infected host may contain as many as 1×10^7 and 2×10^6 *Cryptosporidium* and *Giardia* cysts, respectively (Smith et al., 2006). While there may be several modes of transmission, infection typically occurs following ingestion of water contaminated with *Cryptosporidium* and *Giardia* cysts — even in small doses. Infections in humans have been reported occur in doses as low as 9 and 10 cysts for cryptosporidiosis and giardiasis, respectively (Smith et al., 2006). The cysts are environmentally robust, allowing them to persist for long periods of time outside the host. Their small size allows them to penetrate the physical barriers of conventional water treatment systems. They are also insensitive or resistant to many disinfectants used in the water industry (e.g. chlorine). *Cryptosporidium* and *Giardia* therefore constitute a significant health hazard, even in developed countries.

Between World War I and 2003, a total of 325 recorded water-associated outbreaks of parasitic protozoan diseases occurred (Karanis et al., 2007). North America (Canada and the United States) and Europe (primarily the United Kingdom) accounted for 93% of the reported outbreak (Karanis et al., 2007) — most likely due to reporting bias (Baldursson and Karanis, 2011). Cryptosporidiosis and giardiasis make up nearly all of the reported cases: 51% and 41%, respectively. More recently, at least 199 outbreaks occurred between January 2004 and December 2010 (Baldursson and Karanis, 2011). Again, *Cryptosporidium* (60%) and *Giardia* (35%) were the main etiological agents of these waterborne parasitic outbreaks. Documented cases were mainly reported from North America and Europe, along with 'newcomers', Australia and New Zealand. Reports from these countries/continents make up approximately 96% of the documented outbreaks (Baldursson and Karanis, 2011).

Consideration of these two reviews discloses several important findings. Firstly, *Cryptosporidium* and *Giardia* are dominant causative agents of waterborne disease outbreaks, compared with other protozoan parasites. Secondly, even first world nations with reliable and modern water treatment systems and technology are susceptible to parasitic outbreaks. Thirdly, marked progress has been made in the detection and diagnostic methods, which in turn has resulted in the improvement in surveillance and reporting systems. Finally, there is a lack of research and monitoring in developing countries of Asia, Africa and Latin America. This latter issue is ironic, yet important, because the poorer communities from these regions without reliable water and sanitation facilities are likely more vulnerable to these diseases than those in the developed world where most cases are reported (Hotez et al., 2009; Pruss-Ustun et al., 2008; WHO, 2008b).

Similarly, in the developing nations of Southeast Asia, *Cryptosporidium*- and *Giardia*-related studies are relatively rare compared with their first world counterparts. Wherever available, studies on *Cryptosporidium* and *Giardia* almost always pertain to their prevalence in hosts rather than the environment (Dib et al., 2008; Lim et al., 2010 and the references therein). In Thailand, for example, only five such studies have been published (Anceno et al., 2007; Diallo et al., 2008; Koompapong and Sukthana, 2012; Kumar et al., 2014; Srisuphanunt et al., 2010). These studies typically only investigated the occurrence of *Cryptosporidium* and *Giardia* in the aquatic environment and water resources. Investigations on the factors contributing to their distribution are rare.

Herein, this research void is addressed by investigating the spatial variation of *Cryptosporidium* and *Giardia* in the surface water resources in a rural study area in northern Thailand. The role of seasonality (i.e. dry weather vs. wet weather) is also explored by investigating the association of hydroclimatological factors with the distribution of *Cryptosporidium* and *Giardia* in the environment. In addition, faecal samples from cattle, an important host for *Cryptosporidium* and *Giardia*, were also screened for both protozoa as well as other intestinal parasites. Finally, isolates of both organisms in faecal samples from cattle were molecularly characterised.

3.2 Site Description

The area investigated for this study is centred on the Kuang River Basin, which is located on the eastern bank of the Ping River in Northern Thailand. The catchment area is 1,661 km^2 and has a population of 291,000, of which, half are classified as rural (Ganjanapan and Lebel, 2014). The area spans across the districts of San Sai, San Kamphaeng, Mae On and Doi Saket within the Chiang Mai Province, as well as the districts of Ban Thi, Pa Sang and the capital district (*Amphoe Mueang*) of the Lamphun Province. Forests, mostly deciduous and dry dipterocarp, cover just over half of the drainage basin and are mostly restricted to higher elevations. Approximately one third of the area is devoted to agriculture and about 7– 8% to residential use (Ganjanapan and Lebel, 2014).

The Kuang River is an important tributary to the Ping River, which drains into the Chao Phraya River in Central Thailand (Figure 3.1). The Ping River basin is the largest (catchment area of over 35,000 km^2) in the Chao Phraya River basin. It is a vital source of water not only in the northern region, but also to the nation's capital, Bangkok, as well as many parts of Central Thailand for domestic, agricultural and industrial uses (Thomas, 2005).

The Lai River, Pong River and San River are major tributaries to the Kuang River. The former, together with the upper reach of the Kuang River, form the primary inflows to the Mae Kuang Reservoir. With a water storage capacity of approximately 260 million m^3 and a catchment area of over 550 km^2, the Mae Kuang Reservoir is a major source of irrigation, domestic and drinking-water supply for many locations in the provinces of Chiang Mai and Lamphun (Chansribut, 2002; Nutniyom, 2003).

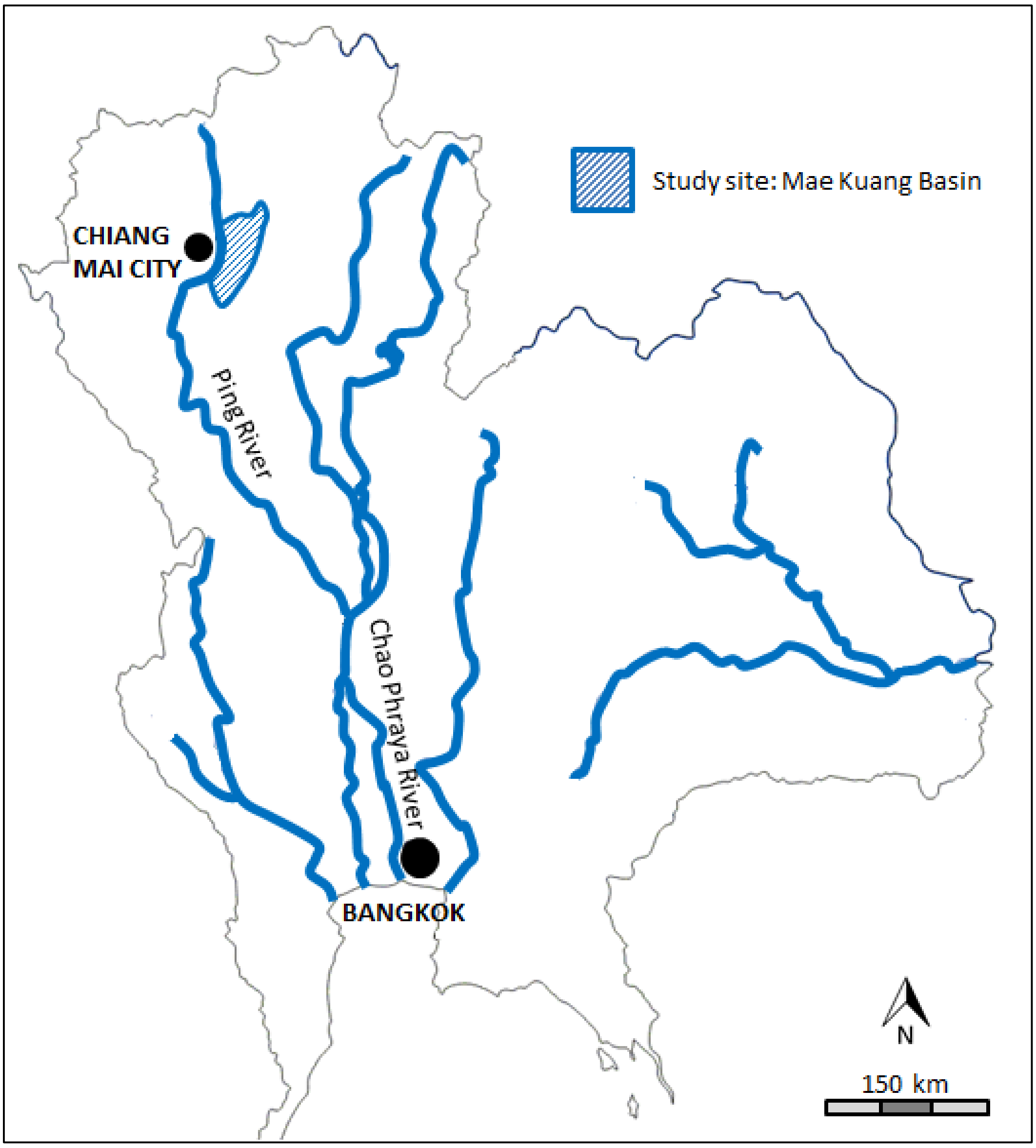

Figure 3.1: The location map of the study area, the Mae Kuang Basin

A complex network of canals also distributes surface water across the study site. Importantly, the Mae Taeng-San Sai Canal, constructed and managed by the Royal Irrigation Department of Thailand, is a 40-km long, trapezoidal concrete canal that receives water from the Ping River, immediately downstream of the Mae Ngat Reservoir, and conveys water to the San Sai District from the Mae Taeng District, to the north. The water from this canal is typically used from irrigation purposes. The canal can also be used for recreational purposes where we observed villagers bathing in the waters particularly during the dry season.

Chiang Mai and Lamphun have a tropical wet and dry climate (Köppen *Aw*), typical of the northern region of Thailand. Annual rainfall in Northern Thailand is approximately 1,200 mm with seasonal rainfall, between May and October, accounting for almost 92% of the annual total (Wood and Ziegler, 2008; Lim et al., 2013).

The livestock sector contributes approximately 2.3% to Thailand's gross domestic product (GDP) (FAO, 2005). The cattle farming industry has been growing steadily over the years with an increase of nearly 46% in the number of cattle between 1961 and 2013 (FAO, 2015b). Correspondingly, milk as well as beef production had also increased during this period. Milk production increased nearly 550 fold from 2,000 tonnes in 1961 to 1,095,000 tonnes in 2013 (FAO, 2015b). Growth in the beef production was lower (56%) with 70,000 tonnes of beef produced in 1961 compared to 160,000 tonnes in 2013 (FAO, 2015b). Approximately 26% of the total number of cattle in Thailand is found in the northern region whereby over 70% are from small-scale farms with less than 10 cows (FAO and APHCA, 2002).

3.3 Materials and Methods

3.3.1 Water Samples

3.3.1.1 Sample Collection

A total of 120 water samples were collected from 60 sampling sites from natural (52) and manmade (8) surface water bodies at the study area which include the following: the Kuang River (K1 – K18), the Upper Kuang River (uK1 – uK4), the Lai River (L1 – L7), a Lai River tributary (tL1 – tL3), the Pong River (P1 – P9), the Upper Pong River (uP1 – uP3); a Pong River Tributary (tP1 – tP3), the San River (S1 – S5) and the Mae Taeng-San Sai Canal (C1 – C8). Sampling was carried out twice at each sampling site: (i) near the end of dry season (April – May, 2014) when water levels are at the lowest; and (ii) during the peak of the rainy season (July – August, 2014). For each sampling occasion, 40 L of river/canal water were collected directly from water bodies with a bucket and stored in two 20-L plastic bottles before transferring to the laboratory for analyses. For the dry weather samples, no rain events were recorded at least one week preceding the days of collection. For the wet weather samples, collection was carried out on five separate occasions – 15th July, 16th July, 14th August, 16th August and 18th August. The total rainfall three days preceding the days of collection were 258 mm, 63 mm, 99 mm, 124 mm and 11 mm, respectively. Rainfall data were obtained from our weather station in San Sai District.

3.3.1.2 Sample Analyses

The procedures employed for detection and enumeration of *Cryptosporidium* and *Giardia* processes followed those developed and validated by the United States Environmental Protection Agency (USEPA, 2012): (i) filtration; (ii) elution (wash); (iii) concentration; (iv) purification (immunomagnetic separation); (v) staining; and (vi) immunofluorescence assay microscopy. Briefly, 40 litres of water sampled from each site were transported immediately to a private laboratory in Chiang Mai. The samples were filtered on the same day of collection using Filta-Max® foam cartridge filters (IDEXX Laboratories, Inc., Westbrook, ME, USA), which retain *Cryptosporidium* or *Giardia* cysts. Filtration was assisted by a motorised pump located on the inlet side (upflow) of the filters.

Following filtration, the filters were transferred to a Filta-Max® manual wash system (IDEXX Laboratories, Inc., Westbrook, ME, USA) to elute all cysts retained. To do so, the filters were washed with 600 mL of phosphate buffered saline (PBS) (10 mM) with 0.01% Tween® 20 (PBST). Following washing, the concentrator tube containing the eluate was transferred onto a magnetic stirring plate. While stirring (to ensure that any cysts present stay afloat), the tube was drained from its base to concentrate the samples to approximately 20 mL. The filters were washed for the second time with 600 mL of PBST. The concentrates from the first wash were pooled with those from the second wash and then concentrated to a final volume of approximately 20 mL. Any cysts retained on the filter membranes from the base of the concentrator tube were washed off with PBST by transferring the filter membranes into a small sealable plastic bag with approximately 5 mL of PBST and then manually kneading the membranes to remove cysts retained. The wash products from the membranes were pooled together with the primary concentrated eluates in centrifuge tubes (final volume of approximately 25 mL) and stored in the dark at 4 ^{o}C before transferring to the laboratory at the Department of Parasitology, University of Malaya, Kuala Lumpur (Malaysia), for the subsequent procedures.

Before immunomagnetic separation (IMS), the samples were centrifuged at 1500 g for 15 min. The supernatants were then carefully aspirated to 5 mL above the pellets. The samples were re-suspended vigorously to ensure complete homogenisation before transferring to Leighton tubes for IMS. For each sample, 1 mL of 10× SL-buffers A and B and 100 µL of *Cryptosporidium* and *Giardia* IMS beads (Dynabeads® GC-Combo, Invitrogen Dynal AS, Oslo, Norway) were added then mixed with a rotating mixer at approximately 18 rpm for 1 hour at room temperature (~25 °C). The Leighton tubes were then placed in a magnetic particle concentrator and gently rocked at an angle of 90° for 2 min at 1 tilt/sec. The supernatants were decanted before removing the tubes from the magnetic particle concentrator. The samples were gently rocked to re-suspend the bead-cyst complexes with 1 mL of 1× SL-buffer A before transferring to labelled 1.5-mL polypropylene centrifuge tubes. The tubes were then placed in a second magnetic particle concentrator and rocked for 1 min to aspirate the supernatants before removing the magnet.

For the disassociation of the bead-cyst complexes, 50 mL of 0.1 N HCl were added to each sample, which was then vortexed for 50 s then allowed to stand for at least 10 min in an upright position. The samples were vortexed for a further 10 s, replaced in the magnetic particle concentrator and left undisturbed for at least 10 s. At this point, the beads were collected at the back of the tube and the acidified suspensions were transferred to the wells microscope slides, each containing 5 µL of 1.0 N NaOH.

Before staining, the samples were allowed to dry at 37 °C (max. 1 hr) and then fixed with methanol. Then, 50 µL of 4′,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich Co., Ontario, Canada) solution (2 µg/mL in PBS) were added to each well. After 2 min, the excess DAPI was removed and 50 µL of distilled water were added to wash the wells. After 1 min, the excess water was removed.

Fluorescein isothiocyanate-conjugated anti-*Cryptosporidium* sp. and anti-*Giardia* sp. monoclonal antibodies (FITC-MAb) (EasyStain™, BTF Pty. Ltd., NSW, Australia) were added (50 µL) and the mixture was incubated at room temperature. After 30 min, the excess FITC-MAb was removed and 300 µL of the fixing buffer from the EasyStain™ kit were added to the wells. The fixing buffer was drained after 2 min and 5 µL of EasyStain™ mounting medium were added before sealing the slides with cover slips for subsequent examination.

The slides were scanned at a magnification of 400× by epifluorescence microscopy (Olympus BX51, Tokyo, Japan). *Cryptosporidium* spp. and *Giardia* spp. cysts were first identified and enumerated by immunofluorescence reaction and then confirmed by DAPI fluorescence on the basis of their sizes, morphological features and the presence of nuclei as described in Method 1623.1 (USEPA, 2012).

3.3.1.3 Protozoan Cyst Recovery Efficiency

To establish the ability to demonstrate control over the analytical system as described in the preceding section and to generate acceptable precision recovery, protozoan cyst recovery efficiency tests were conducted. EasySeed™ (BTF Pty. Ltd., NSW, Australia), which contains 100 inactivated *Cryptosporidium* oocysts and 100 inactivated *Giardia* cysts, was spiked into 10 L of deionised water. The samples were then processed in accordance to the procedures described above for the detection and enumeration of *Cryptosporidium* and *Giardia* cysts.

The cyst recovery efficiency test was replicated 6 times. The mean recovery for *Cryptosporidium* oocysts and *Giardia* cysts was 39% and 45%, respectively. The acceptance criteria for the mean recovery of *Cryptosporidium* and *Giardia* as specified by the USEPA are 38% and 27% (minimum), respectively (USEPA, 2012). The mean recovery of cysts is comparable to those from other recent studies: 41 – 55% for *Cryptosporidium* and 31 – 41% for *Giardia* (Budu-Amoaka et al., 2012; Castro-Hermida et al., 2015; Sato et al., 2013; Xiao et al., 2012). The precision (as relative standard deviation) of the recovery was 36% and 37% for *Cryptosporidium* oocysts and *Giardia* cysts, respectively. The acceptance criteria for the precision of *Cryptosporidium* and *Giardia* recovery system as specified by the USEPA are 37% and 39% (maximum), respectively (USEPA, 2012). With a recovery efficiency of less than 100%, it is important to consider that the cysts concentrations in water samples reported in this study are likely underestimated.

3.3.2 Faecal Samples

3.3.2.1 Sample Collection

A total of 126 faecal samples were collected from Brahman beef cattle (n = 64) and Holstein-Friesian dairy cattle (n = 62) between May and July 2014. All samples were collected from Chiang Mai Province. Dairy cattle samples were collected directly from four farms in the San Sai District while samples from beef cattle were collected from free-ranging herds in the grazing fields along the Mae Taeng-San Sai canal. Approximately 10 g of faeces were collected using disposable plastic spoons and stored in sterile plastic containers. Although collection of faeces directly from the rectum of individual animals would have minimised the potential for cross-contamination, the lack of a trained animal handler prevented us from doing so. However, to minimise the contamination of samples and to ensure that repeat samples did not occur, only fresh and wet samples were collected – often immediately following defecation. Collected samples were preserved in 2.5% potassium dichromate solution before being transported to the Department of Parasitology, University of Malaya, Kuala Lumpur, Malaysia where they were stored at 4 °C until required for subsequent analyses.

3.3.2.2 Identification and Molecular Analyses

All samples were first concentrated using solvent-free faecal parasite concentrators (Mini Parasep® SF, Apacor, Berkshire, United Kingdom) as per the manufacturer instructions. Faecal samples were screened for the presence of *Giardia* and other protozoa (except *Cryptosporidium*) and helminths by smearing the concentrated products onto microscope slides. The samples were stained with Lugol's iodine before viewing under a light microscope at 100× and 400× magnification. Samples were not screened for *Cryptosporidium* by light microscopy because even with staining, the threshold of detection is typically low (Weber et al., 1991).

Molecular techniques were carried out to determine the assemblage of *G. intestinalis* detected in the positive samples via microscopy as well as to determine the presence and species of *Cryptosporidium* in all the faecal samples. Genomic DNA was extracted from all samples using the NucleoSpin® Soil Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany), according to the manufacturer's protocol.

For molecular typing of *G. intestinalis*, a two-step nested PCR and partial sequencing of the triosephosphate isomerase (TPI) gene were performed based on the work of Sulaiman et al. (2003). In the primary reaction, a 605 base-pair (bp) fragment was amplified with the forward primer AL3543 5′-AAATIATGCCTGCTCGTCG-3′ and reverse primer AL3546 5′-CAAACCTTITCCGCAAACC-3′. The PCR reaction consisted of 1.0 µL of DNA, 12.5 µL (2x) of ExPrime Taq Premix (containing ExPrime TaqTM DNA Polymerase 1 unit/10 µL, 20 mM Tris-HCl, 80 mM KCl, 4 mM $MgCl_2$, and 0.5 mM of each dNTP) (GeNet Bio Inc., Daejeon, S. Korea) and 0.25 µM of both the forward and reverse primers. The PCR was performed with an initial denaturation step of 94°C for 5 min followed by 35 cycles of 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 60 s; and a final extension cycle of 72°C for 10 min. For the nested PCR reaction, a PCR product of 530 bp was amplified by using the forward primer AL3544 5′-CCCTTCATCGGIGGTAACTT-3′ and the reverse primer AL3545 5′-GTGGCCACCACICCCGTGCC-3′. The nested PCR mixture consisted of 1.0 µL of the first PCR product, 25.0 µL (2x) of ExPrime Taq Premix, 0.2 µM of both forward and reverse primers. The conditions for the secondary PCR were identical to the primary PCR.

Cryptosporidium species and genotyping were also determined by a two-step nested PCR protocol and sequencing of the partial 18S rDNA gene based on the work by Ryan et al. (2003). For the primary PCR, a PCR product of 763 bp was amplified using the forward primer 18SiCF2 5′-GAC ATA TCA TTC AAG TTT CTG ACC-3′ and reverse primer 18SiCR2 5′-CTG AAG GAG TAA GGA ACA ACC-3′. The PCR mixture consisted of 2.5 µL of the purified DNA, 15.0 µL (2x) of ExPrime Taq Premix, and 10 µM of the forward and reverse primers. The PCR was performed with an initial denaturation of 94°C for 5 min followed by 45 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s and a final extension of 72 °C for 10 min. For the secondary PCR, a fragment of ~587 bp was amplified using the forward primer 18SiCF1 5'-CCT ATC AGC TTT AGA CGG TAG G-3' and the reverse primer 18SiCR1 5'-TCT AAG AAT TTC ACC TCT GAC TG-3'. The secondary PCR mixture consisted of 2.0 µL of the first PCR product, 25.0 µL (2x) of ExPrime Taq Premix, and 10 µM for both the forward and reverse primers. The conditions for the nested PCR were identical to those for the first PCR. Amplicons were analysed by electrophoreses on 1.5% agarose gel and visualised under UV lamp after staining with GelRed (Biotium, Hayward, CA, USA). Amplicons of the expected size were excised from the gel and purified using a QIAquick gel extraction kit (Qiagen, Germantown, MD, USA). Purified PCR products were sent to Axil Scientific for sequencing in forward and reverse directions.

Sequences were initially examined using BLAST (Altschul et al., 1990). Reference *Cryptosporidium* and *Giardia* sequences were downloaded and aligned using MAFFT in Geneious 7.1.6 (Kearse et al., 2012) and then manually curated. FastTree was run to create an initial phylogeny after which redundant sequences were removed (Price et al., 2010). Ambiguous regions were removed for the final *Cryptosporidium* alignment. Model testing and Maximum Likelihood tree inference was performed with IQTREE v 1.3.8 (Nguyen et al., 2015). Based on the best Bayesian Information Criteria (BIC) score, a TN+G4 nucleotide substitution model was selected for *Giardia* sequences and a K3Pu+I+G4 model was selected for *Cryptosporidium* sequences. Branch support values were provided through 10,000 bootstrap repetitions (Minh et al., 2013). The *Cryptosporidium* tree was midpoint rooted, while the *Giardia* tree was rooted to a *Giardia muris* sequence. Outgroups were removed in the final tree.

3.4 Results

3.4.1 Water Samples

All four rivers at the study area were contaminated with varying levels of *Cryptosporidium* and/or *Giardia* cysts (Table 3.1 and Figure 3.2). More than half of the 52 river sampling sites (27/52) tested positive for *Cryptosporidium* and/or *Giardia*. *Giardia* was detected in half (26/52) of the river sampling sites while 25% (13/52) of these sites contained *Cryptosporidium* (Figure 3.3a). *Cryptosporidium*-*Giardia* co-contamination occurred in nearly a quarter (12/52) of the monitored river sampling sites. The highest concentration of *Cryptosporidium* (6.50 oocysts/10 L) was detected in P2 at the Pong River during the dry season while the highest concentration of *Giardia* (13.85 cysts/10 L) was detected during the wet season in L7 at the Lai River. Neither *Cryptosporidium* nor *Giardia* was detected in the water samples collected from any of the eight sampling sites of the Mae Taeng-San Sai Canal in either the dry or wet season.

During the dry season, *Cryptosporidium* or *Giardia* were detected in 21% (11/52) of the river sampling sites while the samples containing either protozoa nearly doubled (40%; 21/52) during the wet season (Table 3.1; Figure 3.2). *Giardia* cysts were detected more frequently than *Cryptosporidium* oocysts for both dry and wet seasons (Figure 3.3b). For the dry season samples, 13% contained *Cryptosporidium* (0.25 – 6.50 oocysts/10 L). In comparison, 18% of the tested samples contained *Giardia* (0.25 – 2.94 cysts/10 L). Meanwhile, for the wet season samples, 15% tested positive for *Cryptosporidium* (0.37 – 4.00 oocysts/10 L) and 38% tested positive for *Giardia* (0.28 – 13.89 cysts/10 L).

Table 3.1: Concentration of *Cryptosporidium* and *Giardia* cysts at all river sampling sites for both the dry and wet seasons

River	Sampling site	Dry season		Rain season	
		***Cryptosporidium* (oocyst/10 L)**	***Giardia* (cyst/10 L)**	***Cryptosporidium* (oocyst/10 L)**	***Giardia* (cyst/10 L)**
Kuang River	uK1	–	–	–	–
	uK2	–	–	–	–
	uK3	–	–	–	**0.50**
	uK4	–	–	–	**0.28**
	K1	–	–	–	–
	K2	–	–	–	–
	K3	–	–	–	–
	K4	–	–	–	–
	K5	–	–	–	**4.17**
	K6	–	–	–	–
	K7	–	–	–	–
	K8	–	–	**0.40**	**0.40**
	K9	–	–	–	–
	K10	–	–	–	–
	K11	–	**0.50**	**0.37**	**0.74**
	K12	–	–	–	–
	K13	–	–	–	–
	K14	–	–	–	–
	K15	**0.25**	**0.25**	–	–
	K16	–	–	–	**0.48**
	K17	**0.50**	**0.25**	–	–
	K18	–	–	–	–
Lai River	tL1	–	–	–	–
	tL2	–	–	**4.00**	**1.50**
	tL3	–	–	–	–
	L1	–	–	**1.41**	**2.12**
	L2	–	–	–	**3.23**
	L3	–	–	**1.82**	**4.85**
	L4	**0.25**	–	**0.65**	**10.32**
	L5	–	–	–	**4.28**
	L6	–	–	–	**5.71**
	L7	–	–	–	**13.89**
Pong River	uP1	–	–	–	–
	uP2	–	–	–	–
	uP3	–	–	–	**0.50**
	tP1	**0.74**	**2.94**	–	–
	tP2	–	**0.80**	–	–
	tP3	–	**1.19**	–	–
	P1	–	–	–	**0.50**
	P2	**6.50**	**1.00**	–	**1.00**
	P3	**0.50**	–	–	–
	P4	–	**0.50**	**0.75**	**1.25**
	P5	–	–	–	–
	P6	–	–	–	**1.99**
	P7	–	–	–	–
	P8	**0.50**	**2.50**	**0.57**	–
	P9	–	–	–	–
San River	S1	–	–	–	**2.24**
	S2	–	–	–	–
	S3	–	–	–	–
	S4	–	–	–	–
	S5	–	–	–	–

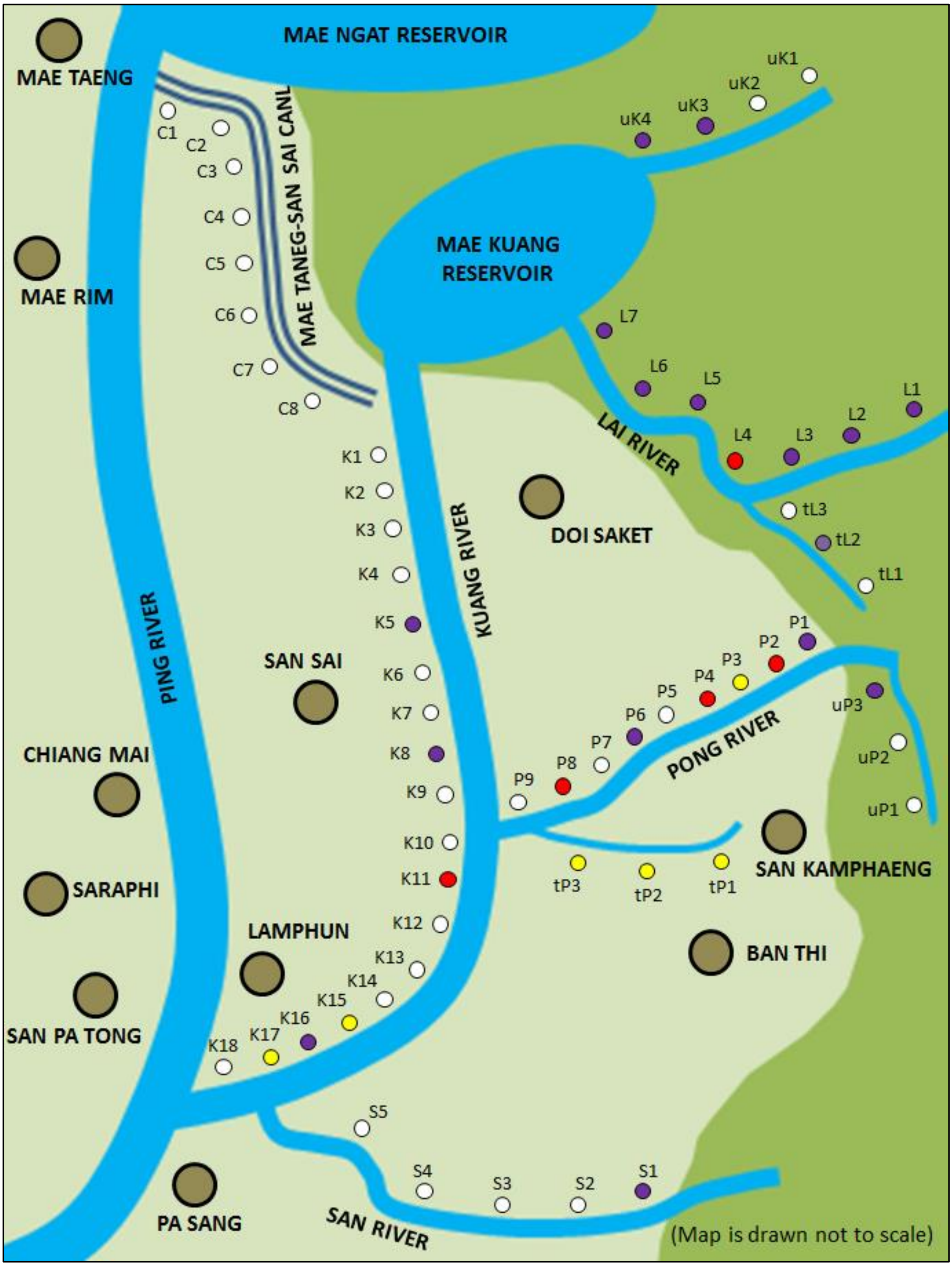

Figure 3.2: Locations of sampling sites denoting absence/presence of *Cryptosporidium*/*Giardia* during dry and wet seasons (RED: Dry and wet season; YELLOW: Dry season only; VIOLET: Wet season only; WHITE: non-detection)

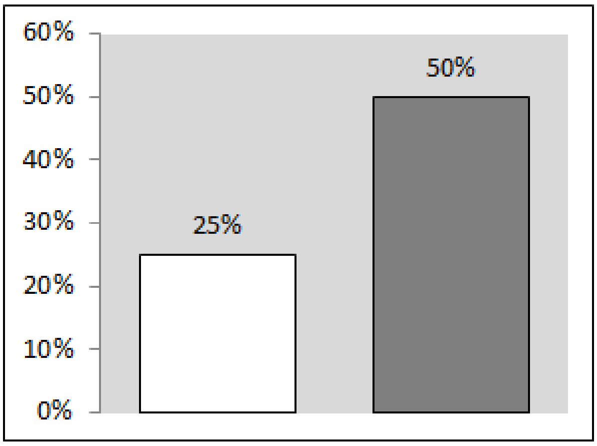

Figure 3.3a: Prevalence of *Cryptosporidium* and *Giardia* in the sampling sites of the rivers at the study area

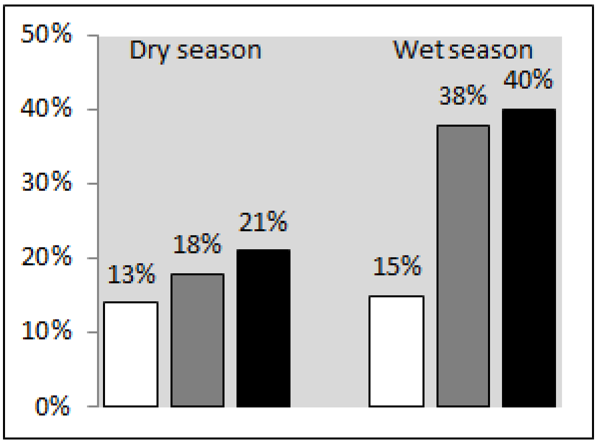

Figure 3.3b: Seasonal variation of *Cryptosporidium* and *Giardia* in the rivers of the study area

3.4.2 Faecal Samples

Both dairy and beef cattle had high parasitic infection rates with 97% of the faecal samples from dairy cattle testing positive for at least one intestinal parasite compared to the 94% in faecal samples from beef cattle. *Entamoeba* (53%) was the most prevalent of the gastrointestinal parasites detected in beef cattle followed by *Eimeria* (42%), *Paramphistomum* (33%), strongyle (25%), *Buxtonella sulcata* (23%), *Giardia* (13%), *Fasciola* (8%), *Cryptosporidium* (3%) and *Dicrocoelium* (2%) (Figure 3.4). Some of the parasites found in beef cattle were also detected in dairy cattle: *Entamoeba* (98%), *Eimeria* (18%), *Paramphistomum* (13%), *Buxtonella sulcata* (5%) and strongyle (3%) (Figure 3.4). *Dicrocoelium*, *Fasciola*, *Giardia* and *Cryptosporidium* were not detected in any dairy cattle samples. Co-infections were observed in 56% (n = 36) of the meat cattle samples and 38% (n = 24) of dairy cattle. Beef cattle showed noticeably higher infection for all parasites except *Entamoeba* where infection in dairy cattle was almost double that in beef cattle.

Giardia cysts were detected in eight of the 64 faecal samples from beef cattle (~13%) through microscopy. PCR was conducted on the *Giardia*-positive samples, but only three were successfully amplified. Three PCR-positive Giardia samples were sequenced and phylogenetic analysis revealed two were from the non-zoonotic assemblage E that only infects hoofed livestock, while the third was identified as assemblage B, which is known to infect humans (Figure 3.5a). Only two (~3%) of the samples from beef cattle tested positive for *Cryptosporidium*. Sequence analysis determined both were *Cryptosporidium rynae, a non*-zoonotic species that infects cattle (Figure 3.5b).

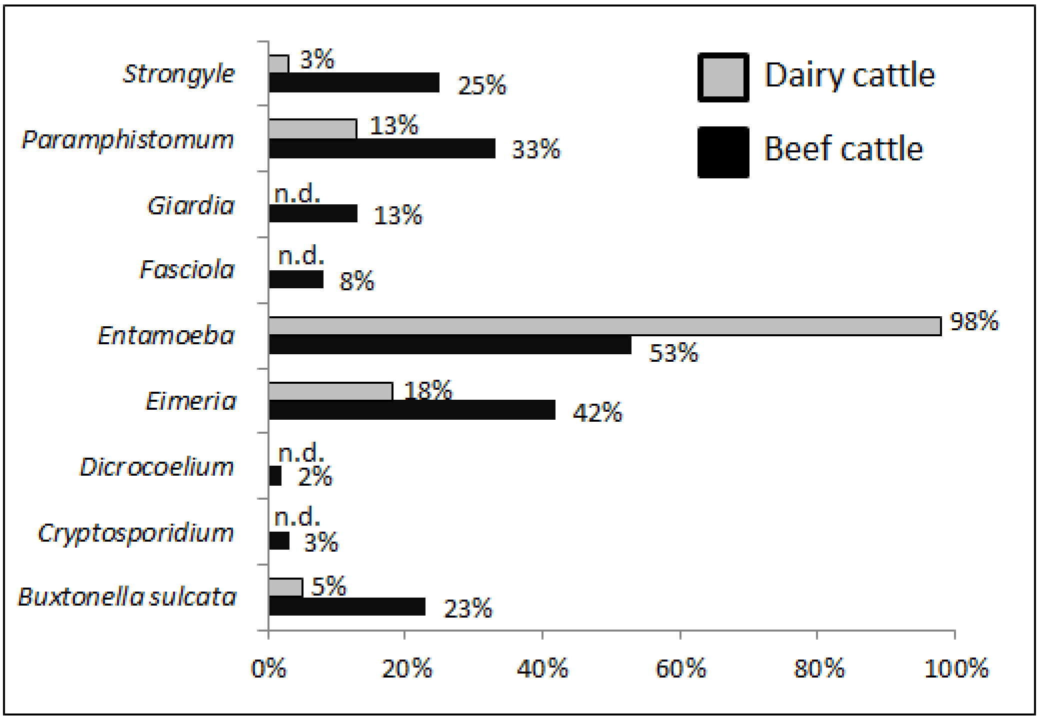

Figure 3.4: Prevalence of *Cryptosporidium*, *Giardia* and other parasites in the faecal samples of beef and dairy cattle

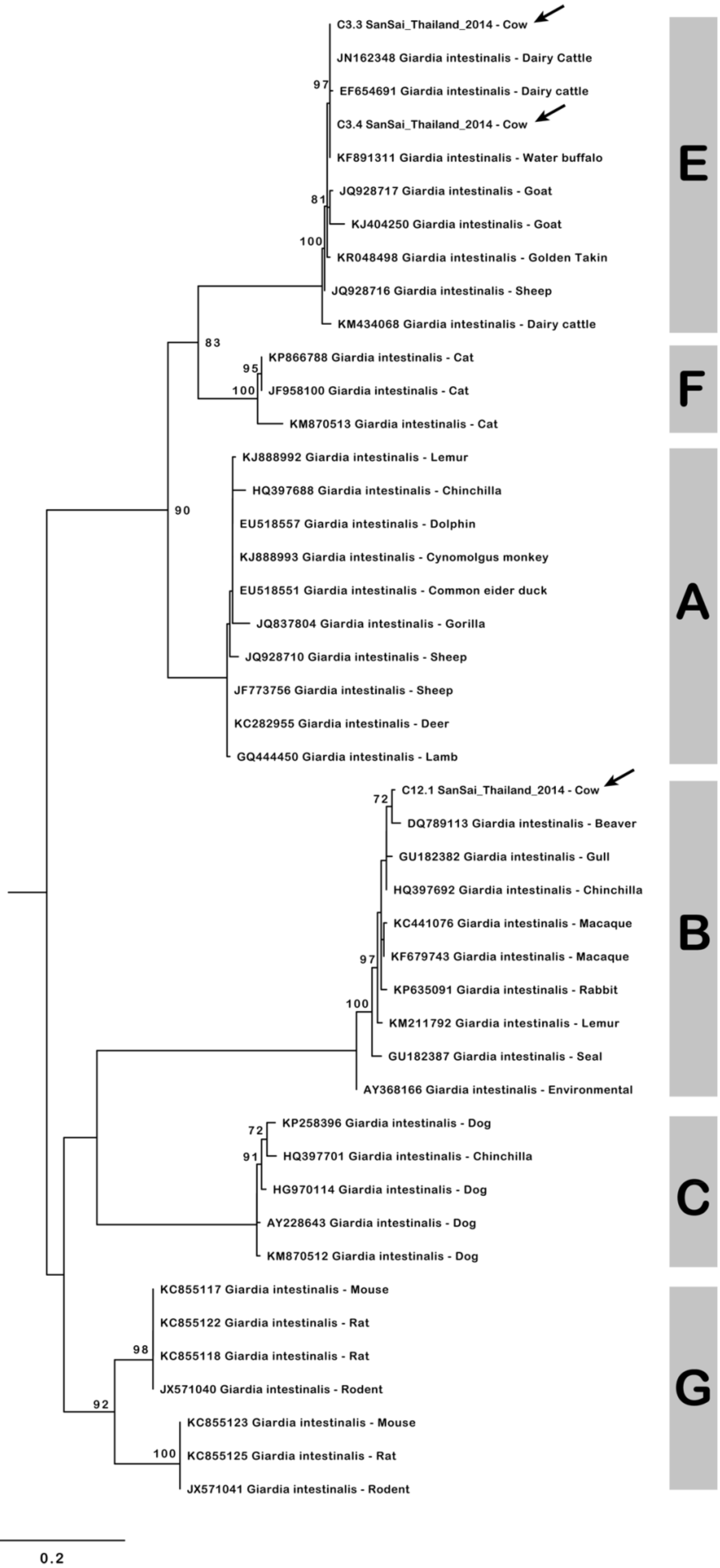

Figure 3.5a: Phylogenetic analysis of the partial sequences of the *Giardia* triosephosphate isomerase gene

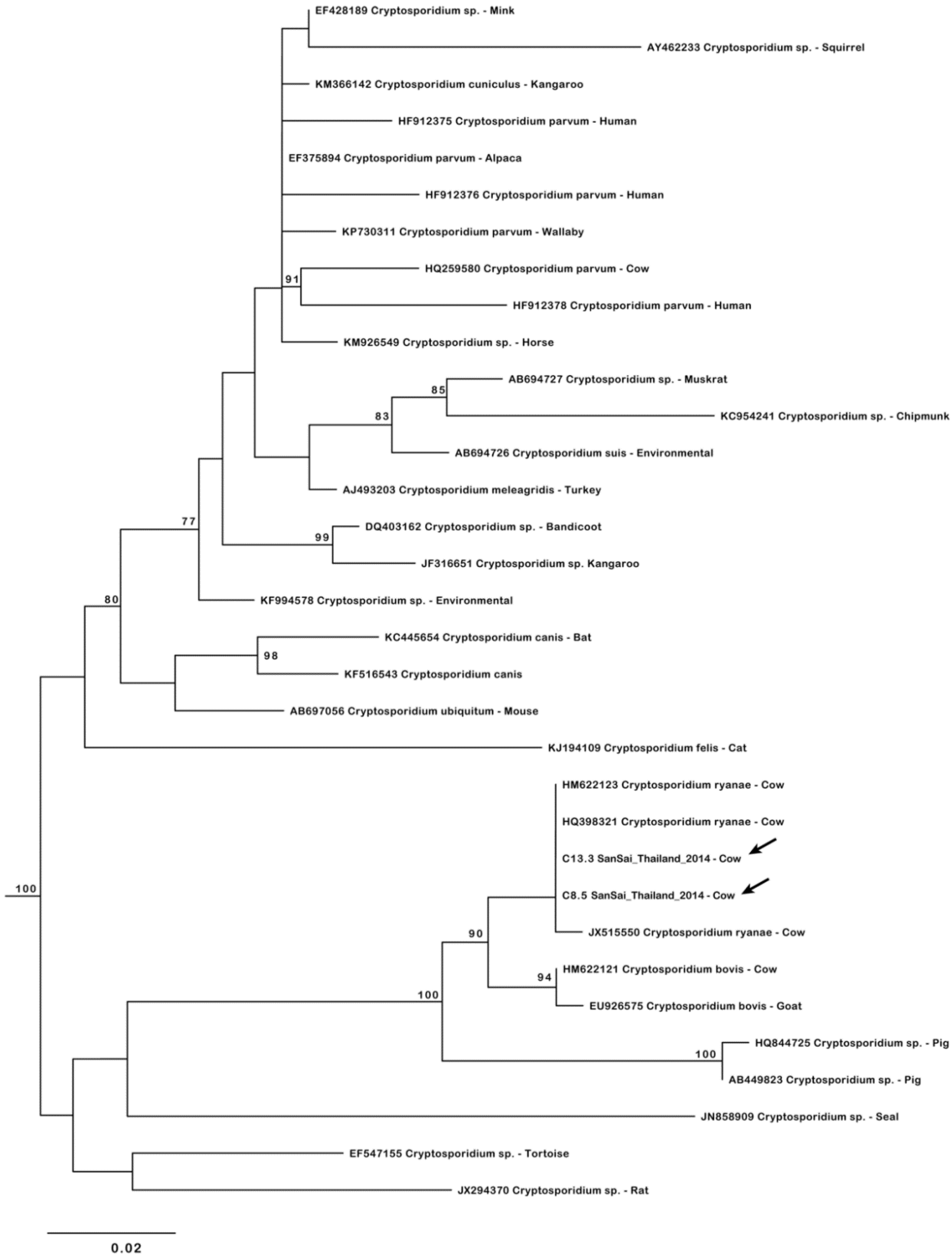

Figure 3.5b: Phylogenetic analysis of the partial sequences of the *Cryptosporidium* 18S ribosomal RNA gene

3.5 Discussion

3.5.1 Environmental Prevalence and Population Vulnerability

Both *Cryptosporidium* and *Giardia* were detected in varying levels in all the rivers at the study area, reflecting their ubiquity, and correspondingly, the associated risk of cryptosporidiosis and giardiasis within the predominantly rural landscape. The findings reflect the water quality in this headwater region draining to the Ping River, and ultimately the Chao Phraya River. These streams and rivers are not only vital drinking-water resources for the local regional communities but also for those downstream in the more densely populated areas, including Bangkok, which rely on the northern region for much of its municipal water. Prior studies have largely focused on the highly polluted surface waters in Bangkok and its vicinities in Central Thailand (Anceno et al., 2007; Diallo et al., 2008; Koompapong and Sukthana, 2012). Anceno et al. (2007) and Diallo et al. (2008), for example, surveyed many canals, some of which function as open conveyance systems for wastewater. Not surprisingly, they frequently detected *Cryptosporidium* and *Giardia* cysts in high concentrations. In another Central Thai study, Koompapong and Sukthana (2012) reported the presence of the zoonotic *C. parvum,* as well as the non-zoonotic *C. meleagridis* and *C. serpentis*, in the surface waters at the mouth of the Chao Phraya.

We sampled the river/stream/canal network systematically at the catchment scale to assess the spatial variability of *Cryptosporidium* and *Giardia* contamination. All the previous studies in Thailand (i.e. Anceno et al., 2007; Diallo et al., 2008; Koompapong and Sukthana, 2012; Kumar et al., 2014; Srisuphanunt et al., 2010) have not been able to assess this aspect. These studies only reported the presence or absence of *Cryptosporidium* and *Giardia* in various water resources without providing the origins of the water samples and other informative details that may be useful for management. Studies conducted in southern Thailand, for example those by Srisuphanunt et al. (2010) and Kumar et al. (2014), tested for *Cryptosporidium* and *Giardia* in numerous samples ranging from raw to processed water. Detailed information regarding the source (e.g. river, aquifer etc.) and the type of treatment (e.g. filtration, disinfection etc.) for processed water like tap and bottled water was however not reported, preventing the tracking of contamination sources and the understanding of processes driving the microbial dynamics (Kay et al., 2007). Elsewhere, the European Union Water Framework Directive and its USA counterpart, the Clean Water Act, require catchment-scale investigations for microbial contamination studies such that programmes of measures can be designed by water resource managers and policy makers to preserve, protect and improve the quality of drinking water resources (Council of the European Communities, 2000; Davison et al., 2005; Horn et al., 2004).

This catchment-scale study provided new and invaluable insight to the spatial prevalence of *Cryptosporidium* and *Giardia* in the surface water bodies of the study area. These findings make it possible to identify and prioritise the next steps for research in order to track the source of contamination and understand the processes that underpin microbial transport. In particular, the detection of *Cryptosporidium* and *Giardia* cysts in the upper reaches of the Kuang River (sites uK3 and uK4), and especially their high prevalence and intensity in the Lai River (sites L1 to L7, tL2) is of concern because these rivers are the two primary inflows to the Mae Kuang Reservoir, the principal source of drinking water to several districts in the provinces of Chiang Mai and Lamphun. In the highly publicised 1993 Milwaukee (USA) cryptosporidiosis outbreak, where over 400,000 people were infected, the concentration of *Cryptosporidium* oocysts in the public water supply ranged between 0.03 and 1.32 per 10 L water (MacKenzie et al., 1994). In the Kuang River Basin, significantly higher levels of *Cryptosporidium* oocysts were recorded in the Lai River. For example, between 0.65 and 4.00 *Cryptosporidium* oocysts per 10 L of water were detected, while the concentrations of *Giardia* cysts were even higher, ranging from 1.50 to 13.89 per 10 L of water.

The drinking water resources in the district centres and major towns/cities of Northern Thailand, including those in the study area, are managed by the Provincial Waterworks Authority (PWA). Water supplies are typically treated using the conventional method of coagulation–sedimentation–filtration–disinfection via chlorination. However, studies have consistently shown that such drinking water treatment procedure are unable to remove these pathogens completely, especially when the raw (incoming) water contains high concentrations of *Cryptosporidium* and *Giardia* cysts (Ali et al., 2004; Castro-Hermida et al., 2008; Castro-Hermida et al., 2014; Hashimoto et al., 2002). With cysts having a generally high-resistance to chlorination, the threat of cryptosporidiosis and giardiasis from drinking-water treated through such means is far from eliminated.

Village water resources, in comparison, are usually managed by the local communities themselves. The financial capacities of these rural populations to operate and maintain their water resource management systems are usually limited. As such, water treatment, whenever available, are generally limited to sand filtration only. Given the limited treatment of drinking-water in these rural communities, the risk of cryptosporidiosis and giardiasis is therefore amplified over the urban communities with better access to treatments facilities.

3.5.2 Seasonal Effects and Transport Processes

The frequency of detection of *Cryptosporidium/Giardia* nearly doubled during the wet season compared to the dry season. Thus, the hydroclimatological factors play a crucial role in the prevalence of *Cryptosporidium* and *Giardia* in surface waters in the study area. Unfortunately, direct comparisons cannot be made with most other temporal-based studies, as they were largely conducted in temperate regions where the seasons (e.g. spring, summer, autumn and winter) do not affect water resources in the same manner as in the tropics (Caccio et al., 2003; Castro-Hermida et al., 2008; Castro-Hermida et al., 2009; Hansen and Ongerth, 1991; Naumova et al., 2005; Wilkes et al., 2009). Nevertheless, most of these studies inferred that *Cryptosporidium* and *Giardia* are generally more prevalent during the wetter periods of the year. Xiao et al., 2013 compared the occurrence of *Cryptosporidium* and *Giardia* between the flood period (rainy season) and the impounding period (dry season) in the Three Gorges Reservoir, China. Their results revealed the reservoir was more prone to *Cryptosporidium* contamination during the flood period. However, the opposite was observed for *Giardia*. Epidemiological studies conducted in the tropics also reported positive correlations between infections and the rainy season. For example, Siwila et al. (2011) reported that both cryptosporidiosis and giardiasis infections in 100 pre-school children aged 3 to 6 years in Kafue, Zambia were significantly more prevalent in the rainy season than the dry season. Wongstitwilairoong et al., (2007) also reported a similar result in their survey of intestinal parasitic infections among 472 pre-school children in Sangkhlaburi, western Thailand.

Several reasons may contribute to the significant increase of *Cryptosporidium* and *Giardia* in the surface waters during the wet season. Firstly, domestic wastewater is an important source of faecal and microbial contaminants in surface water. In Thailand, only a little over 20% of the domestic wastewater generated is directed to central wastewater treatment facilities for treatment; the rest is managed using on-site sanitation systems (Simachaya, 2009; World Bank, 2008). In rural and peri-urban areas of Thailand, open-bottomed, non-watertight cess pits are still commonly used for domestic waste. During the wet season, local water tables may rise and mix with the waste in these pits. Sequestered cysts in these cess pits may then be transported laterally through the soil matrix via groundwater flow, eventually reaching surface waters (Corapcioglu and Haridas, 1984; Abu-Ashour et al., 1993; Torkzaban et al., 2007). Many studies have identified wastewater as an important source of *Cryptosporidium* and *Giardia,* whereby high concentrations of cysts can be detected even in the treated effluents of wastewater treatment plants (Castro-Hermida et al., 2011; Cheng et al., 2009; Kitajima et al., 2014). The untreated, raw wastewater from the prevalent on-site sanitation systems in Thailand therefore likely contribute to the presence of *Cryptosporidium* and *Giardia* in surface waters, especially during wet periods (Figure 3.6).

In addition, during storm events, surface runoff facilitates the transport of animal manure and *Cryptosporidium* and *Giardia* cysts (if the host is infected) from land to surface waters (Ferguson et al., 2003; Pachepsky et al., 2006; Tyrrel and Quinton, 2003). When the hydrologic connectivity between sources and receiving bodies is high, cysts may enter the stream network efficiently (Figure 3.6). For example, the presence of rills, gullies, storm drains and canals will accelerate the transport processes while increased surface roughness associated with vegetated land plots yields an opposite effect (Bracken and Croke, 2007; Darboux et al., 2001; Jencso et al., 2009; Penuela et al., 2015). In grazing pastures, we observed the compaction of soil along animal paths (trails) due to the trampling of cattle herds. The compacted soil of these cattle trails can increase hydrologic connectivity and surface runoff (Batey, 2009; Trimble and Mendel, 1995), which in turn, can lead to an increase of *Giardia* and *Cryptosporidium* contamination in surface waters. Furthermore, soil compaction can also result in the reduction of storm runoff infiltration (Batey, 2009; Trimble and Mendel, 1995). Percolating storm water facilitates the vertical (downward) transport of *Giardia* and *Cryptosporidium* whereby the cysts can be strained by the underlying soil matrix. The decrease of the infiltration capacity therefore reduces the efficiency of this natural filtration system provided by the soil layers that is important for the protection for water resources against contamination.

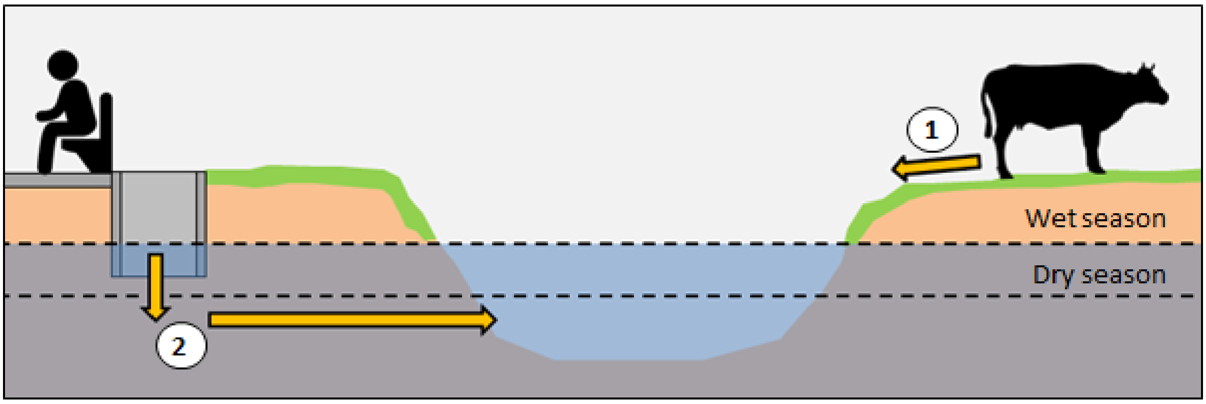

Figure 3.6: Cross sectional profile of a stream and the various sources of *Cryptosporidium* and *Giardia* as well as the processes involved the transport of cysts to surface water. [1] During the dry season, manure from cattle and cysts enter surface waters by direct deposition while manure deposited on land can be washed into stream by surface runoff during the wet season. [2] During the wet season, groundwater may rise, and thereby increasing the proximity of the water table to cess pits. Sometimes, in low-lying areas, groundwater may even flood these on-site sanitation systems. Wastewater, potentially laden with cysts, will contaminate groundwater and transported horizontally via soil matrix to surface waters.

Streambeds are also potentially important repositories of *Cryptosporidium* and *Giardia* (Jamieson et al., 2005; McDonald et al., 1982; Nagels et al., 2002). In the dry season, cysts may be deposited into surface waters by direct defecation or transported from source areas either by surface or sub-surface flow. Under stream baseflow conditions (i.e. low velocity, low discharge rate), cysts in the water column settle to the streambed as both *Cryptosporidium* (specific gravity: 1.009 – 1.080;

settling velocity: 0.35 – 1.31 µm/s) and *Giardia* (specific gravity: 1.013 – 1.117; settling velocity: 0.84 – 1.40 µm/s) have natural propensities to sink in water (Dumetre et al., 2011). Settling rate is further enhanced when cysts in the water column are associated with (adhered to) suspended particles, altering their physical properties. Dai et al. (2004) revealed that hydrophobicity and surface charges of the cysts are important characteristics that are responsible for their adhesion to solid surfaces. Settling column experiments conducted on *Cryptosporidium* oocysts by Searcy et al. (2005) demonstrated that oocysts were removed from suspension at a much higher rate when associated with sediments, whereby the settling rate depended primarily on the type of sediment present in the water.

The rivers in the Kuang River Basin are highly managed. For example, many weirs have been built to impound water during the dry season when water levels are low. Sediments are known to accumulate behind dams and weirs (Lai and Shen, 1996). Thus, in areas where *Cryptosporidium* and *Giardia* are prevalent, cysts may be stored along with river sediments behind (or upstream of) these retention structures. During storm events, turbulent waters may re-suspend and entrain the cysts (Figure 3.7). Jamieson et al. (2005) demonstrated this phenomenon using tracer bacteria in a small alluvial stream, where the increase of the tracer bacteria concentration occurred during the rising limb of storm hydrographs. Nagels et al. (2002) also investigated this process by creating an artificial flood in a stream by releasing water from a supply reservoir during dry weather conditions (i.e. no wash-in from the upper catchment was allowed). Increases and decreases of the faecal indicator organism concentration corresponded with the rising and falling limbs of the hydrograph.

Nagels et al. (2002) suggested that cysts accumulated in the streambeds may be of similar or greater importance than the wash-in from land. This may explain the non-detection of both protozoa in the canal even though *Cryptosporidium* and *Giardia* cysts were detected in faecal samples of beef cattle grazing along it. The flow in the canal is mechanically-controlled at the source (Ping River) which maintains a constant flow rate in the dry season. The continuous flushing of water minimises the settling potential of the cysts into the channel bed. In addition, the low surface roughness of the concrete-lined canal in comparison to those of meandering rivers and streams increases the volumetric discharge rate and velocity of water which in turn also decreases the settling rate of the cysts (Rouse, 1965). However, it may also be that these protozoa were present in the waters of the canal but in much lower concentrations than those in the rivers monitored in this study. Given that the recovery efficiencies were only 39% and 45% for *Cryptosporidium* and *Giardia* respectively, low concentrations of cysts may escape detection altogether thereby yielding the negative result.

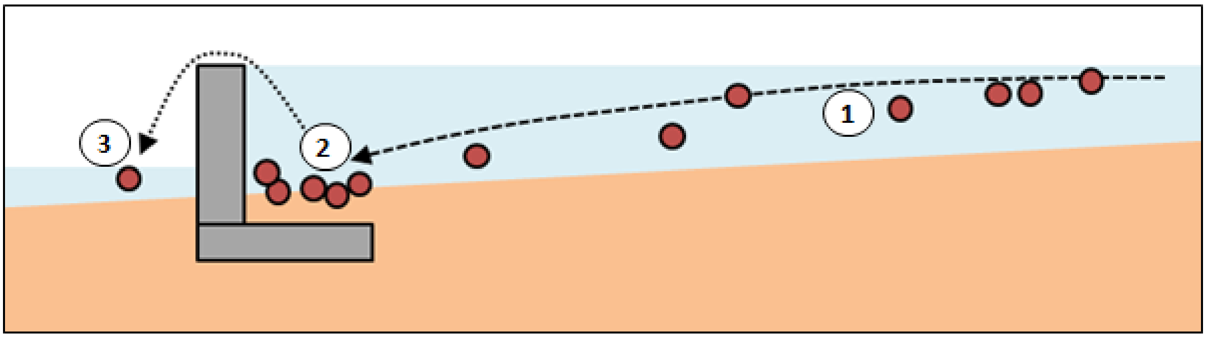

Figure 3.7: Longitudinal profile of a fragmented stream and processes influencing the transport of cyst in surface waters. [1] *Cryptosporidium* and *Giardia* cysts settles from water column to streambed. Settling rate is enhanced when cysts are associated to sediments. [2] A store of cysts may exist behind weirs. [3] During storm events, stream velocity and volumetric discharge rate will increase which causes the re-suspension of cysts from streambed to water column.

3.5.3 Livestock Management

The dairy cattle surveyed in this study were not infected with either *Cryptosporidium* or *Giardia*. However, a high prevalence of infection by other parasites, especially *Entamoeba* spp., reflects the potential risk of parasitic transmission and infection–particularly for the parasites that occur via the faecal-oral route (e.g. *Cryptosporidium* and *Giardia*). In contrast, cysts of both parasites were detected in the faecal samples from the beef cattle, for which, at least one of the samples tested positive for parasites with zoonotic potential (*G. intestinalis* assemblage B). Only one other study on the prevalence of *Cryptosporidium* and *Giardia* infection in beef cattle in Thailand was available for comparison. Kaewthamasorn and Wongsamee (2006) screened 207 faecal samples of beef cattle in Nan Province, Northern Thailand but did not detect either parasite in their samples. While we did not detect either *Cryptosporidium* or *Giardia* in faecal samples of dairy cattle at our study site, other Thai studies have (Inpankaew, et al., 2010; Jittapalapong et al., 2006; Jittapalapong et al., 2011; Nuchjangreed et al., 2008). In Northern Thailand, the seroprevalence of *Cryptosporidium parvum* infection of 642 dairy cows were 3.3%, 5.1% and 3.0% in the provinces of Chiang Mai, Chiang Rai and Lampang, respectively (Inpankaew, et al., 2009).

Several factors associated with cattle farm management may have contributed to the disparity of the *Cryptosporidium*/*Giardia* infection between dairy and beef cattle. The beef cattle from this study area are typically left to graze in pastures where drinking water is available. Transmission and infection may occur through direct ingestion of faecal matter containing *Cryptosporidium*/*Giardia* in the communal fields in which cattle from different herds graze and defecate. Alternatively, beef cattle may also be exposed to these parasites from surface waters (e.g. rivers, streams, canals, ditches etc.) contaminated with *Cryptosporidium* or *Giardia*.

In comparison, dairy cattle are typically housed in shelters and in some instances are separated by stalls. In contrast with free-ranging beef cattle, cattle feed such as cogon grass (*Imperata cylindrica*), stalks of rice and corn are brought to the shelters and placed in troughs or raised surfaces that minimise faecal contamination. The shelters of dairy cattle are cleaned daily, typically up to twice a day before milking. The cow manure is often collected and dried in separate areas to be sold as fertiliser. Furthermore, dairy cattle are usually provided water piped from the local village waterworks system. These water supplies are derived from protected sources such as deep aquifers or have undergone some form of treatment (typically sand-filtration) and therefore less prone to *Cryptosporidium* or *Giardia* contamination. Like the cattle-feed, drinking water for the dairy cattle is placed in a common trough or in individual concrete containers that decreases the chances of faecal contamination.

The management practices for both the beef and dairy cattle may result in the *Cryptosporidium* and *Giardia* contamination of water resources in the study area. For the beef cattle, the contamination process is straightforward. Free-ranging cattle are typically found near water bodies where parasites may be directly deposited into water bodies during defecation. Manure deposited on land may also be washed into surface waters during storm events. Even though most of the manure from dairy cattle was collected for the production of fertiliser, the residual faecal matter left behind after collection is a possible source of *Cryptosporidium* and *Giardia*. During the cleaning of the dairy cattle sheds, faecal residues are washed into ditches which may be ultimately drained into nearby surface waters thereby resulting in *Cryptosporidium* and *Giardia* contamination.

3.6 Conclusion

This novel study reveals for the first time the prevalence and distribution of *Cryptosporidium* and *Giardia* in the water resources of Northern Thailand. Both intestinal parasites were detected in varying levels in all the monitored rivers of the study area. With regards to public health, the detection of these protozoa in high concentrations in rivers upstream of the drinking-water reservoir is of great importance. Immediate precautionary measures must be taken to minimise future contamination of these raw water supplies while treated drinking-water must be thoroughly tested to ensure public safety. Additionally, the frequency of *Cryptosporidium* and *Giardia* detection was found to be higher during the wet season highlighting the importance of water as an agent of transport for *Cryptosporidium* and *Giardia* from sources to water bodies.

We also screened faecal samples from potential *Cryptosporidium* and *Giardia* hosts, i.e. beef and dairy cattle, and detected *Giardia intestinalis* assemblage B, known for its ability to infect humans. We also found that different cattle management strategies employed can influence the transmission of intestinal parasites amongst cattle and potentially to humans. Grazing pastures and water bodies from which free-ranging cattle drink from must be carefully managed to prevent *Cryptosporidium* and *Giardia* contamination of water resources.

The ubiquity of these pathogens in water resources of the study area highlights the potential risks of cryptosporidiosis and giardiasis not only to the local population but also the consumers in urban centres that rely on headwaters areas in Northern Thailand to provide drinking water. As such, monitoring plans for *Cryptosporidium* and *Giardia* are essential for developing programs to ensure clean and safe drinking-water supplies. Finally, as demonstrated in our study, these monitoring plans must take into account the spatial and temporal aspects to provide a better understanding of the contamination sources and important factors which may influence the prevalence and distribution of *Cryptosporidium* and *Giardia* in the local water resources.

CHAPTER 4 – FLUORIDE: A NATURALLY-OCCURRING HEALTH HAZARD IN DRINKING-WATER RESOURCES

4.1 Introduction

Most cases of drinking-water resource degradation are in direct association with the contamination of water as a result of anthropogenic activities, for example pesticides and fertilisers from agriculture, tailings from mining operations, effluents from industrial processes, chemical spills, etc. (Gilbert, 2012; Meybeck and Helmer, 1989; Meybeck, 2002; Vitousek et al., 1997; Vorosmarty et al., 2010). While contaminants of anthropogenic origin will likely continue to be a major cause of the impairment of drinking-water resources, naturally-occurring drinking-water hazards, although less commonly reported, do exist – and they play a substantial part in the threat to public health and the livelihoods of millions around the world each year. One such example is the highly-publicised accidental mass poisoning from the drilling of wells into groundwater containing naturally-occurring arsenic in Bangladesh (Acharyya et al., 1999; Ahmad et al., 1997; Dhar et al., 1997). Between 35 million and 77 million people are at-risk to drinking arsenic-contaminated water (Smith et al., 2000). Another important naturally-occurring drinking water hazard is fluoride, which is the main focus of this study.

The element fluorine is the lightest member of the halogen group and is the most electronegative. As such, it is the most reactive of all elements (Brindha and Elango, 2011). Fluorine does not occur in the environment naturally in its elemental state but rather as the negatively charged fluoride ion, F^-, because of its high tendency to react and combine with other elements forming strong electronegative bonds and producing ionic compounds (Ayoob and Gupta, 2006). Fluoride is therefore mostly retained in minerals and rocks in the lithosphere. Fluoride has an ionic radius very similar to that of a hydroxide ion (OH^-) and substitutes readily in hydroxyl positions in late-formed minerals of igneous rocks (Edmunds and Smedley, 2005). It is widely dispersed, making up 0.06 – 0.09% of the composition of earth's crust. Fluoride concentrations in freshwater bodies such as rivers and lakes are generally less than 0.5 mg/L, while fluoride content of seawater is higher at approximately 1.0 mg/L.

In groundwater, however, significantly higher concentrations of fluoride can occur, especially in areas where fluorine is found in great abundance in local subterranean minerals and rocks (Fawell et al., 2006).

In small amounts, fluoride is beneficial for oral health because it reduces the ability of plaque bacteria to produce acid that damages teeth. Fluoride also improves the chemical structure of the enamel by making it more resistant to acid attack that causes tooth decay (Ayoob and Gupta, 2006). For these reasons, fluoride is added to toothpaste; in some countries, to drinking water (Edmunds and Smedley, 2005). However, prolonged exposure to high doses of fluoride is detrimental because of the risk of fluorosis. The most common symptom of dental fluorosis is mottling, and ultimately, destruction of teeth. With exposure to high concentrations for prolonged periods, fluoride may accumulate in bones, leading to crippling skeletal fluorosis. Once developed, the symptoms of fluorosis are irreversible (Ayoob and Gupta, 2006).

Exposure to fluoride occurs mainly through inhalation or ingestion (Fawell et al., 2006). In areas where solid fuel burning is prevalent for cooking or heating, the concentration of fluoride in the indoor atmosphere can be elevated due to the combustion of coal with high fluoride content, leading to increased exposure through the respiratory route. In China alone, almost 1.5 million cases of dental fluorosis and an estimated 18 million cases of reported skeletal fluorosis were related to fluoride emissions from the burning of coal (Ando et al., 2001; Hou, 1997; Li and Cao, 1994). Worldwide, however, the inadvertent consumption of the colourless, tasteless and odourless fluoride in drinking water is the single largest contributor to daily fluoride intake (Murray, 1986).

Globally, an estimated 200 million people are exposed to high concentrations of naturally-occurring fluoride that exceeds the World Health Organisation's (WHO) guideline value of 1.50 mg/L for drinking water (Ayoob and Gupta, 2006; Fawell et al., 2006). Fluorosis is endemic in at least 25 countries on almost every continent including Asia, Africa, Europe, North and South America (Fawell et al., 2006). For instance, in the Hetao Plain of Inner Mongolia, China, approximately 6 million people are at risk to fluorosis from drinking high-fluoride water. Nearly 2 million of this total has shown signs of dental fluorosis; nearly a quarter of a million are suffering from skeletal fluorosis (Guo et al., 2012; He et al., 2013). In India, where 90% of the rural population rely on groundwater as drinking water sources, more than 60 million people in more than half of the states in the country are at risk to high levels of fluoride exposure (Gupta et al., 2005; Kundu et al., 2009; Viswanathan et al., 2009).

Incidences of fluorosis have also been documented in other countries, including Thailand. One of the earliest reports in Thailand was a nationwide nutrition survey carried out by the United States Inter-Departmental Committee on Nutrition for National Defence in the 1960's (Leatherwood et al., 1965). Cases of dental fluorosis were found in every region of the country, but it was most prevalent in Northern Thailand (61% of 3,614 people surveyed). Further, the fluoride concentrations in drinking water and urine samples of local people in the northern region were also found to be the highest compared to the other regions (Leatherwood et al., 1965). Despite the prevalence and severity of the problem, subsequent scientific studies and reports pertaining to fluorosis have been rare. In one, Ratanasthien (1991) reported severe cases of fluorotoxicosis involving osteosclerosis (or abnormal calcification on various parts of bones) associated with the drinking of fluoride-contaminated groundwater in Chiang Mai Province of Northern Thailand. Also in Chiang Mai Province, Namkaew and Wiwatanadate (2012) found links between lower back pains – a common symptom of acute fluorosis – and the consumption of high-fluoride groundwater in elderly villagers. In another Chiang Mai-based study, McGrady et al. (2012) estimated a three-fold increase of dental fluorosis prevalence (to at least 37%) for subjects ingesting water with fluoride concentrations of 0.90 mg/L or more. Incidences of fluorosis have also been documented in several other provinces in Northern Thailand. In Chiang Rai Province, Noppakun et al. (2000) attributed the mottling of enamel in primary school children to the consumption of drinking waters contaminated with fluoride-enriched waters from nearby hot springs. In Lampang, the prevalence of dental fluorosis among children at the age of 12 was 10% in 1995 (Vuttipitayamongkol, 2000). In Lamphun, Takeda and Takizawa (2008) reported significantly elevated levels of fluoride (up to 4.9 mg/L) in urine samples of school children living in a village supplied with high-fluoride water, compared to the maximum of 0.94 mg/L fluoride in the urine of children utilising low-fluoride water from another village.

Despite the awareness of the potential risk of fluoride contamination in drinking water for half a century, fluorosis still represents a serious and widespread health problem particularly to some rural communities of Northern Thailand. Oddly, studies that identify the extent of high-fluoride areas, the origin, and the transport of fluoride in water sources – all aspects that are crucial for drinking-water resource management and public health safety – are limited. Further, the lack of scientific reporting and public dissemination of health and safety information threatens the ability to manage drinking water resources safely in at-risk areas. For example, the construction of many drinking water wells in locations with high levels of fluoride may have occurred in the past, and may still be occurring now. Our motivation is to contribute to local rural water management in the region by (1) mapping the extent of two high-fluoride endemic areas; and (2) describing the relevant transport processes of fluoride from source to sink.

4.2 Site Description

The study area is located on the eastern part of the Ping River Basin, which is situated between the Khun Tan Mountain range to the east and the Ping River to the west (Figure 4.1). The site extends from Chiang Mai Province in the north to Lamphun Province in the south. In Chiang Mai, the study was carried out predominantly in the districts of Doi Saket, San Kamphaeng, and Mae On. In Lamphun, the capital district of the province (*Amphoe Mueang* Lamphun), the districts of Ban Thi and Pa Sang were covered.

4.2.1 Climate

Annual rainfall in the area ranges from 800 mm in the lowlands (~350 m a.s.l) to 1,500 mm in the highlands (~1,800 m a.s.l.) with seasonal rainfall between May and October accounting for over 90% of the annual total (Lim et al., 2012; Margane and Tatong, 1999; Wood and Ziegler, 2008).

Temperature is typically lowest, 3.7 to 17.2 °C, between the months of November and February based on meteorological records from 1967 to 2001 (Uppasit, 2004). Historical records from the same period show that highest temperatures of the year usually occur in the months of February to April in the range of 32.1 to 41.4 °C (Uppasit, 2004).

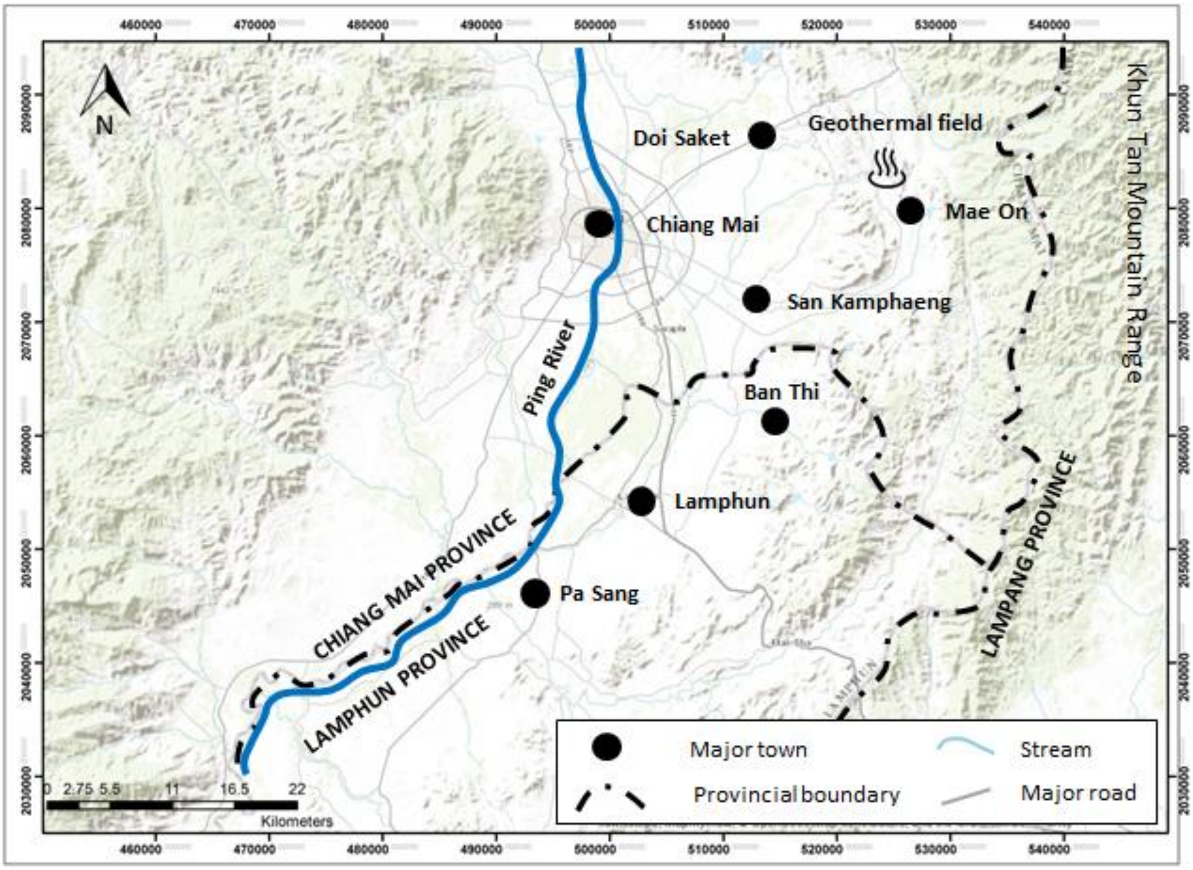

Figure 4.1: Location map of the study site in the Ping River Basin.

4.2.2 Geology

The Ping River Basin is generally regarded as an inter-montane pull-apart basin formed under an extensional tectonic regime between the Late Cretaceous and the Early Tertiary periods following the collision of the Indian with the Eurasian plate (Asnachinda, 1997; Margane and Tatong, 1999). The structural framework of the basin is governed by N-S trending extensional faults that are related to the movement of NW-SE and NNE-SSW trending strike-slip faults which have been active since the Oligocene (Asnachinda, 1997).

Lithologically, the basin can be divided into two parts: (1) the well-indurated rocks from the Paleozoic to the Mesozoic eras; and (2) the poorly-indurated rocks of the Tertiary and Quaternary periods (Wattananikorn et al., 1995; Figure 4.2). Sedimentary Paleozoic and metamorphic Cretaceous (Khorat Group) rocks underlie the basin as well as the western and eastern mountain ranges (Wattananikorn et al., 1995). Of importance to this study is the intrusion of the Late Triassic/Earliest Jurassic granitic rocks of the eastern marginal belt of plutons – the Khuntan Batholith of biotite-granite – in the Palaeozoic rocks on the eastern part of the basin (Yokart et al., 2003). Biotite ($K(Mg,Fe)_3(AlSi_3O_{10})(F,OH)_2$) is a known source of fluoride in the environment (Chae et al., 2006). Fluoride can be transferred from these granitic rocks to groundwater through dissolution (Chae et al., 2007; Nordstrom et al., 1989). Above the sedimentary Palaeozoic and metamorphic Cretaceous rocks is the Tertiary sequence of which the oldest unit is the Mae Sod Formation of the Mio-Pliocene (Wattananikorn et al., 1995).

4.2.3 Hydrogeology

Unconsolidated Quaternary alluvium deposits are important aquifers and lie unconformed over older rock formations, covering most of the Ping River Basin (Wattananikorn et al., 1995). The Quaternary deposits that are of relevance to the study area can be categorised into two geomorphological units: (i) flood plain alluvial deposits; and (ii) low-terrace colluvial deposits. Holocene alluvial deposits are restricted to the floodplains and meander belts of the Ping River that cover the central part of the basin. The formation is composed mostly of well-sorted sand and gravel overlain by a few metres of clay. This area has the highest groundwater exploitation potential in the basin with well yields greater than 20 m^3/hour (Intrasutra, 1983). The Middle-Upper Pleistocene low colluvial terraces flank the central alluvial plain. These formations are composed of thick beds of fine sediments including kaolinite with intercalating sand and gravel lenses (Intrasutra, 1983). These low permeability layers of fine materials function as aquitards to restrict the flow and mixing of groundwater from one aquifer to another (Suvagondha and Jitapunkul, 1982). At the low-terrace colluvial deposits, well yields vary in the range of 12 to 60 m^3/hour (Intrasutra, 1983).

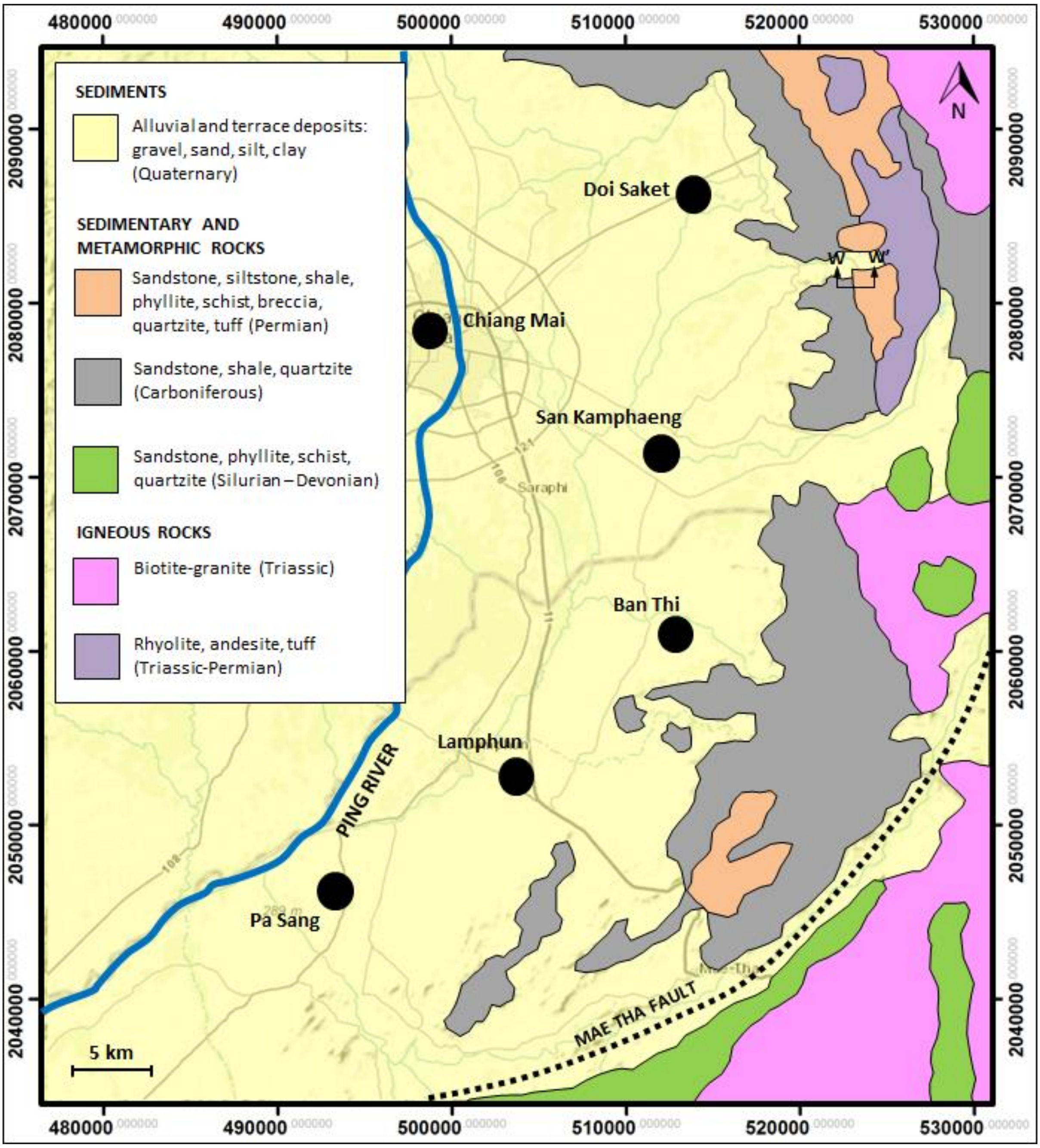

Figure 4.2: Geological map of the study site (after Department of Mineral Resources, 1995). Section W-W’ is detailed in Figure 4.7.

4.2.4 Geothermal Source

A section of the north-eastern part of the study site falls within a geothermal field. The 12-ha geothermal field, which has more than 70 natural hot springs (Singharajwarapan et al., 2012), has been studied for its geothermal energy production potential (Barr et al., 1979; Chuaviroj, 1988; Praserdvigai, 1986; Ramingwong et al., 1978). Geothermal waters from these springs are known to have high levels of fluoride – for example a concentration as high as 42 mg/L has been recorded (Ratanasthien et al., 1987).

4.2.5 Human Activities

Agriculture is very important to the livelihood of the local communities of Chiang Mai and Lamphun. Approximately 34.5% of the population in both provinces are involved in the agricultural sector (Thomas, 2005). An estimated 11% and 18% of the total land areas are used for agricultural activities in the provinces of Chiang Mai and Lamphun, respectively (Thomas, 2005). Besides surface water sources like rivers and canals, farmers also pump groundwater for irrigation. Additionally, the groundwater is also extracted for use by the industrial sector. Anuwongcharoen (1989) reported that the Lamphun Industrial Estate, located to the east of the Lamphun Province's capital district, heavily exploits the local groundwater resources for industrial activities.

4.3 Materials and Methods

Water samples from private and community (village or town) wells from Chiang Mai Province were collected between May 2013 and December 2013. Sampling in Lamphun Province was carried out from January 2014 to April 2014. A total of 175 and 301 samples were collected from deep and shallow wells in Chiang Mai, respectively. At the Lamphun study site, 301 and 218 samples were collected from deep and shallow wells respectively. Due to the proximity of the geothermal field (where waters from hot springs are known to contain high levels of fluoride), we also collected samples from surface waters (e.g. streams, rivers) to investigate the influence of these high-fluoride geothermal waters on the local surface water geochemistry in the Chiang Mai study area. In addition, water samples were also collected directly from the hot springs. A total of 121 surface water samples as well as 6 geothermal water samples were collected.

Water samples from wells with depths of 30 m or less (typically hand-dug wells or borewells from private residences) are categorised as shallow wells. Water samples from wells with depths greater than 30 m (typically community borewells that supply water village-wide) are designated as deep wells. Water samples from hand-dug wells were collected using a 2-L bucket lowered from the top of the well with a rope. Water samples from borewells were collected directly from taps. The depths of hand-dug wells were obtained by lowering a weighted measuring tape to the base of wells; borewells depths were obtained by interviewing the owners (for private wells) or the caretakers of village water supply systems (for community wells). Samples sent for laboratory analyses were stored in distilled water-rinsed, 250-mL polyethylene bottles and stored in the dark at approximately 4 °C.

Specific electrical conductivity and pH of water samples were determined on site using a handheld multi-parameter probe (YSI 556 MPS, Yellow Springs, OH, USA). Concentrations of fluoride from water samples were first determined on site by colorimetry (SPADNS method, upper limit: 2.00 mg/L F^-) with a portable spectrophotometer (Hach® DR2800™, Loveland, CO, USA). Prior to processing, raw samples were filtered through a 0.45-μm nylon-membrane to remove suspended particles that may interfere with the colorimetry determination. Samples with fluoride concentrations of at least 2 mg/L (upper detection limit for SPADNS), as well as all samples from deep wells, were (re)analysed for fluoride in the GEOLAB at the Department of Geography, National University of Singapore, using a high-pressure ion chromatography system (Dionex™ ICS-5000, Thermo Scientific™, Sunnyvale, CA, USA). Concentrations of major groundwater cations and anions including Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-} from a subset of well water samples were also determined by ion chromatography.

To understand the spatial variation of fluoride concentration in water sources, sampling sites with the corresponding fluoride concentrations (represented with different colours for various concentration groups) were mapped using a geographic information system (GIS) software, ArcGIS

(Esri, Redlands, CA, USA). The range (minimum and maximum concentration), median, mean and standard deviation of the measured parameters were calculated and tabulated. Regression analysis was performed to determine the correlation between fluoride and the other water quality parameters.

4.4 Results

4.4.1 Spatial Distribution of Fluoride

4.4.1.1 Chiang Mai Province

As expected, water samples collected from the geothermal springs contained the highest concentrations of fluoride (n = 6; mean = 17.03 mg/L; range: 12.30 – 19.89 mg/L). A total of 121 surface water samples were collected. Surface water samples with the highest fluoride concentrations were found in streams close to the geothermal field to the northeast of the site (Figure 4.3). The maximum fluoride concentration recorded in surface water was 18.84 mg/L where the sample was collected from a stream draining the geothermal field. The concentrations of fluoride in surface water gradually decreased away from geothermal field in the south-westerly direction following the flow of the local stream system.

A total of 175 and 301 water samples were collected from deep and shallow wells, respectively. Approximately 18% of the water samples from deep wells contained fluoride with concentrations greater than 1.50 mg/L, the guideline value recommended by the WHO for safe (long-term) drinking-water consumption (WHO, 2011; Figure 4.4). More shallow wells contained unsafe levels of fluoride than deep wells (Figure 4.5). Approximately 31% of these water samples were found to contain fluoride exceeding the WHO guideline threshold. Comparison of fluoride concentrations in water from paired deep and shallow wells of the same locality (collected no more than 50 m from each other) demonstrated that the shallow wells in this area have a relatively higher susceptibility to high-fluoride water intrusion (Figure 4.6a). In 61% of the pairs, shallow wells had higher concentrations of fluoride than deep wells.

The spatial patterns of fluoride distribution in deep and shallow wells revealed a linear-shaped, high-fluoride ($F^- > 1.50$ mg/L) anomalous zone within the study area, similar to that as observed in the surface waters (Figures 4.4 and 4.5). This zone extends from the geothermal fields in the northeast to the edge of San Kamphaeng town centre in the southwest of the Chiang Mai study site.

We did not find any relationships between the concentrations of fluoride and either the physicochemical water quality parameters (pH and specific electrical conductivity) or the major ions (Na^+, K^+, Mg^{2+} and Ca^{2+}) of the water samples from shallow and deep wells (Table 4.1).

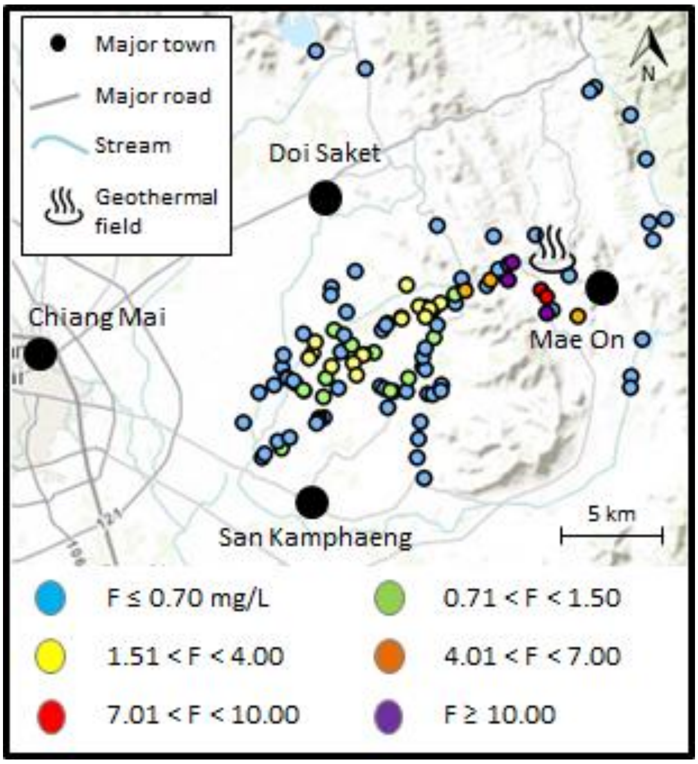

Figure 4.3: Sampling locations of surface waters and corresponding fluoride concentrations in Chiang Mai Province

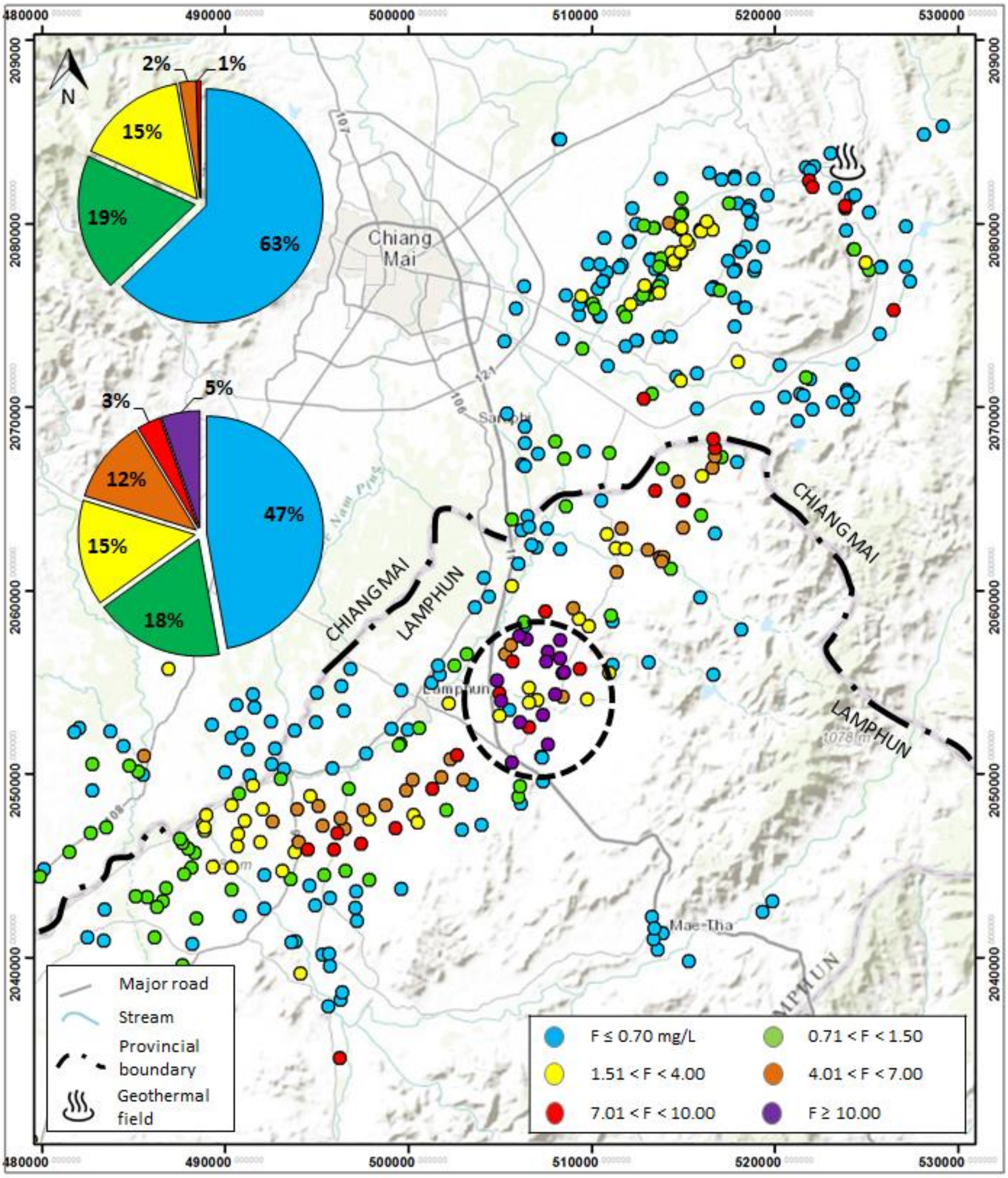

Figure 4.4: Locations of sampled deep wells and corresponding fluoride concentration ranges. Pie charts show statistical summary of fluoride concentration ranges in deep wells at study sites in Chiang Mai (top) and Lamphun (bottom), respectively. Black dotted circle: 'Hot spot' in high-fluoride anomalous zone.

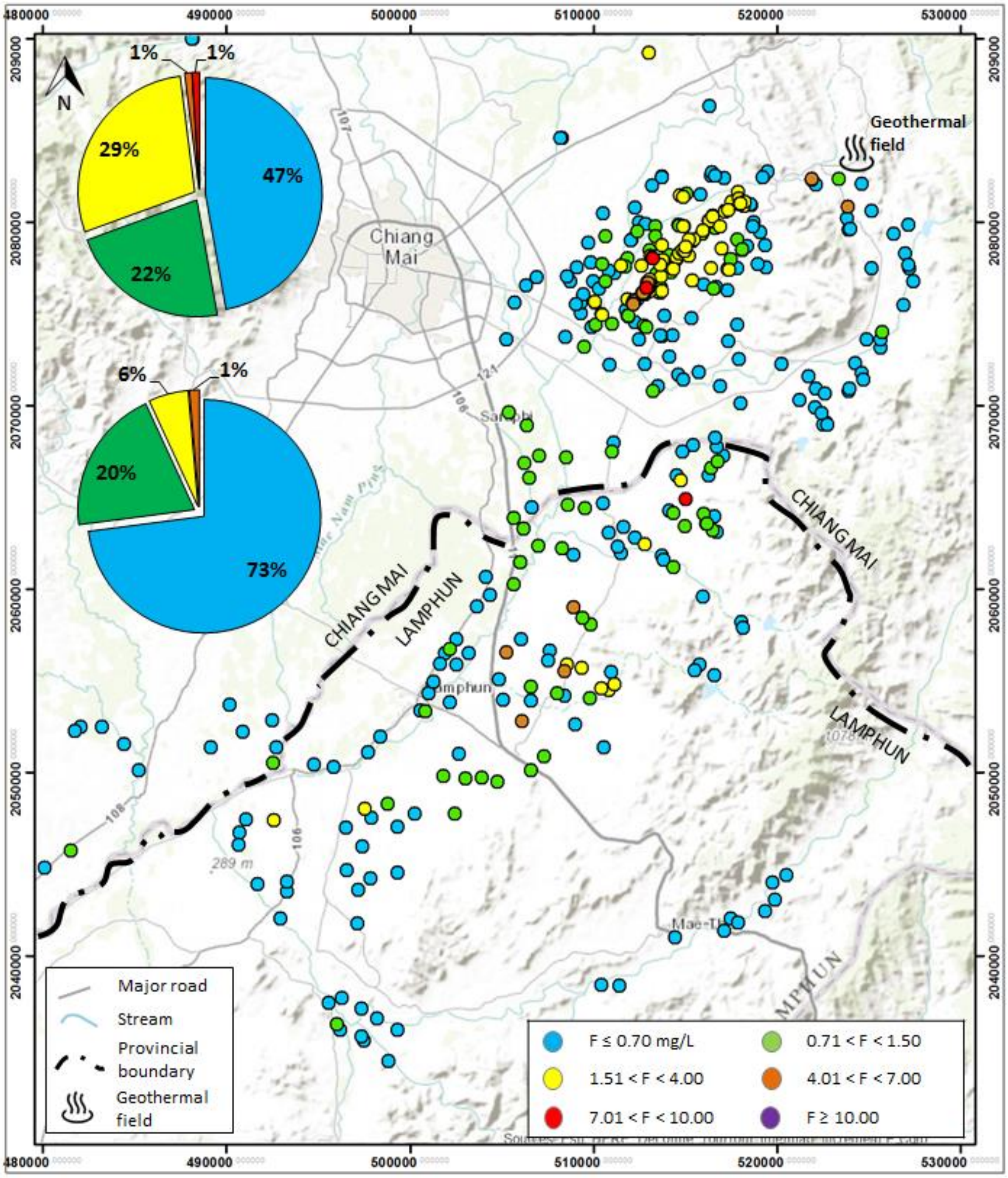

Figure 4.5: Locations of sampled shallow wells and corresponding fluoride concentration ranges. Pie charts show statistical summary of fluoride concentration ranges in shallow wells at study sites in Chiang Mai (top) and Lamphun (bottom), respectively.

Table 4.1: Statistical summary of water quality parameters from deep and shallow wells at the Chiang Mai study area

Parameter	Deep well samples						Shallow well samples					
	n	Min.	Max.	Median	Mean	S.D.	n	Min.	Max.	Median	Mean	S.D.
pH	175	4	9.85	7.06	7.04	0.78	301	4.45	9.24	7.12	7.11	0.69
SEC (µS/cm)	175	18	3,900	488	527	438	301	30	4,413	743	887	662
F^- (mg/L)	175	0.01	9.60	0.48	0.92	1.28	301	0.01	8.48	0.75	1.16	1.17
Na^+ (mg/L)	62	0.20	578.14	38.45	52.28	80.98	93	0.20	668.90	38.51	65.36	100.71
K^+ (mg/L)	62	0.20	9.68	0.51	1.52	1.88	93	0.20	210.10	0.95	17.47	43.62
Mg^{2+} (mg/L)	62	1.16	238.30	27.62	33.37	32.68	93	0.20	106.15	31.44	36.12	22.92
Ca^{2+} (mg/L)	62	0.30	138.80	33.20	37.10	25.72	93	0.30	139.70	47.53	52.19	31.87

Note: SEC – Specific electrical conductivity; S.D. – Standard deviation; Cl^- and SO_4^{2-} were not measured for this site

Table 4.2: Statistical summary of water quality parameters from deep and shallow wells at the Lamphun study area

Parameter	Deep well samples						Shallow well samples					
	n	Min.	Max.	Median	Mean	S.D.	n	Min.	Max.	Median	Mean	S.D.
pH	301	4.81	10.89	7.99	7.93	0.59	218	5.95	9.82	8.02	7.92	0.59
SEC (µS/cm)	301	36	1,440	471	480	214	218	43	2,854	556	647	463
F^- (mg/L)	301	0.01	14.12	0.76	2.21	3.17	218	0.01	5.63	0.44	0.65	0.76
Na^+ (mg/L)	275	2.16	214.15	55.24	66.90	50.35	108	3.43	688.96	59.05	98.67	123.30
K^+ (mg/L)	275	0.61	33.64	4.24	5.86	4.80	108	0.22	178.87	9.59	20.93	33.15
Mg^{2+} (mg/L)	275	0.20	55.23	11.02	13.20	9.03	108	0.86	58.70	14.64	17.74	12.61
Ca^{2+} (mg/L)	275	1.22	151.00	35.98	41.96	28.76	108	5.20	170.95	48.65	51.92	28.17
Cl^- (mg/L)	275	0.33	177.24	5.89	13.71	21.11	108	0.69	727.01	20.70	51.41	96.34
SO_4^{2-} (mg/L)	275	0.02	4231.00	4.03	26.12	255.05	108	0.02	252.72	25.41	39.28	47.74

Note: SEC – Specific electrical conductivity; S.D. – Standard deviation

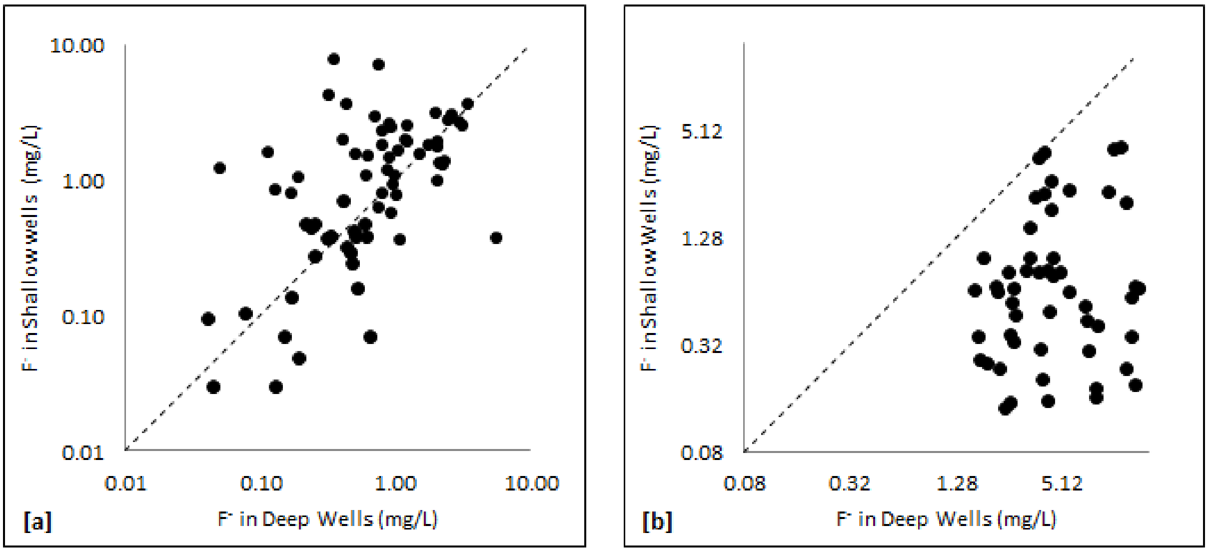

Figure 4.6: Comparisons of fluoride concentrations between shallow and deep wells at the high-fluoride anomalous zones in Chiang Mai [a] and Lamphun [b]. All data represent paired samples. The dotted line indicate the 1:1 line. Values falling below this line indicate the fluoride concentrations from the deep well samples are greater than the shallow well samples.

4.4.1.2 Lamphun Province

A total of 301 water samples were collected from deep wells at the Lamphun study site. Approximately 35% of these water samples had concentrations of fluoride greater than the recommended WHO drinking-water quality threshold value of 1.50 mg/L (Figure 4.4). Up to 5% from these samples had fluoride concentrations of more than 10.00 mg/L. The highest recorded value was 14.12 mg/L. Of the 218 shallow well water samples collected, only 7% had concentrations greater than the recommended WHO drinking-water quality threshold (Figure 4.5). Concentrations of fluoride in water samples from shallow wells were generally lower; the highest recorded value was 5.63 mg/L. Comparison of fluoride concentrations in the water samples from paired deep and shallow well samples (collected no more than 50 m from each other) showed that deep wells were more influenced by high-fluoride waters than the shallow wells – a result in contrast with that at the Chiang Mai site (Figure 4.6b). All deep wells contained higher levels of fluoride than the shallow wells.

A well-defined, curvilinear high-fluoride anomalous zone can be identified on the map showing fluoride concentrations in sampled deep wells (Figure 4.4). This zone trends in the northeast-southwest direction, with an eastward arc extending from the villages in the northeast of Ban Thi

District to the east of Pa Sang town centre. A 'hot spot' of the high-fluoride zone occurs immediately east of the Lamphun town centre (demarcated with a black dotted circle in Figure 4.4). Concentrations of fluoride in many deep wells within this hot-spot are in excess of 10 mg/L. Fluoride concentrations in deep wells appear to decrease gradually along this arcuate zone with distance away from the hot spot. In contrast, concentrations of fluoride decrease sharply in deep groundwater at areas immediately to the east and west of the high-fluoride anomalous zone.

Only a limited number of shallow-well samples (15/218) contained high concentrations of fluoride with concentrations of more than 1.50 mg/L (Figure 4.5). These wells were found sporadically within the curvilinear high-fluoride anomalous zone from Ban Thi District in the northeast to Pa Sang District in the southwest. The concentrations of fluoride in water samples from these shallow wells ranged from 1.81 to 5.63 mg/L.

In a comparison of fluoride concentrations with physicochemical parameters of water (pH, specific electrical conductivity) and important groundwater ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-}), only Na^+ in the deep wells was correlated with fluoride (coefficient of determination, R^2 = 0.70) (Table 4.2).

4.5 Discussion

4.5.1 Source and Transport of Fluoride

4.5.1.1 Chiang Mai Province

The gradual decrease of fluoride concentration from the northeast to the southwest, as depicted in Figure 4.3, suggests that the geothermal field is a common origin of fluoride at the study site. Fluoride-enriched geothermal waters are discharged from the hot springs in the area and transported across the study site, creating the linear zone of high-fluoride. Other researchers (e.g., Ratanasthien and Ramingwong, 1982; Ratanasthien et al., 1987; Ratanasthien, 1991) have also reported that the elevated groundwater fluoride levels were due to the intrusion of geothermal waters, but they did not explain the transport processes.

Evidence suggests that the fluoride-enriched waters from the hot springs are a result of deep-circulating, locally-derived and low-fluoride meteoric water that originates from a higher altitude (Praserdvigai, 1986; Ramingwong et al., 1978). This water percolates into deep granitic geothermal reservoirs formed within a complex, high-faulted graben structure where it is heated to 180-200 °C, as estimated by Na-K-Ca geothermometer (Praserdvigai, 1986; Ramingwong et al., 1978; Wood and Singharajwarapan, 2014). The descending water not only encounters a heat source, it also acquires chemical constituents, including fluoride, from the surrounding biotite-bearing plutons (Figure 4.7). Fluoride is transferred from rock into water via the dissolution from biotite, which contains fluorine at the OH^- sites of the octahedral sheet (Chae et al., 2007; Nordstrom et al., 1989).

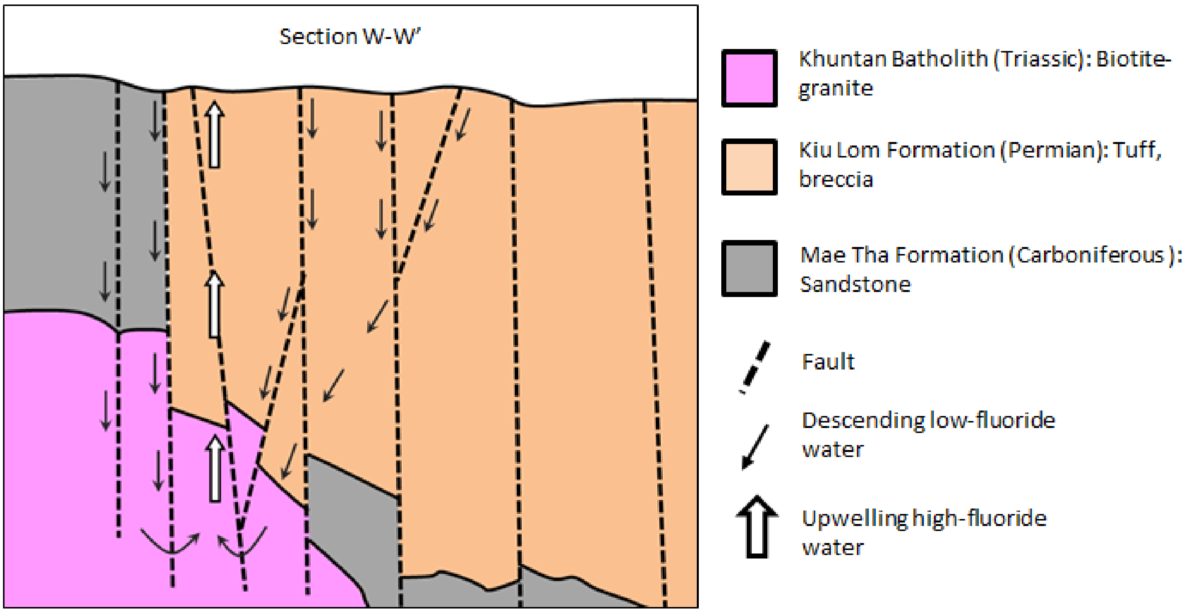

Figure 4.7: Model cross-section of geologic stratigraphy (after Praserdvigai, 1986) at the geothermal field and the genesis of high-fluoride geothermal water – Section W-W' from Figure 4.2.

To elaborate this water-rock/mineral interaction, we refer to the work by Chae et al. (2006) who performed laboratory experiments on batch dissolution of granite and biotite at room temperature (25 °C). They found that, for granite, concentrations of fluoride in water doubled in approximately 500 hours, while dissolution of biotite resulted in a 100% increase of fluoride in less than 200 hours. Additionally, dissolution of fluorine-bearing minerals in rocks was enhanced with increasing temperature or residence time (Chae et al., 2007; Kim and Jeong, 2005; Nordstrom et al., 1989; Saxena and Ahmed, 2003). These findings support our belief that heating in the deep granitic reservoirs at the study site facilitates the release of fluoride.

Chuaviroj (1988) described the presence of a second reservoir that also may yield geothermal fluids. This reservoir is made up of faults, fractures and zones of lateral continuity in sedimentary rocks situated above the primary granitic reservoir. This secondary reservoir likely channels geothermal waters laterally across the study site (Figure 4.8). A detailed record of the faults and fractures in these sedimentary rocks were not available to us and therefore, we were unable to elaborate more pertaining to this secondary reservoir and its association with the high-fluoride zone at the Chiang Mai site.

Lateral flow of geothermal water also occurs in the alluvial and terrace deposits that overlay these sedimentary rocks as described above. In this layer, the flow direction of geothermal fluids follows the local groundwater flow pattern and therefore results in the intrusion of fluoride-enriched waters, especially in shallow wells down-gradient of the hot springs (Figure 4.8).

To understand how the hydrological controls of the shallow aquifers affect fluoride distribution, we compared the zone of high-fluoride to a piezometric contour map of the Ping River Basin based on the work of Intrasutra (1983) (Figure 4.9). The study area is located in the northernmost section of the basin where excessive pumping of groundwater for irrigation, an anthropogenic process, has lowered the water table (Intrasutra, 1983). The extent of the high-fluoride endemic areas aligns with the hydrogeological gradient of the groundwater. Fluoride is transported from the source (the geothermal field) where the piezometric head is the highest to the end of the high-fluoride anomalous zone where the piezometric head is the lowest. The shallow wells we sampled with the highest levels of fluoride ($F^- > 4$ mg/L) were located at the end of the zone where the piezometric head was lowest. This finding of an accumulation of fluoride in an area where the water table is lowest is based on three groundwater surveys conducted between 1981 and 1982. As we were unable to acquire more survey data, there is some uncertainty in this interpretation.

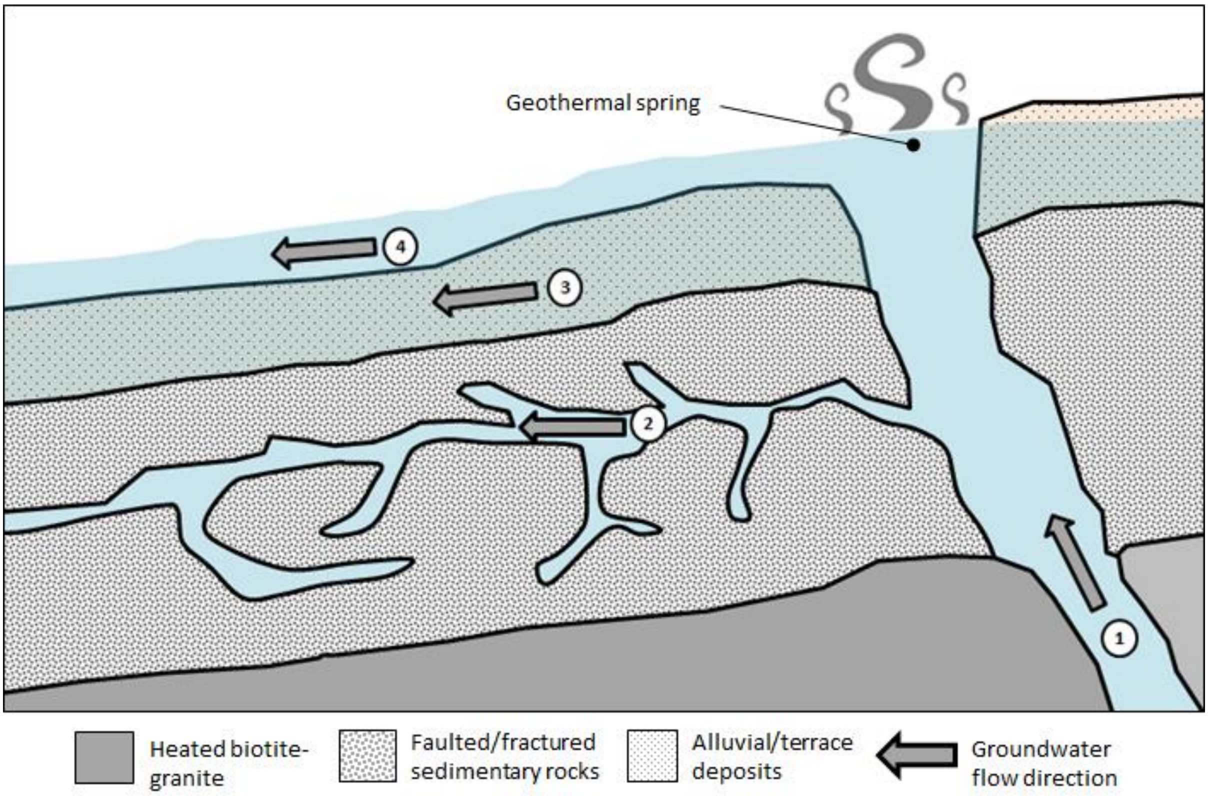

Figure 4.8: Model longitudinal section of the Chiang Mai study area showing the multiple modes of the transport of fluoride-enriched geothermal waters from the source. [1] Upwelling of geothermal fluids with high concentration of fluoride acquired from the dissolution of biotite in the primary, heated granitic-reservoirs made up of high-angular faults. [2] Secondary reservoirs formed by faulting and fracturing in the sedimentary rocks facilitate deep lateral transport. [3] Lateral transport through highly-permeable alluvial and terrace deposits following the groundwater flow direction. [4] Geothermal waters emerge to the surface and distributed across the study area by local streams and rivers.

To further explain the spatial distribution of fluoride at the site, we recognise that geothermal fluid flows upward through narrow fissures, emerging at the surface as hot springs (Figure 4.8). Above ground, fluoride-enriched fluids move across the study area in the south-westerly direction, according to the flow of the local streams which aligns with the linear zone of high-fluoride as observed. The general flow direction of these streams is similar to the groundwater flow direction. The gradual decrease in concentrations of fluoride with increased distance from the geothermal field is probably the result of dilution as stream water originating from the fluoride source (geothermal field) mixes with other surface waters with low fluoride concentrations. Some artificial enhancement of surface water may take place following the extraction of fluoride-rich groundwater for irrigation, discarding of fluoride-rich wastewater from reverse osmosis filtration facilities, and diversion within the extensive canal system in the area.

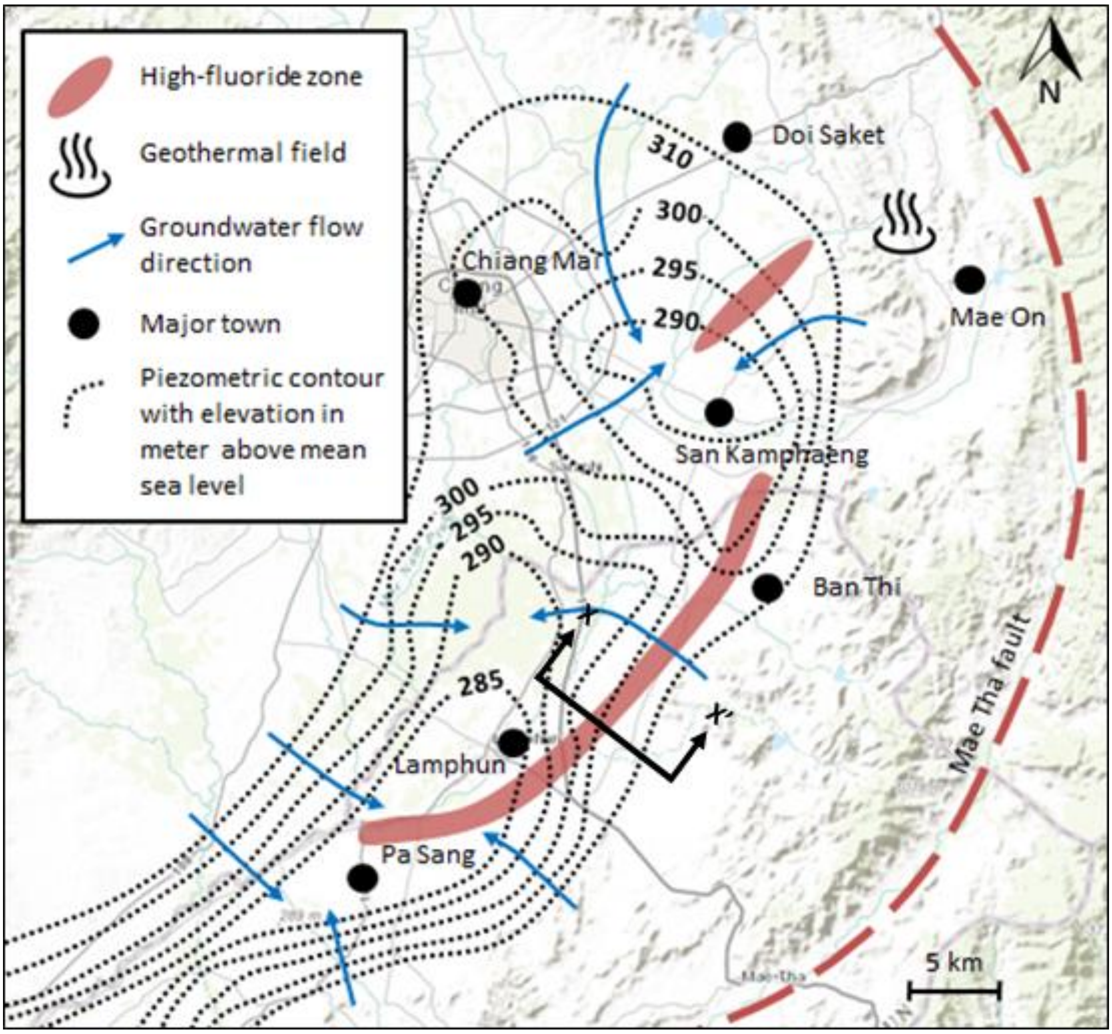

Figure 4.9: Piezometric contour map (after Intrasutra, 1983) showing groundwater flow in the Ping River Basin and the high-fluoride anomalous zones in Chiang Mai and Lamphun. The Mae Tha fault which has a similar alignment with the high-fluoride anomalous zone of Lamphun is also shown. Section X-X' is detailed in Figure 4.10.

4.5.1.2 Lamphun Province

In contrast with the study site at Chiang Mai Province, the deeper wells of the study site at Lamphun Province had higher levels of fluoride than shallow wells. At this site, different factors control the transport and distribution of fluoride in the groundwater. Shallow wells with high fluoride content generally coincide with the areas where the highest concentrations of fluoride are also found in deep groundwater, implying a connection between the two. This connection may be mixing of normally low-fluoride shallow groundwater during deep groundwater abstraction of deep fluoride-enriched water. Suvagondha and Jitapunkul (1982) noted a layer of impermeable clay at an average depth of 60 m between two principal aquifers in the study area. This impermeable layer is an aquitard that prevents the mixing of groundwater between the two aquifers. However, the construction of deep wells potentially breaches the aquitard, creating a portal allowing the intrusion of deep, fluoride-enriched groundwater into the shallow aquifer. In addition, fluoride from deep sources may also enter the shallow groundwater system via the screens of borewells (Figure 4.10).

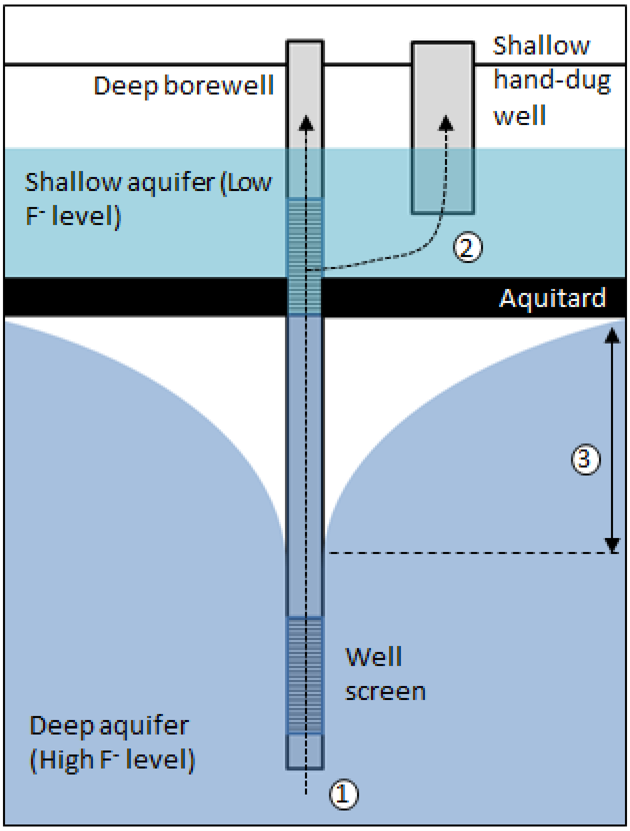

Figure 4.10: Cross sectional profile of the Quaternary alluvium deposits at the Lamphun site. [1] Abstraction of high-fluoride water by deep wells. [2] Intrusion of high-fluoride water from deep to shallow aquifer. [3] Well drawdown: intensive groundwater extraction may have caused a depression in water table, thus creating the high-fluoride hotspot as observed at the Lamphun site.

The curvilinear and eastward-trending, convex zone of high-fluoride concentrations in deep wells aligns (in direction and shape) with the conspicuous Mae Tha fault to the east of the Ping River Basin, as well as other minor faults in the area (Department of Mineral Resources, 1995; Figure 4.9). This alignment supports the existence of a previously unmapped, blind fault buried beneath the basin fill, as well as its likely association with a geogenic source of fluoride related to the faulted zone. A gravity survey of the Ping River Basin by Wattananikorn et al. (1995) supports the existence of this fault. The gravity anomaly map showed a belt of steep-to-moderate gravity gradients, reflecting boundary fault zones, on the east side of the basin near the eastern mountain range (Wattananikorn et al., 1995). The location of this 'inferred' fault coincides with the high-fluoride zone found in this study (Figure 4.11).

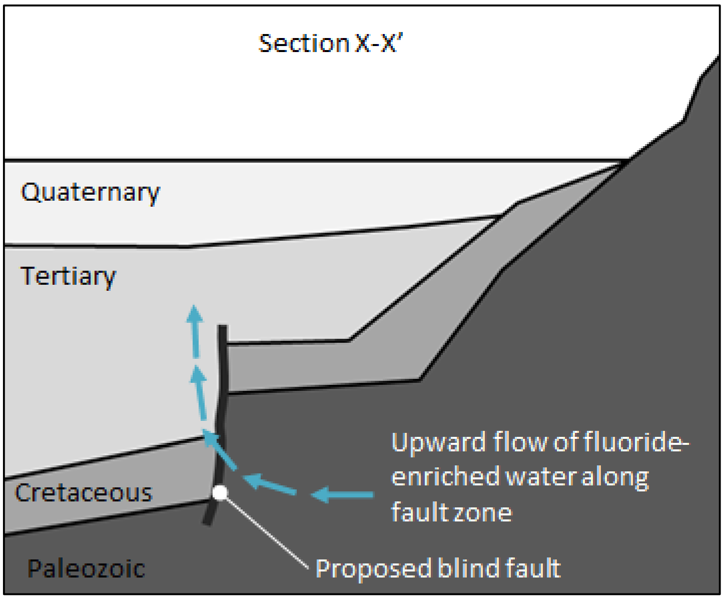

Figure 4.11: Stratigraphic section of the Ping River Basin at the high-fluoride anomalous zone in Lamphun – Section X-X' from Figure 4.8 (after Wattananikorn et al., 1995).

Kim and Jeong (2005) concluded a similar fault-fluoride association in the south-eastern part of the Korean peninsula where 10% of the surveyed public water supply wells contain fluoride exceeding the safe drinking water limit of 1.50 mg/L. They described two environmental processes that have contributed to the occurrence of high-fluoride distributed along major faults: (i) the weathering (dissolution) of fluoride-bearing rocks in faults; and (ii) the upward flowing of deep fluoride-enriched groundwater along the fault zone. We believe both of these processes are crucial in the genesis and transport of fluoride-enriched waters in the geothermal field at the Chiang Mai site. The same processes are therefore probably applicable to the Lamphun site, with the fluoride-bearing biotite-granite intrusion in the Palaeozoic rocks as the plausible source.

An alternative explanation of the occurrence of the high fluoride levels in Lamphun is that Ca^{2+} ions are removed from the groundwater by replacing Na^{+} ions from clay minerals with high cationic exchange capacities, thereby preventing the precipitation of the highly insoluble calcium fluorite (CaF_2), resulting in the accumulation of F^- in the groundwater (Asnachinda, 1992). While this process is plausible, it is unlikely to be the dominant control because it can neither account for the discernible differences of fluoride concentrations between deep (high fluoride) and shallow (low fluoride) groundwater, nor the curvilinear shape of the high-fluoride anomalous zone. Moreover, we did not find a relationship between Ca^{2+} and F^- from the water samples of both deep and shallow wells.

We did, however, observed a positive correlation between Na^{+} and F^- in water samples from the deep wells at the study area in Lamphun. The source of the Na^{+} may originate from the granitic rocks from which the F^- was derived. Chae et al. (2006) demonstrated in their batch granite dissolution experiments a simultaneous increase of Na^{+} and F^- concentrations due to the progressive dissolution of granite.

Finally, it is plausible that the occurrence of the fluoride 'hot spot' in Lamphun may be caused by a similar human-influenced hydrogeological factor that influenced the extent of the high-fluoride anomalous zone at the Chiang Mai site. In addition to the extraction for agriculture, a part of the hotspot also coincided with the Lamphun Industrial Estate where heavy extraction of groundwater also occurs (Anuwongcharoen, 1989). The extraction of groundwater may have also changed the hydraulic gradient of local groundwater thereby creating a local depression in the groundwater level at the hotspot where fluoride can potentially accumulate (Figure 4.10). Unfortunately, we do not have current survey data of the piezometric surface to verify this assertion.

4.5.2 Risk of Fluorosis

In recent years, many rural villagers have been informed of the risks of fluorosis from drinking high-fluoride water through health education programs in schools for children, as well as other outreach programs conducted by government health workers. Most villagers have responded by using bottled water instead of water piped from village water supplies (but also in response to unclean water from other sources such as pathogens from faecal sources and agrochemicals such as pesticides). Yet, the risk of fluorosis persists. Takeda and Takizawa (2008) found that many of the subjects from their study in the Lamphun Province still had high levels of fluoride in their urine despite drinking fluoride-free bottled water. They attributed this to the ingestion of rice cooked with high-fluoride water in the local piped-water supply. In many parts of Thailand, including Northern Thailand, glutinous rice or 'sticky rice' (*Oryza sativa var. gluotinosa*) is the main staple food. During preparation, this rice is washed several times and then soaked in water overnight before steamed. In laboratory experiments, Takeda and Takizawa (2008) demonstrated that fluoride content in rice was proportional to the concentration of fluoride in water used for soaking, as well as the duration of the soaking.

Traditionally, the rice fields in Northern Thailand were rain-fed, and therefore, harvest in the past was only once per year. More recently, farmers have used groundwater for irrigation to produce a second growing season. We observed an abundance of irrigation wells in rice fields and farms in the high-fluoride anomalous zones in both Chiang Mai and Lamphun. We assume, based on laboratory experiments, the use of high-fluoride water for irrigation contributes to an increase in fluoride intake in the area. In one example, Jha et al. (2013) conducted a pot culture experiment to evaluate the bioaccumulation of fluoride in rice using irrigation water with different levels of fluoride concentration. Fluoride content in rice grains increased by 41%, 59% and 96% when irrigated with water containing fluoride concentrations of 2, 4 and 8 mg/L, respectively, in comparison with rice irrigated with fluoride-free water.

4.5.3 Drinking-Water Management Implications

Numerous low-pressure, reverse osmosis (RO) membrane filtration water treatment plants have been built in Thailand to provide clean drinking water. These plants are either operated by the communities themselves or by private companies. The community-managed public plants produce between 1 to 5 m^3 of drinking-water per day, while the private plants typically produce up to 50 m^3 per day (Matsui et al., 2006). Reverse osmosis membrane filtration is often preferred over other treatments because of its ease of operation and relatively lower cost of drinking-water production. More importantly, especially for the sites in this study, this method of drinking-water treatment is attractive because it efficiently removes fluoride, even when present in high concentrations.

There are however flaws with the applicability of RO membrane filtration for drinking-water production. In their study in Lamphun Province, Matsui et al. (2006) noted that despite investments made for the construction of these drinking-water treatment facilities, one of the nine plants studied was in not operation due to the poor and unfavourable quality of the local raw water for treatment. In particular, the polyamide composite membranes used in RO membrane filtration process were prone to fouling from calcium carbonate ($CaCO_3$) precipitation that occurs in the raw alkaline groundwater at their study site.

The average water recovery rate for these plants is approximately 40% (Matsui et al., 2006); therefore, less than half of the groundwater abstracted is converted into drinking water that can be consumed safely. The remainder is wastewater. The inefficiency of this process is somewhat unsustainable in terms of local water resource management, particularly if water is extracted from confined aquifers where recharge rate is low. Monitoring wells have already shown significant lowering of groundwater of up to 1.0 – 1.5 m per year (Intrasutra, 1983; Margane and Tatung, 1999), indicating the recharge rate is much lower than the rate of groundwater abstraction for a variety of agricultural, industrial, and domestic purposes.

As the chemical characteristics of the reject-brine from RO filtration reflect the feed water source (Squire, 2000), as much as 6 L of wastewater, highly enriched in fluoride, is generated for every

4 L of potable water produced. There are multiple options for the proper disposal of the reject-brine, including direct discharge to sewer systems, deep-well injection, and evaporation ponds (Ahmed et al., 2001; Squire et al., 1996; Squire, 2000). However, none of these options are currently available to the rural communities we visited. The wastewater is typically released on-site untreated. Developing safe waste-management infrastructure is costly and requires technical expertise for operation and maintenance – luxuries many rural communities do not possess. While impacts of the disposal of reject-brine water directly into the environment (e.g. streams, groundwater) is not known, it contributes to the (re)distribution of fluoride-rich water throughout the study area. In doing so, it likely increases the concentrations of fluoride, potentially to hazardous levels, in some water bodies that otherwise might be safe drinking-water resources.

4.6 Conclusion

Our analysis of more than a thousand surface and sub-surface water sources shows that high levels of fluoride in confined areas of Chiang Mai and Lamphun are not solely functions of distance from a nearby geothermal field. Multiple modes of transport of sub-surface and surface water, as well as water interaction with geological features, create/maintain these anomalous zones. In addition, anthropogenic activities influence the distribution of fluoride in surface waters in the area.

This complexity in fluoride genesis and transport creates a challenge for managing water resources for safe consumption in affected areas. As we have demonstrated, water at different depths may have different, unpredictable levels of fluoride. The simple assumption that deep water is safer than shallow water is not valid. This is demonstrated in this study where we found that more shallow wells in the Chiang Mai zone had higher concentrations of fluoride than deep wells; the opposite relationship was found in the Lamphun zone. Regardless of location, groundwater at any depth should always be tested before the construction of wells to provide water for domestic use.

Existing wells abstracting from high-fluoride aquifers need not be abandoned if the water is otherwise uncontaminated. Reverse osmosis filtration is a viable treatment to remove fluoride but it is expensive to install/maintain and it generates substantial wastewater that requires proper disposal. A simple solution to managing fluoride-rich water for domestic use is dilution with water of low fluoride concentration. Dilution could be achieved by mixing fluoride-rich water with groundwater abstracted from depths with low fluoride levels – although constant monitoring would be needed to ensure the mixture remained below the recognised risk threshold.

Finally, because the risks of fluorosis still exists in communities in zones of high-fluoride, particularly in areas where insufficient resources hinder the ability to obtain sufficiently treated water for drinking and food preparation, continuing (re)education is needed to inform community members of the risk of long-term consumption of fluoride in local water resources.

CHAPTER 5 – EPILOGUE

The degradation of water quality is a serious threat to the drinking-water availability as well as to the public health in Thailand. In this study, examples of drinking-water quality problems of immediate concern to the health of vulnerable populations, particularly those in the rural areas, were highlighted. Both surface and sub-surface water resources were investigated. The origins of these health hazards in drinking-water supplies derived from anthropogenic activities (e.g. faecal wastes, pathogens) as well as those occurring naturally (e.g. fluoride) were investigated.

Although several substantial drinking-water quality problems that still persist today, Thailand has done remarkable work to provide basic drinking-water service to both the urban and rural communities (UNICEF and WHO, 2015). The United Nations Millennium Development Goals (MDGs) included Target 7c which aimed to '*halve the proportion of the population without sustainable access to safe drinking-water*' between 1990 and 2015 (JMP, 2015). The Joint Monitoring Programme for Water Supply and Sanitation (JMP) of the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) were tasked to report the progress towards meeting this goal (Onda et al., 2012). The corresponding MDG indicator is the '*proportion of households using water from an improved source*' (Bain et al., 2012; Onda et al., 2012). An 'improved source' is defined as '*one that, by nature of its construction or through active intervention, is likely to be protected from outside contamination*' (JMP, 2015). Examples of drinking-water sources that fall in this category include water piped into dwellings, tube wells, protected dug wells, protected springs and harvested rain water (JMP, 2015). Today, nearly all of Thailand (98%) has drinking-water access from such improved sources (UNICEF and WHO, 2015).

However, as demonstrated in the case studies presented in Chapters 2, 3 and 4, an improved source of drinking-water is not necessarily synonymous with a safe drinking-water source. The villages of Bo Hin and Pa Kang in the San Sai District of Chiang Mai Province rely on groundwater from hand-dug wells and borewells that are contaminated with faecal matter originating from poorly managed on-site sanitation systems (Chapter 2). Faecally contaminated water may contain harmful and potentially life-threatening waterborne pathogens such as *Cryptosporidium* and *Giardia* (Chapter 3). Many households in the provinces of Chiang Mai and Lamphun have access to piped water supplies; although, some may tap from sources with hazardous levels of naturally-occurring fluoride (Chapter 4). As such, the use of the MDG indicator – improved source – to determine if a water source is safe can lead to substantial overestimates of the population with access to safe drinking-water and, consequently, also overestimate the progress made towards the 2015 MDG target (Bain et al., 2012). In 2010, the JMP estimated that 783 million people do not have access improved sources of drinking-water. An independent study by Onda et al. (2012) re-evaluated the estimate by JMP, accounting for microbial water quality and sanitary risk. They found that 1.8 billion people (28% of the global population) in 2010 were using unsafe water supplies. This figure could be even higher when chemical hazards (e.g. fluoride) in drinking-water are considered.

Therefore, while the vast majority of the Thai population today has access to basic drinking-water (i.e. improved sources of drinking-water), they are not necessarily invulnerable to an array of potential drinking-water health hazards. A number of challenges largely arising from various anthropogenic factors including population growth, economic expansion and human-induced climate change must be addressed such that sufficient clean and safe drinking-water can be provided to all now and in the future.

5.1 Challenges

5.1.1 Socio-Economic Development

In 2011, the world population breached the 7 billion mark, which is more than 250% of the global population in 1950 (UN-DESA, 2015). Meanwhile, the population of Thailand has tripled, from nearly 21 million in 1950 to almost 68 million today (UN-DESA, 2015). The increase of population has resulted in an increase of water demand. Between 1950 and 2000, the amount of fresh water withdrawn for use per capita has increased by over 20% worldwide (Shiklomanov, 1999; USCB, 2012; Figure 5.1).

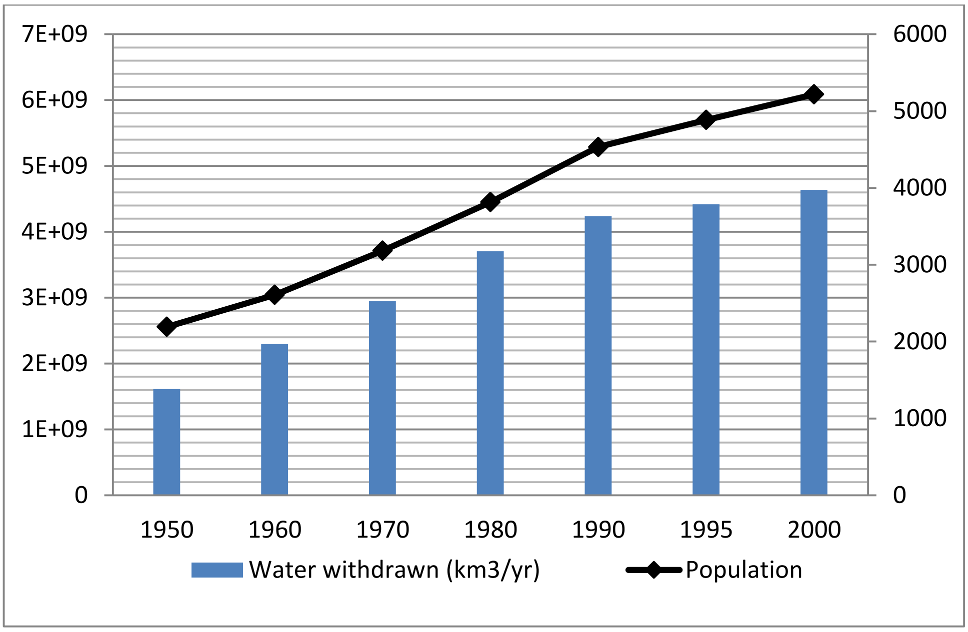

Figure 5.1: Global population growth (left vertical axis) and water withdrawal (right vertical axis) from 1950 to 2000

Presently, of the 25 river basins in Thailand, 16 have populations exceeding one million (World Bank, 2011). As discussed in Chapter 1, the Chao Phraya River Basin is already facing severe water scarcity. During the dry season, the demand of water can be 6 times more than the available renewable water resources (Hoekstra and Mekonnen, 2011). The demand of water will likely be increased in all sectors. It is estimated that the total demand of water supply from the domestic, agricultural and industrial sectors may increase by 35% in 20 years between 2004 and 2024 (World Bank, 2011; Figure 5.2).

A growing population will directly influence the additional water required for personal and household use (i.e. drinking and domestic use). An increased population will also cause the surge of food demand thereby resulting in a corresponding increase of water use for the production of food. Additionally, rampant urbanisation will exacerbate the stress on the water resources further. In 2014, almost half of the Thai population lives in urban areas compared to the 29% in 1990. By 2050, nearly three quarters of the total population will be living in urban areas (UN-DESA, 2014). Records have shown that an average urban household typically uses much more water than its rural counterpart. In

Thailand, the domestic water use in rural areas is estimated to be 50 L per person daily, while urban dwellers use approximately five times (250 L) more water every day (World Bank, 2011).

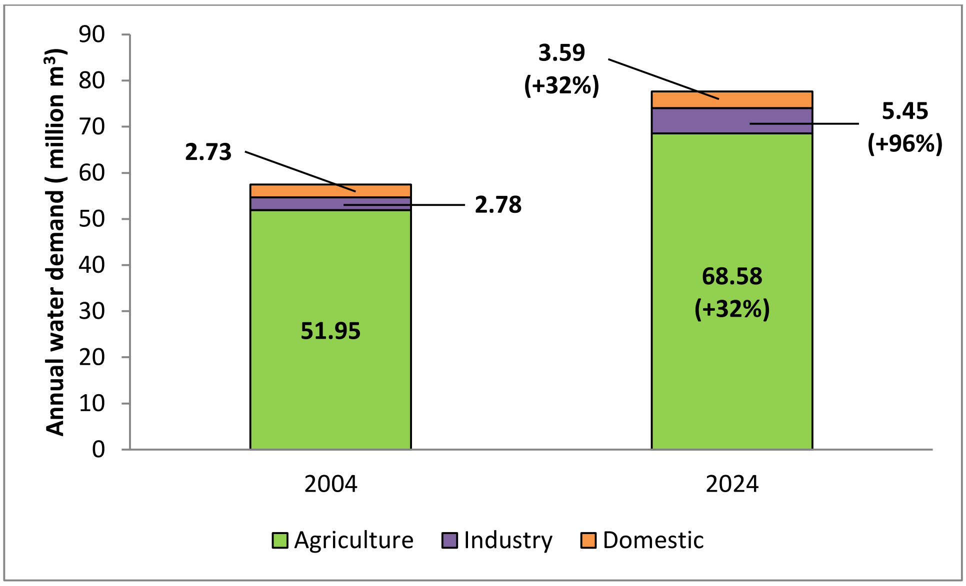

Figure 5.2: Annual water demand in the agricultural, industrial and domestic sectors of Thailand in 2004 (historical) and 2024 (predicted)

In 2011, Thailand became an upper-middle income economy (World Bank, 2015). In four decades, Thailand has made remarkable social and economic progress, ascending from a low-income to an upper-income country in less than a generation. Concomitantly, poverty has also declined substantially over the last 30 years – from 67% in 1986 to 11% in 2014 as incomes rose (World Bank, 2015). In tandem with the development of the national economy is growth of the industrial sector. In general, an increase in industrial activities increases the demand for water. Compared with the domestic and agricultural sectors, the increase of water demand due to the expansion of the industrial sector is estimated to be the largest. In a period of 20 years, between 2004 and 2024, annual water demand in the industrial sector is expected to increase by 96% (World Bank, 2011).

Economic development and the subsequent improvement in individual wealth will also incur a dietary shift – a shift from a primarily plant- and starch-based diet to one that has a higher proportion of meat and dairy products, which requires more water to produce. Producing 1 kg rice, for example, requires about 3,500 L water while 1 kg beef requires 15,000 L (Hoekstra and Chapagain, 2008). This dietary shift is the greatest to impact on water consumption over the past 30 years worldwide, and is likely to continue well into the middle of the twenty-first century (FAO, 2006).

Population and economic growth will not only increase water demand but will also increase waste generation, which can result in the degradation of the quality of water resources if not properly managed. This increase of waste generation will not merely be restricted in quantity but also in the variety. In this dissertation, only a few water quality parameters were studied because of constraints in time and resources. However, in reality, scientific studies and government surveys (although limited in numbers) have consistently shown that the quality of many water resources in Thailand is degraded by a variety of human-related sources and activities. For example, while domestic wastewater is often associated with faecal matter (Chapter 2) and pathogens (Chapter 3), another important emerging class of water contaminants – pharmaceuticals and personal care products (PPCP) – can also be found in domestic wastewater. PPCPs include a diverse group of chemical substances – for example, pharmaceuticals include human and veterinary drugs used to prevent or treat diseases, whereas personal care products are those that are used to improve the quality of daily life, including cosmetics, fragrances, body cleaning (hygiene) products etc. (Daughton and Ternes, 1999). Numerous studies have shown that conventional drinking and wastewater treatment plants cannot completely remove many PPCPs (Snyder et al., 2003). While there is no confirmed adverse human health effects associated with PPCPs in drinking water to date, their presence is a significant concern. For instance, many PPCPs have been identified as endocrine-disrupting compounds (Daughton and Ternes, 1999). Endocrine disruptors are synthetic chemicals that block or mimic natural hormones in the body, disrupting normal organ function. Even at extremely low concentrations, these endocrine disruptors can have effects on the human endocrine system (Daughton and Ternes, 1999).

The anticipated expansion in the industrial and agricultural sectors, along with population and economic growth, will also result in the increase in the use of chemicals to improve productivity. Correspondingly, an increase in the generation of wastewater containing these chemicals will almost likely be observed. Based on the National Research Centre for Environmental and Hazardous Waste

Management, Thailand's import of industrial and agricultural chemicals increased a hundred fold in just 25 years from 600,000 tonnes in 1978 to 60,000,000 tonnes in 2003 (Kan-atireklap et al., 2007). Alarmingly, many of these chemicals are considered hazardous.

Between 1970 and 1985, the number of industries registered for the use of hazardous chemicals increased from 19,700 to 51,500. In 2005, this number again increased by more than a 100% to 122,300 (Kan-atireklap et al., 2007). In the agricultural sector, the Office of Agricultural Economics and the Office of Agriculture Regulation estimated that pesticide use increased by a factor of four in ten years between 2002 and 2012 (Panuwet et al., 2012). About 70,000 tonnes of pesticides, composed of 265 individual active ingredients, were imported into Thailand in 2010, making the country one of the largest users of pesticide in Southeast Asia, second only to Malaysia (Panuwet et al., 2012; FAO, 2015c). When classified based on WHO's Recommended Classification of Pesticides by Hazard, about one third of the pesticides imported into Thailand in 2010 were either Class I (Highly to Extremely Hazardous) or Class II (Moderately Hazardous) pesticides (Panuwet et al., 2012). Additionally, some of the imported pesticides are classified as potential carcinogens (cancer-causing agents) or suspected endocrine disruptors (Panuwet et al., 2012).

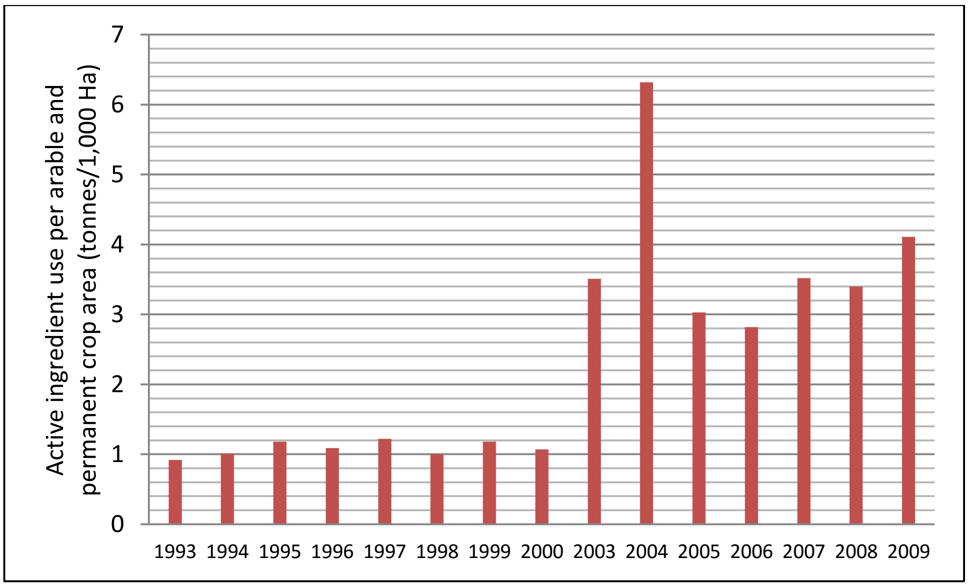

Figure 5.3: Pesticide use in Thailand between 1993 and 2009 (FAO, 2015c)

5.1.2 Climate Change

Climate change is another significant challenge of increasing importance to the management of water resources worldwide (Arnell, 1999). The major alterations to the hydrological cycle due to the increase of ambient temperature will likely result in the increased incidence of extreme hydrological events (IPCC, 2014). The hydrological cycle will intensify, with more evaporation and more precipitation occurring in some parts of the world. Elsewhere, significant reductions in precipitation will be experienced, resulting in frequent and prolonged droughts (IPCC, 2014). Additionally, the timing of the wet and dry seasons may be altered (Arnell et al., 1999; Sharma and Babel, 2013). These changes to the hydrological processes will in turn influence the physical availability of water resources. Although not as extensively studied, climate change may also impact the quality of water resources (Delpla et al., 2009).

The main climate change determinants affecting water quality are (i) precipitation and (ii) ambient temperature. Based on global and regional climate models, forecasted precipitation trends in Thailand and the Southeast Asian region are generally in concurrence with one another: precipitation is predicted to increase during the wet season and decrease during the dry season (Chotamonsak et al., 2011). In other words, the wet seasons will become wetter while the dry seasons are expected to be drier (Chotamonsak et al., 2011). Changes in precipitation can affect water quality by modifying concentrations of water quality constituents through dilution or concentration. An increase in precipitation will result in the increase of surface and sub-surface water volume, which will consequently produce a diluting environment in water bodies. The decrease in precipitation, on the other hand, will reduce the volume of water, thereby creating a concentrating environment.

Dilution and concentration effects were demonstrated in Chapter 2. For example, the effects of faecal contamination were diluted during the rainy periods in some of the shallow wells at the study site in the villages of Bo Hin and Pa Kang of the Chiang Mai Province. The concentration effect was observed particularly during the transition period from the dry to the wet season when the detected highest levels of faecal contamination coincided with the period when the water table was lowest (i.e.

the volume of water in well was at a minimum). Dilution and concentration effects will almost certainly become more pronounced with the corresponding changes of precipitation in the wet and dry seasons.

In addition, as an important agent of transport, water from precipitation will invariably play an important role in affecting the quality of water resources. Surface runoff can mobilise biological or chemical constituents deposited on land surfaces, washing them to surface water bodies. Meanwhile, percolating rainwater facilitates the vertical conveyance to groundwater. Contamination occurs when potential hazards are transported from their sources into water resources. These transport phenomena were demonstrated in Chapters 2 and 3. As described in Chapter 2, several hand-dug wells of the study area exhibited elevated levels of faecal contamination corresponding with the rainy season, as rainwater enhanced the vertical transport of waste from on-site sanitation systems into the underlying aquifer. Meanwhile, as presented in Chapter 3, the frequency of *Cryptosporidium* and/or *Giardia* detection in rivers of the study site nearly doubled during the wet season, compared to the dry season, reflecting the crucial role of overland transport of the protozoan cysts from their sources (e.g. cattle manure on grazing pastures) into surface water bodies. Without adequate protection of drinking-water resources and improvement to waste management, the increase of precipitation will surely lead to the deterioration of water quality.

Besides precipitation, the change in ambient temperature can also influence the quality of water. Continued emissions of greenhouse gases will cause further warming and changes in all components of the climate system (IPCC, 2013). By the end of the 21st century, the Inter-governmental Panel on Climate Change predicted a likely increase of ambient temperature by 0.3 to 4.8 ^{o}C (IPCC, 2013). A rise in temperature will favour all physico-chemical reactions, including dissolution, mineralisation, and complexation (Arnell et al., 1999). For example, an increase in dissolution potential and rate will result in the increase of total dissolved solids (mainly calcium, magnesium, sodium, and potassium cations and carbonate, bicarbonate, chloride, sulphate anions). Consequently, an increase of the salt content and salinity in the affected water body may potentially be observed. In drinking-water, dissolved solids are not typically hazardous to health. However, the presence of dissolved solids in water may affect its taste. Based on its palatability, the WHO (2003) rates drinking-water with total dissolved solids of less than 300 mg/L as excellent and concentrations greater than 1,200 mg/L as unacceptable.

Alterations to the ambient temperature can also affect biological reactions in environmental microorganisms which may indirectly affect water quality. As an example, an escalating soil temperature can enhance the mineralisation of nitrogen (conversion of organic-nitrogen to inorganic nitrogen, ammonium) by soil microbes, which many lead to eutrophication in nearby surface waters (Ducharne et al., 2007). In addition, changes in temperature may also affect the survival of microorganisms in water. While most studies indicate that survival of enteric pathogens are generally reduced at higher temperatures, there are also some suggestions that increased water temperatures will lead to more prolonged survival of pathogens (Hunter, 2003). In the latter case, the risk of waterborne diseases can be increased.

5.2 Towards Safe Drinking-Water

5.2.1 Water Quality Monitoring

The measurement of the physical, chemical, and biological characteristics of water bodies provides crucial information for identifying and addressing water quality problems. By observing trends over time and making comparisons between different water bodies, water quality data can help to: (i) define the suitability for specific uses (e.g. drinking, recreation, agriculture etc.); (ii) determine the impacts of human activities or natural processes; (iii) assess the effectiveness of policies and management plans; (iv) develop water management models; and (v) prioritise where management effort should be concentrated. However, there are currently large gaps in monitoring efforts and data related to water quality worldwide. With limited financial and technical means, water quality information in poorer and developing nations like Thailand is scarce. In March of 2012, the United Nations Educational, Scientific and Cultural Organisation (UNESCO) launched the fourth World Water

Development Report at the World Water Forum in Marseilles, France. Although the document contains a plethora of water-related facts and figures, its authors argue that a lack of reliable data on water quality has become a stumbling block for efforts to strengthen policies and enforce regulations (Gilbert, 2012).

Even for water quality data sets that do exist, there are limits to what is measured and for how long, thereby restricting beneficial use. Typically, only a few basic water quality parameters are consistently measured – and even among these, the measurements are limited in duration. As an example, while the Thai Pollution Control Department (PCD) carries out routine water quality checks in the major rivers from hundreds of monitoring stations nationwide, only five water quality parameters are tested: Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Ammoniacal-Nitrogen (NH_3-N), Total Coliform Bacteria (TCB) and Faecal Coliform Bacteria (FCB). Moreover, measurements are only made four times annually (i.e. once every three months) (PCD, 2013).

In reality, these parameters are merely indicators used to assess the general status of the monitored water bodies. For example, TCB and FCB are indicators of faecal contamination; DO and BOD, organic matter contamination; NH_3, agricultural or domestic wastewater contamination. Many other measurements that reflect the actual presence and/or concentration of a potential drinking-water hazard (chemical and biological) are not made. Protozoan parasites, *Cryptosporidium* and *Giardia*, investigated in Chapter 3 are just two of many other biological drinking-water hazards that exist. Other etiological agents including various protozoa (e.g. *Acanthamoeba castellanni*, *Entamoeba histolytica*), helminths (e.g. *Ascaris lumbricoides*, *Dracunculus medinensis*), bacteria (e.g. *Legionella* spp., *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*) and viruses (e.g. adenovirus, rotavirus, norovirus) are also important waterborne pathogens that affect the health of millions worldwide (Ashbolt, 2004).

Similarly, there are also many potential chemical hazards in drinking-water. In addition to fluoride, arsenic is also another common naturally-occurring drinking-water hazard affecting the health of many worldwide (Gordon et al., 2004). In Thailand, groundwater containing hazardous levels of arsenic (> 10 µg/L) has been recorded in the city of Hat Yai of the Songkhla Province (Lawrence, et al., 2000). The number of human-derived, synthesised chemical hazards in drinking-water is much greater. Pharmaceuticals and personal care products (Section 5.1.1) are examples of chemical hazards in drinking water. Another important group of chemical substances that may cause adverse effects on humans is Persistent Organic Pollutants (POPs). These are organic (carbon-based), chemical substances that possess a particular combination of physical and chemical properties that, once released into the environment, remain intact for exceptionally long periods of time (Wania and Mackay, 1996). POPs are hydrophobic ('water-hating') and lipophilic ('fat-loving'). These chemical substances can accumulate in the fatty tissue of living organisms, and are known to be toxic to humans (Jones and de Voogt, 1999). Due to these reasons, the usages of many POPs have been banned or restricted in many countries including Thailand (Table 5.1). Although their use has been controlled, these chemicals continue to persist in the environment because of their low-degradability.

Table 5.1: The original twelve Persistent Organic Pollutants, coined 'The Dirty Dozen', recognised by the Stockholm Convention in 2001 in causing adverse health effects on humans

Persistent Organic Pollutant	Use
Aldrin	Pesticide
Chlordane	Pesticide
Dichlorodiphenyltrichloroethane (DDT)	Pesticide
Dieldrin	Pesticide
Endrin	Pesticide
Heptachlor	Pesticide
Hexachlorobenzene	Pesticide, Industrial chemical, By-product*
Mirex	Pesticide
Toxaphene	Pesticide
Polychlorinated biphenyls (PCBs)	Industrial chemical
Polychlorinated dibenzo-p-dioxins	By-product*
Polychlorinated dibenzofurans	By-product*

*By-product refers to compounds unintentionally produced due to various industrial processes for the manufacture of other synthetic chemicals

The presence of these biological and chemical hazards (e.g. pathogens, arsenic, PPCPs, POPs etc.) has been recorded in various water resources in Thailand, implying the health risks they pose to the consumers (Anceno et al., 2007; Boontanon et al., 2013; Diallo et al., 2008; Kunacheva et al., 2011; Kwan et al., 2014; Lawrence et al., 2000; Tewari et al., 2013). However, very little is known about the distribution of these health hazards in drinking-water resources nationwide. As such, a water quality monitoring plan that considers these parameters would be beneficial. Various water treatment methods exist (Ray and Jain, 2011) and no single practical technology can be the universal solution to the remove the numerous hazards that may be present in water. Therefore, the knowledge of the prevalence of these drinking-water hazards is particularly important such that the appropriate technologies can be applied to treat and produce safe drinking-water. The awareness of the presence of these hazards coupled with the understanding of their origins as well as the relevant factors affecting their mobility are also vital so that necessary measures can be taken to control them from further degrading the quality of water resources.

Besides the limitation in the types of parameters monitored, many data sets also do not reflect the temporal changes of water quality. As evidenced in the previous sections, the quality of water resources can be highly variable in time due to natural (climate) or anthropogenic (e.g. human activities) factors. Again, drawing upon the case study in Chapter 2, the conflicting hydroclimatological conditions (i.e. rainfall distribution, water table level) between the dry and wet seasons were shown to affect the level of faecal contamination in the groundwater at the study area. Changes of water quality may also occur diurnally. In their study in Singapore, Ekklesia et al. (2015a) showed that levels faecal contamination in surface waters originating from leaking sewer pipes peaked twice a day (in the day time from 10:00 a.m. to 2:00 p.m. and in the night time around 8:00 p.m.), presumably in response to periods of highest domestic wastewater flow. For sources of drinking-water supplies, temporal concentration changes of any hazard influence the risk of exposure and therefore the vulnerability of consumers to health concerns at specific periods of time. This information will especially be most beneficial to the sections of populations in areas without access to reliable central drinking-water treatment facilities such that supplementary precautions (e.g. boiling) can be taken at the household level during high-risk periods.

5.2.2 Wastewater Management

Finally, preserving good quality drinking-water supplies requires effective control and management of wastewater in all sectors. Sustained impairment of streams, rivers and aquifers will in turn reduce the availability of the increasingly limited freshwater resources for the production of clean and safe drinking-water.

Since 1992, the five-year National Economic and Social Development Plans have emphasised environmental conservation in Thailand (World Bank, 2011). Many policies and legislations (e.g. Enhancement and Conservation on National Environmental Quality Act) have been enacted to protect and rehabilitate the water resources of the country. However, the inappropriate disposal of wastewater still occurs, as the enforcement of these environmental laws is limited by the lack of political will, inadequate coordination among fragmented agencies and institutions, low technical coordination for proving violations, and limited access to information (World Bank, 2001).

Although various wastewater treatment technologies exist (Sonune and Ghate, 2004), the facilities are severely inadequate in Thailand (Simachaya, 2009). For example, approximately 14 million m^3 of domestic wastewater is generated every day nationwide but only a little over one fifth of this total is conveyed to wastewater treatment plants (World Bank, 2008). Even so, wastewater entering these facilities often leave inadequately treated as virtually all plants experience varying degrees of operating problems which adversely affect the effectiveness and the efficiency of treatment (Simachaya, 2009). The remaining ~80% of generated wastewater not served by centralised wastewater treatment systems is discharged into on-site sanitation systems whereby, especially in rural areas, the wastewater is allowed to be released into the environment effectively untreated leading to the degradation of the quality of receiving waters (Chapter 2).

Thus, unless significant improvements are made to current wastewater management practices, viable sources of drinking-water supplies – especially surface waters and shallow aquifers – will continue to deteriorate. Greater impairment will incur greater costs of treatment to return water resources to useable qualities. Diseases will ensue and public health will be affected through the inadvertent consumption of inadequately treated contaminated water that may contain various health hazards. Granted, deep sub-surface water resources (e.g. confined aquifers) are potential alternative sources of drinking-water as they are typically devoid of health hazards of anthropogenic origin. However, in some areas, high levels of drinking-water hazards of geogenic origin such as fluoride (Chiang Mai, Lamphun – Chapter 4) and arsenic (Hat Yai – Lawrence et al., 2000) may occur naturally in these deeper groundwater sources and hence, are unsafe for consumption without treatment. Furthermore, some deep resources (e.g. fossil water) receive little to no significant recharge, effectively making groundwater in these aquifers a non-renewable resource. The depletion of these water resources will occur when the extraction rate is greater than the recharge rate.

Hence, with ever increasing water demand, in parallel with an increasing population as well as a burgeoning economy, all sources of drinking-water supplies – surface and sub-surface – must not be taken for granted and measures must be implemented so that these limited natural resources are adequately protected. All aspects of the wastewater management system in every sector, from the investment and provision of treatment facilities to law enforcement, must continue to improve. Uncontrolled disposal of wastewater can no longer be tolerated and must be addressed to ensure a sustainable supply of drinking-water resources.

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