

**DRINKING WATER QUALITIES FOR PRIMARY SCHOOLS
IN BANGKOK**



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OF THE REQUIREMENTS FOR
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Thesis
Entitled

**DRINKING WATER QUALITIES FOR PRIMARY SCHOOLS
IN BANGKOK**



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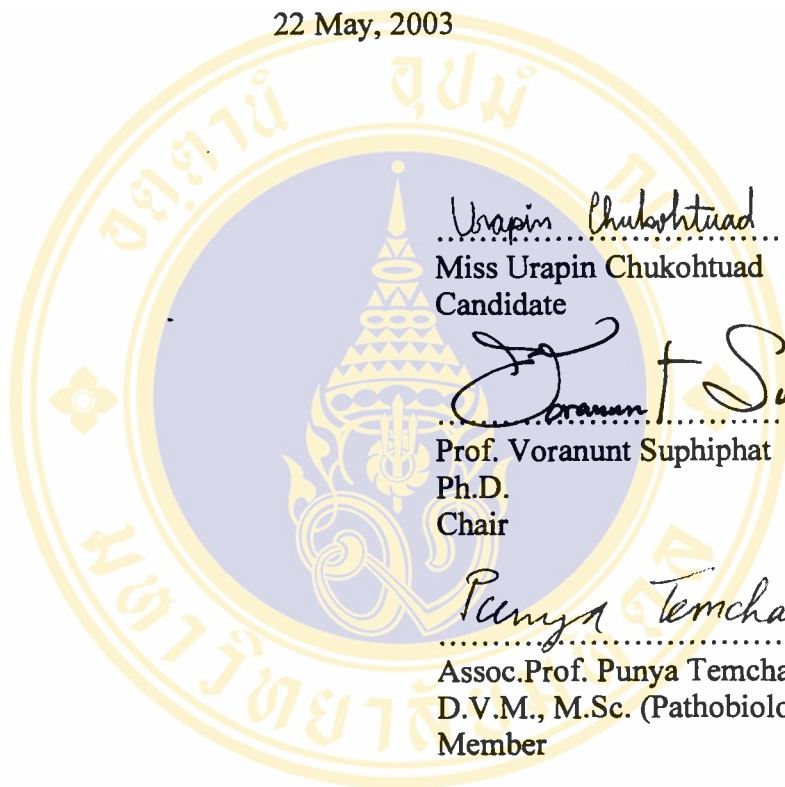
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DRINKING WATER QUALITIES FOR PRIMARY SCHOOLS IN BANGKOK.

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ABSTRACT

Waterborne diseases are now known to be caused by several microorganisms; mainly by bacteria and parasitic protozoa. In recent years, several outbreaks of *Cryptosporidium* spp. waterborne transmission have been reported from several parts of the world. In Thailand even though the drinking water, supplied by 4 water treatment plants in Bangkok, is known to be clean and safe to drink after being passed through the water distribution system, enteric bacteria and parasitic protozoa e.g. *Cryptosporidium* spp. have never been surveyed in the drinking water collected from the water supply pipeline at primary schools in area of Bangkok. This study was designed to investigate the quality of drinking water in terms of the waterborne microbe and the microbiological contaminations. In addition, the physical and chemical qualities were also determined. Drinking water collected from 50 districts in Bangkok, comprising 54 schools was investigated.

Using centrifugation and acid-fast staining method, no *Cryptosporidium* oocysts were found in any of the raw water or tap water samples but they were observed in 7.4% (4/54) of the drinking water from the 54 schools with a mean of 0.74 oocyst per litre. Sources of oocyst contamination could not be identified in this study. Thus, contamination of oocyst into the drinking water might have occurred after water treatment process.

Total coliforms, *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens*, and *Yersinia enterocolitica* were found in each of the 4 raw water samples but not in drinking water. No microbiological contaminations were found in tap water. Cadmium, cyanide, and phenols were not detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants. The highest lead (3.50 µg/L) and zinc (0.244 mg/L) contents were found in drinking water samples from Mahasawat and Samsen respectively, however, the values were lower than the maximum acceptable level for drinking water standards.

KEY WORDS: DRINKING WATER / PRIMARY SCHOOLS / *Cryptosporidium*

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บทคัดย่อ

น้ำเป็นแหล่งแพร่ระบาดของโรคติดเชื้อในลำไส้ที่เกิดจากแบคทีเรียและปรสิตพวกโปรโตซัว โดยมีรายงานสนับสนุนว่าการแพร่ระบาดส่วนใหญ่มีสาเหตุมาจากคริปโตสปอริเดียม สำหรับประเทศไทยนั้นยังไม่เคยมีรายงานว่าพบคริปโตสปอริเดียมในตัวอย่างน้ำดื่มจากท่อส่งน้ำของโรงเรียนระดับประถมศึกษาในพื้นที่กรุงเทพมหานคร การศึกษานี้มีวัตถุประสงค์เพื่อตรวจวิเคราะห์การปนเปื้อนของแบคทีเรียและคริปโตสปอริเดียม รวมทั้งการตรวจวิเคราะห์คุณภาพของน้ำดื่มทั้งทางกายภาพและทางเคมี โดยวิเคราะห์ตัวอย่างน้ำดื่มจากโรงเรียน 54 โรงเรียน จาก 50 เขตการปกครองของกรุงเทพมหานคร

ผลการศึกษาโดยใช้เทคนิคการปั่นด้วยความเร็วสูงและการย้อมสีด้วยเทคนิค acid-fast ไม่พบโอโอซิสต์ของคริปโตสปอริเดียมในตัวอย่างน้ำดิบและน้ำประปาก่อนสูบน้ำ แต่พบโอโอซิสต์ของคริปโตสปอริเดียมในตัวอย่างน้ำดื่มจาก 4 โรงเรียน ของทั้งหมด 54 โรงเรียน คิดเป็น 7.4% หรือ 0.74 โอโอซิสต์ต่อน้ำ 1 ลิตร ซึ่งการศึกษานี้ไม่ได้ศึกษาลงไปถึงสาเหตุของการปนเปื้อนนี้ ระบุได้เพียงว่าการปนเปื้อนนี้เกิดขึ้นหลังจากสูบน้ำออกจากโรงกรองน้ำแล้วเท่านั้น

มีการตรวจพบโคไลฟอร์มอีโคไล ซัลโมเนลลา คลอสตริเดียมและเยอร์ซีเนีย ในตัวอย่างน้ำดิบ แต่ไม่พบในตัวอย่างน้ำดื่ม ส่วนแคดเมียม ไซยาไนต์ ฟีนอล ตรวจไม่พบเช่นกันในน้ำดิบ น้ำประปาก่อนสูบน้ำและน้ำดื่ม แต่พบตะกั่ว 3.5 ไมโครกรัม/ลิตร และสังกะสี 0.244 มิลลิกรัม/ลิตร ในน้ำดื่มที่ผลิตจากโรงกรองน้ำมหาสวัสดิ์และสามเสนตามลำดับ อย่างไรก็ตาม ตัวเลขที่พบต่ำกว่าค่าสูงสุดที่ยอมให้มีได้ตามมาตรฐานน้ำดื่ม

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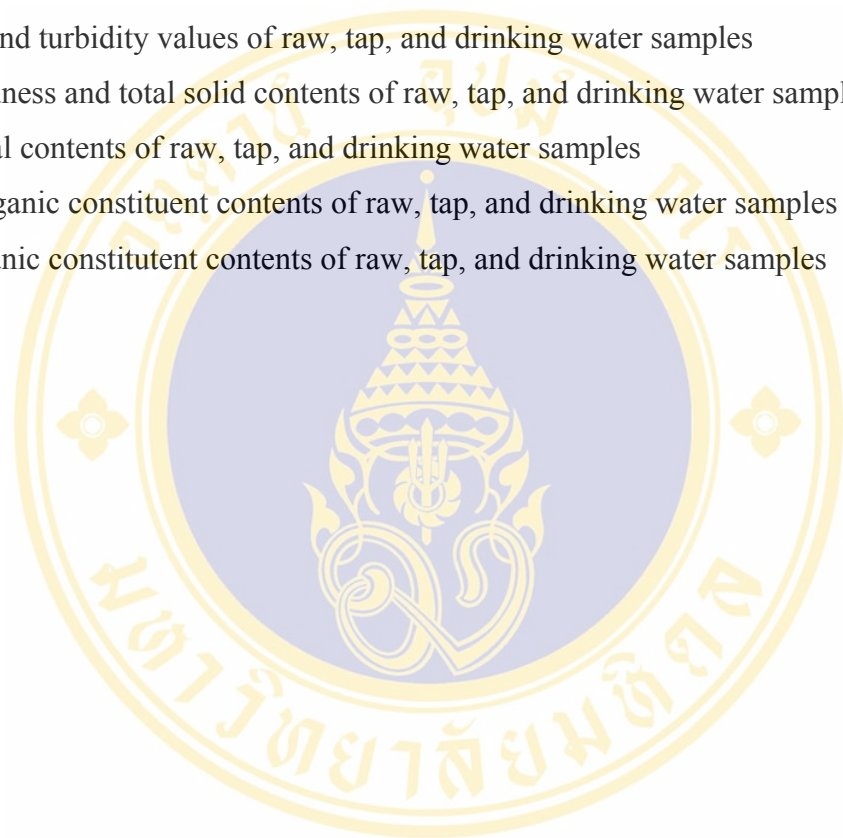
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CHAPTER I

INTRODUCTION

1.1 Statement of problem:

Water is essential to support life; and, therefore, every effort should be made to achieve as high quality drinking water as possible. Failure to do so exposes the population to the risk of disease, particularly the very young, the elderly, the sick, and those who live in a substandard sanitary condition [WHO, 1993]. Ideally, water destined for human consumption should be free from microorganisms; however, water which is microbiologically pure when it enters a water distribution system may become contaminated before it reaches the consumer's tap. Waterborne diseases are now known to be caused by a broader spectrum of microorganisms than by solely enteric bacteria. In recent years, there have been increasing reports of waterborne outbreaks largely as a result of protozoan in water [Subramanyam et al., 1989; Soliman, 1992; Nimri et al., 1994; Okafor et al., 1996; Yamazaki et al., 1997; Suárez-Hernández et al., 1997; Newman et al., 1999; Akyön et al., 1999; Leach et al., 2000; Solorzano-Santose et al., 2001; Iqbal et al., 2001].

Cryptosporidium species are protozoan parasites and have been detected in a wide range of water sources. *Cryptosporidium* infection in children has been reported in eastern India 1989 [Subramanyam et al., 1989]; rural Alexandria, Egypt 1992 [Soliman, 1992]; northern Jordan 1994 [Nimri et al., 1994]; Enugu State, Nigeria 1996 [Okafor et al., 1996]; Saitama Prefecture, Japan 1997 [Yamazaki et al., 1997]; Ciego de Avila Province, Cuba 1997 [Hernández et al., 1997]; northeastern Brazil 1999 [Newman et al., 1999]; a children's hospital in Ankara, Turkey 1999 [Akyön et al., 1999]; Texas-Mexico border 2000 [Leach et al., 2000]; rural Mexico 2000 [Solorzano-Santose et al., 2001]; and a hospital in Kuwait 2001 [Iqbal et al., 2001].

Because of the global presence of *Cryptosporidium* in water sources and because Bangkok has never had a waterborne outbreak of cryptosporidiosis, the school children of Bangkok may be at risk for exposure to *Cryptosporidium*, even though the

drinking water in Bangkok is known to be pure when it enters the water distribution system.

1.2 Purpose of study:

The purpose of this study was to determine if oocysts of *Cryptosporidium* species is present in the drinking water provided to primary school children in Bangkok.

1.3 Specific objectives:

The specific objectives of the study were:

- 1) to investigate the waterborne microbe and the microbiological contaminations in drinking water, and
- 2) to determine the physical and the chemical qualities of drinking water.

CHAPTER II

LITERATURE REVIEW

2.1 *Cryptosporidium*

2.1.1 Taxonomy

The protozoan *Cryptosporidium*, which means “hidden spore” in Greek, is classified as a eukaryote in the phylum Apicomplexa (possesses an apical complex), Class Sporozoasida (reproduces through both sexual- and asexual-cycle oocyst formations), Subclass Coccidiasina (involves a life cycle with merogony, gametogeny, and sporogeny), Order Eucoccidiida (produces schizogony), Suborder Eimeriina (develops both microgamety and macrogamety), Family Cryptosporidiida (contains 4 naked sporozoites within each oocyst, but the life cycle of *Cryptosporidium* is absent of sporocysts) [Fayer et al., 1990].

Like enteric coccidia found in vertebrates, *Cryptosporidium* has a monoxenous life cycle that is completed primarily within the gastrointestinal tract of a single host. However, many unique features distinguish *Cryptosporidium* from enteric coccidia, the most obvious of which are lack of both host and organ specificity, resistance to antimicrobial agents, ability for autoinfection, and curious location within the membrane of the host cell [Levine, 1985].

The taxonomy of *Cryptosporidium* at the species level is much less definitive and less widely agreed upon [Spano et al., 2000]. Originally, it was thought that 21 species existed [Levine, 1984]. However, after a series of transmission experiments comparing the 21 species, 10 have come to be recognised [Fayer et al., 2000; O’Donoghue, 1995; Tzipori et al., 2000] (Table 1). The current differentiation of isolates into valid species is based on (a) host specificity, (b) oocyst morphology, and (c) site of infection [Tzipori et al., 2000].

Table 1. Species of *Cryptosporidium*

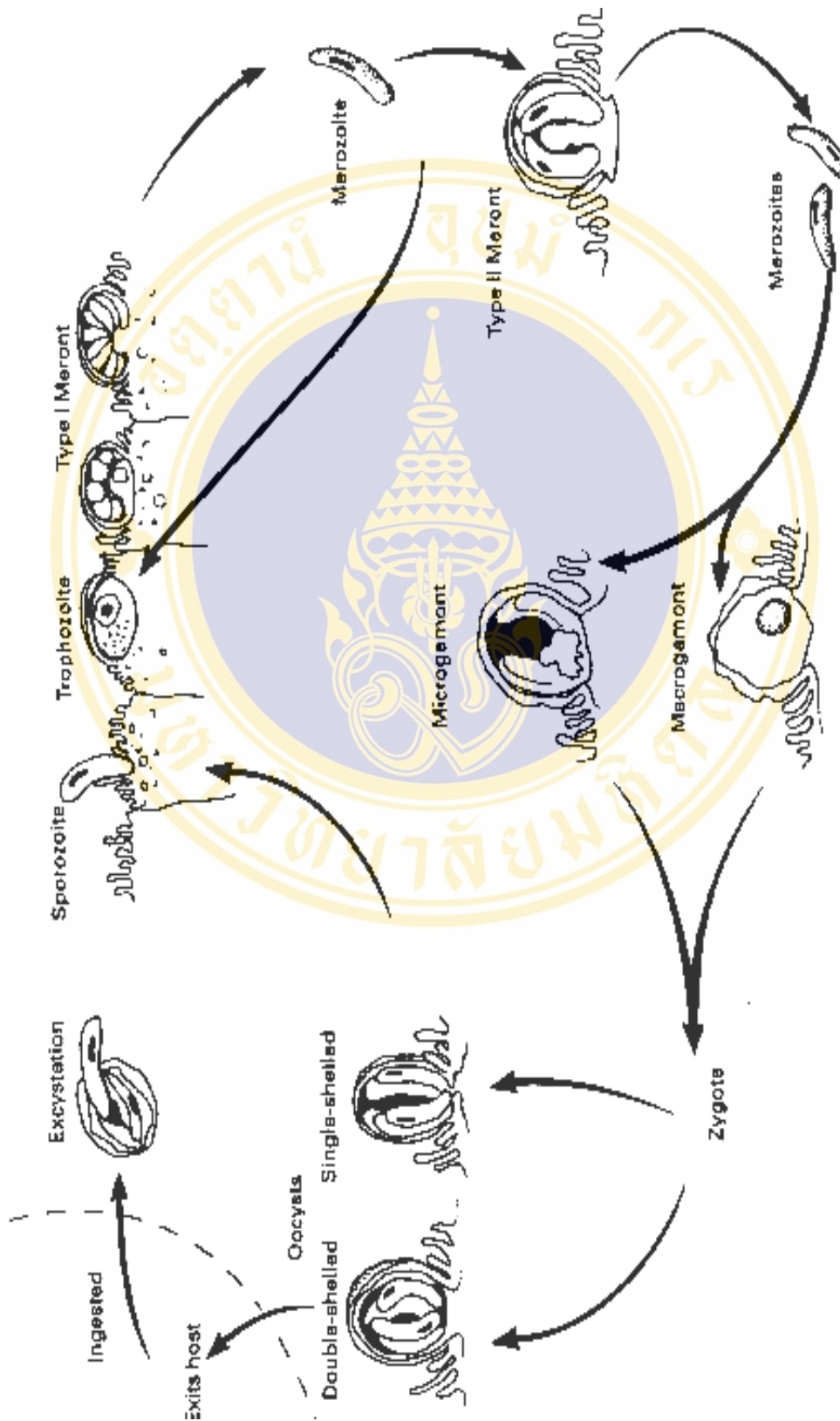
Species name	Common host
<i>C. andersoni</i>	cattle
<i>C. baileyi</i>	chicken
<i>C. felis</i>	cat
<i>C. meleagridis</i>	turkey
<i>C. muris</i>	mouse
<i>C. nasorum</i>	fish
<i>C. parvum</i>	mouse
<i>C. saurophilum</i>	skink
<i>C. serpentis</i>	reptile
<i>C. wrairi</i>	guinea pig

Note: Adapted from Fayer, Morgan & Upton.
Epidemiology of *Cryptosporidium*: transmission, detection
and identification. Int J Parasitol 2000;30:1305-22.

2.1.2 Life Cycle

The life cycle of *Cryptosporidium* (Figure 1) begins with the ingestion of oocysts by the host, followed by 4 sporozoites spontaneously being released from a single oocyst inside the host. Each of these sporozoites reaches and attaches to the epithelial microvillous border of the small intestine; and, after internalization by the host cell's microvillous membrane, the sporozoites become trophozoites. Three divisions of the nucleus within a trophozoite result in 8 first-generation merozoites, and the trophozoite then is classified as a Type I meront. Each of the 8 merozoites either recycles to form another Type I meront, or to form a Type II meront. The Type II meront has only 2 nuclear divisions, resulting in 4 second-generation merozoites. Half of the second-generation merozoites in the Type II meront presumably form either a microgamont which may contain up to 16 microgametes, or a macrogamont which contains a single macrogamete. The fertilization of the macrogamete by microgametes results in the formation of a zygote. While still in the host, two types of sporulated oocysts are developed from the zygote. The majority of sporulated oocysts are released into the faeces as double-shelled and environmentally-resistant oocysts,

while a minority are single-shelled and initiate the life cycle within the host [Tzipori et al., 2000].



(Illustration: Eric Peterson, Tufts University School of Veterinary Medicine).
Figure 1. Life cycle of Cryptosporidium

Cryptosporidium infections have now been recorded in over 170 different host species in tropical to temperate zones, representing over 50 countries throughout the world. All infections presumably have been acquired from the ingestion or the inhalation of infective oocysts which had been excreted by infected hosts. Prior to its description in humans, *Cryptosporidium* was regarded exclusively as an animal pathogen. One- to three-week-old domesticated animals (i.e., piglets, calves, lambs, and kids) are commonly infected with *Cryptosporidium* and either remain asymptomatic or develop a clinical illness that is either self-limiting or fatal to the host [Tzipori et al., 1983].

Cryptosporidium was initially thought to be pathogenic for immunocompromised persons and only a rare cause of disease in immunocompetent individuals. *Cryptosporidium* now is recognised worldwide as a leading protozoal cause of diarrhoea. One study of the infection included persons of all ages, including a 3-day-old infant and a 95-year-old grandparent, both of whom were immunocompetent [Bossen et al., 1985].

The prevalence of human cryptosporidiosis, however, is not yet known, but the infection appears to be more prevalent in developing countries [Farthing, 2000]. Recent investigations of two day-care centers in the United States have documented prevalences of 36% and 43% [Alpert et al., 1986; Taylor et al., 1985]. It has been demonstrated epidemiologically that *Cryptosporidium* infections are now more common and widespread than previously had been thought. While serological surveys may give a better indication of the level of exposure within a given population, antibody titres are insufficient to distinguish either between recent and past infections or between clinical and asymptomatic infections [O'Donoghue, 1995].

2.1.4 Transmission

Cryptosporidium is spread by faecal matter from an infected host and is ingested orally by the recipient during the oocyst stage. Transmission can be animal-to-human or person-to-person, while the source of transmission can be waterborne (drinking water or recreational water), foodborne, and possibly airborne [Fayer et al., 2000].

Animal-to-human transmission has been reported by Current et al. (1983) and Pohjola et al. (1986) and various livestock have been implicated as sources of human infection. The high prevalence of cryptosporidiosis in neonatal calves, the close human contact during their care, and the human contact during the milking of the mother provide opportunities for transmission to farmers, handlers, and veterinarians.

Person-to-person transmission in hospitals, day-care centers, and households appears to be a major source of transmission from and to health care personnel, patients, child-care workers, children, and family members. *Cryptosporidium* is spread probably hand-to-mouth by bathing or cleaning an infected person, or by contact with contaminated clothing, bed linens, potty chairs, bed pans, toys, etc. [Fayer et al., 1990].

Faecal contamination of soil may lead to contamination of drinking water, recreational water, and fresh foods [Anguish et al., 1997; Walker et al., 1998]; however, due to the difficulties of detection and the enumeration of oocysts in soil samples, the movement of *Cryptosporidium* oocysts from faeces to soil has received little attention [Kuczynska et al., 1999].

Both drinking water and recreational water can serve as vehicles for transmission during the oocyst stage. Large-scale U.S.A. outbreaks of cryptosporidiosis in San Antonio, Texas [D'Antonio et al., 1985]; Carrolton, Georgia [Hayes et al., 1989]; and Milwaukee, Wisconsin [MacKenzie et al., 1994] have been associated with contamination of drinking water. As reported by Craun (1988), water used for recreational activities (i.e., swimming, water skiing, surfing, fishing, etc.) has also been a source of transmission for *Cryptosporidium* because of faecal contamination found in highly populated recreational areas as well as oocyst resistance to chlorine [Craun, 1988].

Food can be contaminated by *Cryptosporidium* oocysts via animal fertiliser, contaminated water used to irrigate or moisten produce, contaminated food packages, or poor hygiene of workers, produce handlers, or food handlers. In Costa Rica, *Cryptosporidium* oocysts were found on cilantro roots and leaves, lettuce, carrots, cucumbers, radishes, and tomatoes [Monge et al., 1996]. In United Kingdom, a *Cryptosporidium* infection outbreak, which included 50 school children, was

associated with milk processed by an on-farm pasteuriser [Gelletlie et al., 1997]; and in a Washington DC university, 4 infected cafeteria foodhandlers were responsible for 88 students being diagnosed with cryptosporidiosis [Quiroz et al., 2000].

Airborne transmission of the oocyst is recognised as a source of respiratory cryptosporidiosis because *Cryptosporidium* oocysts also have been found in the pulmonary systems of 6 Danish HIV-positive patients [Hojlyng et al., 1988]. Also, in another study, *Cryptosporidium* pulmonary infection was acquired by a veterinary scientist who was wearing gloves and protective clothing while caring for a calf that was infected with *Cryptosporidium* [Hojlyng et al., 1987].

2.2 Cryptosporidiosis

2.2.1 Human cryptosporidiosis

Cryptosporidium infections were first diagnosed in 1976 in two human patients, both with severe watery diarrhoea. One was an immunocompetent child [Nime et al., 1976]; the other, an immunocompromised adult [Meisel et al., 1976]. Two additional cases, both involving immunocompromised patients, occurred over the next two years and were reported in 1979 [Lasser et al., 1979; Weisburger et al., 1979], causing the scientific community to think *Cryptosporidium* to be a rare opportunistic pathogen. Then, in 1982, two cases of *Cryptosporidium* occurred – one case involving 12 immunocompetent persons who came in close contact with infected calves [Current et al., 1983]; the other, 21 HIV patients [Anon, 1982] – thereby confirming *Cryptosporidium* as a new human pathogen associated with both immunocompromised and immunocompetent patients.

2.2.2 Clinical presentation

Cryptosporidium is now recognized as a well-established cause of both acute and chronic diarrhoea in humans. However, both the severity and the duration of the illness depend on several host factors – the most important of which is the host's immune status, but also included are age, route of inoculation, etc. Undernutrition also appears to promote a more severe and prolonged diarrhoeal illness [MacFarlane et al., 1987].

2.2.2.1 *Cryptosporidiosis in immunocompetent humans*

Cryptosporidiosis in the healthy host is usually a self-limiting illness which may last only a few days or may persist as long as 4-7 weeks.

2.2.2.1.1 Adults

The most complete data on clinical presentation is provided in several studies that have been performed using immunocompetent individuals [Farthing, 2000] (Table 2). Wolfson et al. (1985) reported 28 patients with *Cryptosporidium* infection which had been identified by the Parasitology Laboratory of Massachusetts General Hospital. Jokipii et al. (1986), conducting a detailed investigation of the clinical histories and performing parasitological follow-ups of travellers in southern Finland who had been infected with *Cryptosporidium*, found the incubation period of infection to be 7.2 ± 2.4 days and also identified an average period of 6.9 days for asymptomatic carriage of the organism after resolution of symptoms. In the well-documented Milwaukee outbreak of 1993, MacKenzie et al. (1994) found that 93% of those infected had watery diarrhoea, and more than 80% had abdominal cramps and anorexia. Therefore, based on the 3 foregoing studies [MacKenzie et al., 1994; Wolfson et al., 1985; Jokipii et al., 1986] and that of Farthing

Table 2. Clinical features of laboratory-confirmed cryptosporidiosis in immunocompetent individuals

Clinical feature	Wolfson et al. 1985 n=28	Jokipii et al. 1986 n=50	MacKenzie et al. 1994 n=285
Diarrhoea	24(86)	50(100)	285(100)
Watery diarrhea	24(86)	NR	265(93)
Abdominal cramps	5(18)	44(88)	238(84)
Fatigue	NR	NR	247(87)
Anorexia	5(18)	NR	230(81)
Weight loss	3(11)	NR	150/200(75)
Nausea	3(11)	NR	199(70)
Vomiting	1(4)	NR	136(48)
Cough	NR	NR	68(24)
Fever	3(11)	30(60)	162(57)

Notes: 1) Figures in parentheses are percentages, 2) n=number of observations 3) NR=not reported
4) Adapted from Farthing MJG. Clinical aspects of human cryptosporidiosis. In: Petry F, editor. Cryptosporidiosis and microsporidiosis. Basel(Switzerland): Karger; 2000. p. 50-74.

(2000), who conducted a comprehensive literature review, it can be concluded that there are 3 major presentations of cryptosporidiosis in the immunocompetent adult: (1) acute diarrhea which is usually watery, (2) persistent diarrhea which may continue for several weeks, and (3) asymptomatic carriage.

2.2.2.1.2 Infants and children

Cryptosporidiosis is rare among breast-fed infants. In one perinatal patient, a *Cryptosporidium* infection apparently had been acquired from a symptomatic infected mother; however, the infant behaved neither as an immunocompromised individual nor as a patient with cryptosporidiosis, but as an asymptomatic carrier [Lähdevirta et al., 1987].

High morbidity and mortality in infants and children in the developing world almost certainly relates to both background undernutrition and its effect on the host's immune response. Persistent cryptosporidiosis in infants and young children contributes to impaired growth and development [Molbak et al., 1997]. Studies in Venezuela [Perez-Schael et al., 1985], United Kingdom [Isaacs et al., 1985], and Ghana [Addy et al., 1986] clearly indicate that, while asymptomatic infection occurs in children, both acute and chronic diarrhoea due to *Cryptosporidium* often result in high morbidity and mortality. A West Africa study of 1,064 children less than 3 years of age showed that cryptosporidiosis acquired by an infant results in significant weight loss and impaired linear growth, suggesting that cryptosporidiosis acquired in early life can have a permanent effect on growth and development [Molbak et al., 1997].

2.2.2.2 *Cryptosporidiosis in immunocompromised humans*

Cryptosporidiosis in the immunocompromised individual has been studied most extensively in patients with HIV infection. Two differences in clinical presentation contrast the immunocompromised and the immunocompetent host, i.e., severity and chronicity of the diarrhoeal disease in the former [Blanshard et al., 1992; Flanigan et al., 1992; Guarino et al., 1997] and possible extra-enteric manifestations in the latter [Quiroz et al., 2000; Blanshard et al., 1992; Berk et al., 1984; Godwin, 1991; Gross et al., 1986; Hasan et al., 1991; Vakil et al., 1996; Kline et al., 1993; Kocoshis et al., 1984; Travis et al., 1990].

2.2.2.2.1 Severity and chronicity

Blanshard *et al.* (1992) studied *Cryptosporidium* infection in 128 HIV-infected patients. They identified 4 clinical patterns of the disease in the following order of frequency: (1) chronic diarrhoea which lasts 2 months or more with persistence of the *Cryptosporidium* in stool and/or biopsy specimens; (2) transient infection in which the diarrhoea lasts less than 2 months and there is a complete remission of symptoms and a loss of *Cryptosporidium* from faecal specimens occur; (3) fulminant infection in which the patient passes daily at least two litres of watery stools; and (4) asymptomatic infection in which the patient has no change in bowel habit and passes daily less than 3 stools.

A study in the U.S.A. (Cleveland, New York, Atlanta, San Diego, and Philadelphia) has shown the relationship between the severity of the intestinal cryptosporidial infection and survival. In this study, 8 HIV-infected patients with cryptosporidiosis and CD4 counts of 180 cells/mm³ or more spontaneously cleared the infection within 4 weeks. In contrast, of 39 patients with CD4 counts of less than 140 cells/mm³, 34 (87%) developed persistent disease [Flanigan *et al.*, 1992].

Immunocompromised children usually have protracted diarrhoea with dehydration and severe weight loss [Guarino *et al.*, 1997].

2.2.2.2.2 Extra-enteric manifestations

By the early 1990s, it had been well established that *Cryptosporidium* infection in the immunocompromised host can involve esophagus, gastrointestinal tract, hepatic bile duct, pancreas, pancreatic duct, stomach, and intestine. [Berk *et al.*, 1984; Godwin *et al.*, 1991; Gross *et al.*, 1986]. In addition, numerous *Cryptosporidium* were seen attached to biliary epithelium of a 41-year-old white homosexual man who had been admitted to the Veterans Administration Hospital in Miami [Hasan *et al.*, 1991]. In the 1993 Milwaukee outbreak, of the 24 patients who had been identified as having biliary symptoms (8.42%), only 4 were alive at one year [Vakil *et al.*, 1996]. Kline *et al.* (1993) found extensive squamous metaplasia of the bile duct epithelium of a patient who had a chronic *Cryptosporidium* infection.

There is clear evidence that *Cryptosporidium* is detectable in sputum [Kocoshis et al., 1984], bronchial aspirates [Travis et al., 1990], and bronchial epithelium [Blanshard et al., 1992]. Respiratory involvement is characterised by cough, dyspnoea, and fever and is often associated with other respiratory pathogens including cytomegalovirus, *Pneumocystis carinii*, and *Mycobacterium*. This multiple-infection presentation makes it difficult to evaluate the significance of pulmonary cryptosporidiosis [Hojlyng et al., 1988].

2.2.3 Diagnosis

2.2.3.1 Microscopic staining techniques

In 1995, O'Donoghue, in a review of the literature, compiled many techniques that were being used to stain the walls and/or the contents of *Cryptosporidium* oocysts [O'Donoghue, 1995] (Table 3). Direct staining techniques (e.g., safranin-methylene blue stain [Baxby et al., 1984] and acid-fast stains [Henricksen et al., 1981; Ma et al., 1983]) are time consuming and vary in sensitivity and specificity [Smith et al., 1989]. Of the direct staining techniques, the fluorochrome [MacPherson et al., 1993] are more sensitive, but also more complex; and, therefore, the oocyst-like structure in faecal debris often takes up the stain. Negative staining techniques with iodine [Ma et al., 1983], methanamine silver [Angus et al., 1981], periodic acid-Schiff [Garcia et al., 1983], and phosphotungstic acid [Baxby et al., 1984] stain background yeasts, but not oocysts.

2.2.3.2 Immunologically-based techniques

Through the use of immunoserological test procedures, specific antibodies against *Cryptosporidium* have been detected in host serum samples. In 1981, the first *Cryptosporidium* specific antibody test which employed an indirect fluorescent technique was developed by Tzipori et al. (1981). More recently, immunologically-based techniques including a polyclonal fluorescent antibody assay [Stibbs et al., 1986], a monoclonal fluorescent antibody (mAbs) assay [Garcia et al., 1987], an enzyme-linked immunosorbent assay (ELISA) [Ungar, 1990], a reverse

Table 3. Staining techniques for *Cryptosporidium* oocysts

Technique	Appearance of oocysts	Appearance of yeasts
Direct staining		
Giemsa	blue	blue
Gram	red	purple
Kohn's stain	dark green	grey
Methylene blue	light blue	dark blue
Aniline-carbolmethyl violet	blue	unstained
Safranin+Methylene blue	orange	blue
Acid-fast stains		
Kinyoun	red	counterstained
Ziehl-Neelsen	red	counterstained
DMSO-carbol fuchsin	red	counterstained
Fluorochrome staining		
Auramine-rhodamine	orange	unstained
Auramine-carbol fuchsin	orange	unstained
Acridine orange	orange/green	orange
Diamidino-phenylindole	blue	unstained
Mepacrine	orange	unstained
Propidium iodide	red	unstained
Negative staining		
Carbol fuchsin	unstained	blue
Iodine	unstained	brown
Light green	unstained	green
Merbromine	unstained	orange
Methanamine silver	unstained	black
Nigrosin	unstained	unstained
Periodic acid-Schiff	unstained	red
Phosphotungstic acid	light brown	black
Uranyl acetate	light brown	black

Note: Adapted from O'Donoghue PJ. *Cryptosporidium* and cryptosporidiosis in man and animals. Int J Parasitol 1995;25(2):139-95.

passive haemagglutination (RPH) assay [Farrington et al., 1994], and a solid-phase qualitative immunochromatographic assay [Garcia et al., 2000] have been developed for the detection of cryptosporidiosis.

Due to possible crossreactivity between *Cryptosporidium* oocysts and other microorganisms, non-specificity of antibody-based methods used for the detection of *Cryptosporidium* can be problematic. For example, in a study of gills and haemolymphs in oysters that harboured *Cryptosporidium* oocysts, a variety of multi-sized and multi-shaped organisms and particulate material were observed fluorescing as bright as the oocysts [Fayer et al., 1999].

2.2.3.3 Molecular techniques

The polymerase chain reaction (PCR) technique has provided the basis for the development of a molecular technique for *Cryptosporidium* diagnostics. The advantages of PCR for the detection of *Cryptosporidium* are: sensitivity, ease to use, relatively low cost, ability to speciate, strain-typing potential, and the ability to analyse simultaneously large numbers of samples [Morgan et al., 1998].

Immunomagnetic separation (IMS) has been used in PCR-based techniques to increase high sensitivity [Johnson et al., 1995]. Problems, particularly crossreactivity and antigenic variability of *Cryptosporidium* isolates, are associated with antibody detection and the process of PCR and IMS coupling [Clancy et al., 1994]. In addition, turbid solutions and environmental waters that contain iron compounds inhibit the affinity of magnetic particle antibodies [Newman, 1995].

The nested PCR technique has been applied to *Cryptosporidium* to increase both specificity and sensitivity [Meyer et al., 1996], and appears to be the ideal method for a rapid screening of large numbers of samples as this method has high reproducibility, technique simplicity, and cost effectiveness [Monis et al., 2001].

By the late 1990s, a reverse transcription PCR (RT-PCR) technique had been developed for detecting low numbers of viable *Cryptosporidium* oocysts that had been spiked into environmental water [Stinear et al., 1996; Kaucner et al., 1998].

2.2.3.4 Oocyst infectivity and viability determination techniques

Because a common PCR technique can not determine the viability of oocysts and because live organisms carry out transcription to form messenger

ribonucleic acid (mRNA), the RT-PCR technique must be used to evaluate the viability of oocysts via the detection of mRNA [Morgan et al., 1998].

Vital dyes – propidium iodide (PI) and 4, 6, diamino-2'-phenylindole (DAPI) – which overestimate the viability of *Cryptosporidium* oocysts have been reported [Black et al., 1996]. In vitro excystation is an inaccurate measure of both viability and infectivity because oocysts that failed to excyst in vitro were found to be infectious in vivo [Neumann et al., 2000]. Furthermore, sporozoites that appear viable after having been excysted from oocysts can be non-infectious [Fayer et al., 1998]. In addition, other molecular tests developed for viability testing include fluorescent in situ hybridisation (FISH) [Vesey et al., 1998] and cell culture followed by RT-PCR [Di Giovanni et al., 1999; Rochelle et al., 1999].

2.2.4 Treatment

At present, there is no effective treatment available for cryptosporidiosis. Supportive care – with (1) oral liquids and/or intravenous solutions for the replacement of fluids and electrolytes, (2) nutritional support, and (3) antidiarrhoeal drugs – is beneficial in alleviating the dehydration that accompanies acute diarrhoea while awaiting spontaneous recovery [O'Donoghue, 1995].

2.2.4.1 General supportive therapy

2.2.4.1.1 Fluid and electrolyte replacement

The only treatment for cryptosporidiosis in the immunocompetent patient is supportive care, consisting of rehydration for repletion of electrolyte loss by either an oral or an intravenous route. The severe diarrhoea of up to 20 litres a day that occurs in patients with AIDS often requires intensive support. Aggressive efforts at oral rehydration should be administered with a rehydration solution which contains glucose, sodium bicarbonate, and potassium. If the patient is unable to receive orally food and rehydration solutions, intravenous replacement of fluids and electrolytes is required to correct loss of bicarbonate, potassium, magnesium, and phosphorus [Flanigan et al., 1993].

2.2.4.1.2 Nutritional support for the immunocompromised patient

Weight loss and malnutrition are common features in patients with severe cryptosporidial diarrhoea. In nutritional research for the HIV-infected person, it has been difficult to demonstrate if the clinical outcome is altered significantly when nutritional supplements have been provided oro-enterally [Farthing, 2000].

Diet should be individualised to fit each patient's preference, but fatty foods and dairy products should be avoided. The patient should be offered oral nutritional supplements in liquid form because they are easier than solid foods to ingest and to assimilate. Oral supplements may be administered appropriately by feeding slowly via either an intragastric or a duodenal fine-bore feeding tube, and should be considered before resorting to intravenous nutrition [Farthing, 2000].

2.2.4.1.3 Antidiarrhoeal drugs

Natural and synthetic opiates, which alter intestinal motility, often are helpful for the immunocompetent patient. Loperamide and codeine phosphate are first-line antidiarrhoeal drugs, but are ineffective against *Cryptosporidium* in an HIV patient [Masuda et al., 1991]. Case reports and uncontrolled pilot studies have showed that somatostatin and its analogues (octreotide and vapreotide), when used for persistent AIDS-related diarrhoea, have either limited success or no therapeutic effect [Cello et al., 1991; Girard et al., 1992].

However, the antidiarrhoeal drug acetorphan acts as an enkephalinase inhibitor by increasing the bioavailability of endogenous enkephalin, the antidiarrhoeal properties of which are mediated through opiate receptors [Turvill et al., 1997]. A preliminary study of AIDS patients who had refractory diarrhoea suggests that acetorphan may be a useful alternative to octreotide in the management of refractory diarrhoea in AIDS patients [Beaugerie et al., 1996].

2.2.4.2 Antimicrobial chemotherapy

More than 100 antimicrobial agents, the majority of which were suppressive and not curative, have been tested for efficacy against cryptosporidiosis in humans, but with no significant success [Farthing, 2000].

An early report indicated that the macrolide antibiotic spiramycin may be effective [Portnoy et al., 1984]; but two double-blind, placebo-controlled, clinical trials since have indicated that oral or intravenous treatment had little or no effect on a *Cryptosporidium* infection either in infants [Wittenberg et al., 1989] or in AIDS patients [Flanigan et al., 1993].

An aminoglycoside antibiotic, paramomycin, may be an effective agent in the treatment of cryptosporidiosis. However, positive effects of paramomycin are short-lived in some patients [Fichtenbaum et al., 1993].

More recently, as reported by Doumbo et al. (1997), several studies have suggested that nitazoxanide is active against *Cryptosporidium*, both in vitro and in vivo; and, in addition, nitazoxanide is effective against a wide range of other parasites including other protozoa, nematodes, cestodes, trematodes, anaerobic obligate and facultative bacteria, and some aerobic bacteria.

Kelly et al. (1998) studied the efficacy of albendazole in 94 AIDS patients at the University Teaching Hospital, Lusaka, Zambia, finding that albendazole eradicated completely some *Cryptosporidium* infections [Kelly et al., 1998].

In an uncontrolled study of 24 AIDS patients with cryptosporidial diarrhoea, treatment with roxithromycin produced symptomatic improvement for the most adverse effects which were abdominal pain and elevated hepatic enzymes [Sprinz et al., 1998].

Clarithromycin and rifabutin prophylaxis are highly protective agents against cryptosporidiosis in immunocompromised patients [Holmberg et al., 1998].

2.2.4.3 Immunotherapy

2.2.4.3.1 Passive immunotherapy

Using hyperimmune bovine colostrum (HBC), Tzipori et al. (1987) successfully treated 3 patients with persistent cryptosporidial diarrhoea. All 3 patients recovered within 3-5 days and remained free of diarrhoea. In addition, HBC in powder form appears promising in the treatment of severe cryptosporidial diarrhoea because HBC has no side effects; but the optimal dosage, duration of therapy, and efficacy are yet to be fully determined [Greenberg et al., 1996].

In 1990, there was a study which showed that oral immune bovine dialyzable leukocyte extract (DLE) induced a favorable effect on the course of cryptosporidiosis in patients with AIDS. DLE caused improvement in the consistency of stools, the frequency of bowel movement, and the body weight in 6 of 7 patients [McMeeking et al., 1990].

2.2.4.3.2 Combination antiretroviral therapy

Treatment with protease inhibitors – when combined with nucleoside analogues – (1) clear spore sheddings in stools, (2) induce lasting remission of microsporidiosis in a patient with an HIV infection, and (3) facilitate clinical improvement with cessation of diarrhoea, all of which normally contribute to a gain in weight [Goguel et al., 1997]. In addition, protease inhibitors, when combined with non-nucleoside analogues, can suppress viral replication and increase CD4 cell counts [Palella et al., 1998].

Several studies have confirmed that highly active antiretroviral therapy (HAART), when combined with protease inhibitors, improves cryptosporidiosis-related diarrhoea in HIV patients [Carr et al., 1998; Foudraine et al., 1998; Le Moing et al., 1998].

2.2.5 Prevention and Control

The key, both for the prevention of and for the control of *Cryptosporidium*, is preferably to eliminate but at least to reduce exogenous oocysts from the environment. It is paramount that veterinary, medical, and laboratory personnel avoid contact with material contaminated with *Cryptosporidium* oocysts. For patient care, standard enteric precautions by health-care personnel are indicated to include handwashing, using gloves and gowns, and providing private rooms for patients with poor hygiene. Potentially contaminated equipment should be sterilised [Fayer et al., 1986].

There are possibly 20 commercial disinfectant which are able to inactivate *Cryptosporidium* oocysts [Campbell et al., 1982] (Table 4). In another study, Peeters et al. (1989) indicated that the 2 most effective agents for the elimination of oocysts are chlorine dioxide and ozone. The treatment of water

containing 10^4 oocysts/ml with 0.4 mg of chlorine dioxide/L significantly reduced infectivity within 15 min of contact. Also, 1.11 mg of ozone/L for 6 min totally eliminated the infectivity of oocysts for neonatal mice.

Table 4. Disinfectants tested for the eradication of *Cryptosporidium* oocysts

Disinfectant	Concentration (%)
Ammonia	1.0, 5.0
Benzylkonium chloride	5.0
Campden solution	0.5
Chloramine B	3.0
Cresylic acid	3.0
Ethanol	3.0
FAM 30	1.0
Formaldehyde	1.0, 5.0
Formol saline	10.0
Glutaraldehyde	2.0
Hypochlorite	1.0
Hypochlorite pH7.6	10.0
Iodophore	4.0
Isopropanol	90.0
Lysol	4.0
Paracetic acid	0.2
Phenol	1.0
Potassium permanganate	1.0
Propanol	90.0
Providone iodine	10.0

Note: Adapted from Campbell I, Tzipori AS, Hutchison G, Angus KW.
Effect of disinfectants on survival of *Cryptosporidium* oocysts.
Vet Rec 1982;111:414-5.

2.3 Water Quality Standard

2.3.1 Raw Water Quality Standard

Raw water quality is the one important factor for drinking water production. Raw water quality which is lower than source water class 3 [National Environmental Board, 1994] will effect to the quality of drinking water and consumer's appreciation. In addition, the cost of drinking water production will be higher.

There are 2 major raw water sources for Thailand drinking water production:

- 1) Chao Phraya River, Pathumthani Province
- 2) Tha Chin River, Nakhon Pathom Province

2.3.1.1 Water Supply Restricted Zone

There are 2 restricted zones for protecting of the Bangkok Metropolitan Region (BMR) Water Supply

1) Restricted Zone for Protecting of the BMR Water Supply B.E. 2522 (1979) and 2531 (1988)

The Cabinet decided to protect the source of the Bangkok Metropolitan Region (BMR) Water Supply by expanding the restricted zone of Samlae Catchment area from 200 square kilometers to 350 square kilometers in accordance with the recommendation of NEB (National Environmental Board). This regulation requires that in the restricted zone, the building an expansion of factories which discharge wastewater containing toxic substances listed below or organic matter exceeding one kilogram of BOD per day, will not be permitted. The mentioned toxic substances include

- a) Heavy metal : Zinc, Chromium, Copper, Mercury, Manganese, Cadmium, Lead, Nickel, Barium, and Iron
- b) Other Toxic Substances : PCB(Polychlorinated biphenyl), Cyanide, Arsenic , Selenium, Phenols, etc.

Reference: The Cabinet Resolution dated April 17, B.E. 2522 (1979) and January 12, B.E.2531 (1988)

2) Restricted Zone for Protecting of BMR Water Supply at the Western part of the Chao Phraya River B.E.2535 (1992)

The cabinet decided to protect the sources of the Bangkok Metropolitan Region (BMR) Water Supply by expanding the restricted zone of the western part of the Chao Phraya River which effects water quality at Klong Mahasawas and the Thachin River which have an area of 485 square kilometers, on recommendation of NEB. The areas covered Bangkok Metropolitan, Nontaburi Province and Nakornpatom Province. The pollution sources controls are as follows:

- a) Industrial activities containing high concentration of toxic substances are not permitted to be established and/or expanded
- b) Domestic wastewater must be treated to meet the standards
- c) Agricultural waste must be treated, which include highly polluted sources, such as fish ponds and swine farms

Reference: The Cabinet Resolution dated February 11, B.E. 2535 (1992)

2.3.1.2 MWA Raw Water Quality

Production planning and quality control department monitors the quality of raw water every month. In 2002, MWA reported that the raw water quality of Chao Phraya river (Table 5) was lower than the surface water class 3 standard. The problems of Chao phraya river for drinking water production are as follows:

- 1) high value and high variable of turbidity,
- 2) wastewater drainage from Klong Chiang Rak via Klong Oom,
- 3) agriculture flood drainage from Ang-Thong, Pra Nakhon Si Ayuthya and Pathumthani provinces.

MWA requests the Royal Irrigation Department to close Klong Chiang Rak Yai water gate to protect wastewater pass through Chao Phraya River and increase water entering from Bhumibhol, Sirikitt and Pasak Cholasith Dams to dilute and pull out poor raw water through river mouth. These solutions make raw water quality is better since January 2002 but remain lower than the surface water class 3 standard.

Table 5. Chao Phraya River Water Quality Standards (2003)

Parameters	Unit	Oct 02	Nov 02	Dec 02	Jan 03	Feb 03	Mar 03	Apr 03	May 03	Jun 03	Jul 03	Aug 03	Sep 03	Max.	Min.	Ave.	Std.
Date		1	4	2	6	4	4	3	12	11	9	4	10	-	-	-	-
Time		10.40	9.55	9.00	9.20	20.00	9.52	10.47	10.10	10.03	10.35	12.00	10.00	-	-	-	-
Temp.	°C	30.2	30.5	29.4	27.4	26.6	29.9	31	32.2	31.5	30.2	29.9	29.7	32.2	26.6	29.9	-
Colour	Pt-Co	-/-	-/-	8/-	19/-	6/-	-/-	9/-	8/-	7/-	4/-	8/-	-	29/-	1/-	-	-
Odour		high objectionable		less objectionable		high objectionable	less objectionable		high objectionable	less objectionable		less objectionable	objectionable			-	
Turbidity	NTU	84.1	58.5	39.1	24.3	137.0	93.7	54.0	116.0	88.5	101.0	122.0	145.0	145.0	24.3	88.6	-
pH		7.07	6.98	7.28	7.31	7.36	7.54	7.47	7.58	7.47	7.54	7.53	7.46	7.58	6.98	7.38	5.0-9.0
Total solid	mg/L	181	196	176	208	330	259	187	353	279	262	306	227	353	176	247	-
Hardness	mg/L	90	58	94	100	128	126	84	122	94	88	108	114	128	58	101	-
Chloride	mg/L	5	5	7	14	15	8	2	15	6	10	16	8	16	2	9	-
Sulfate	mg/L	1	1	8	1	1	13	5	19	10	19	26	2	26	1	9	-
Nitrate(as NO ₃ ⁻)	mg/L	0.155	0.189	0.284	0.373	0.663	0.496	0.359	0.528	0.272	0.396	0.459	0.378	0.663	0.155	0.379	5.0
Iron	mg/L	2.41	1.21	1.13	1.26	1.97	1.83	1.13	2.23	1.76	1.83	1.87	1.94	2.41	1.13	1.71	-
Fluoride	mg/L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.06	0.00	0.01	-
Manganese	mg/L	0.05	0.06	0.09	0.08	0.03	0.08	0.06	0.06	0.06	0.06	0.03	0.08	0.09	0.03	0.06	1.0
Mercury	mg/L	ND	ND	-	-	-	-	-	ND	-	-	-	-	ND	ND	ND	0.002
Arsenic	mg/L	ND	-	-	-	-	-	-	ND	-	-	-	-	ND	ND	ND	0.01
Copper	mg/L	ND	-	-	-	-	-	-	ND	-	-	-	-	ND	ND	ND	0.1
Zinc	mg/L	ND	-	-	-	-	-	-	ND	-	-	-	-	ND	ND	ND	1
Lead	mg/L	ND	-	-	-	-	-	-	ND	-	-	-	-	ND	ND	ND	0.05
Chromium	mg/L	ND	-	-	-	-	-	-	ND	-	-	-	-	ND	ND	ND	0.05
Cadmium	mg/L	ND	-	-	-	-	-	-	ND	-	-	-	-	ND	ND	ND	0.005
Total Coliform	MPN/100 mL	50,000	3,000	2,300	5,000	2,300	2,300	2,200	30,000	7,000	50,000	1,700	2,300	50,000	1,700	13,175	20,000
<i>Escherichia coli</i>	MPN/100 mL	1,700	1,700	400	2,300	2,300	800	800	2,200	5,000	14,000	1,100	800	14,000	400	2,758	4,000

Note: Pt-Co = Platinum Cobalt Scale

NTU = Nephelometric Turbidity Unit

Std. = Standard of surface water class 3 as Notification of the Ministry of Industry, No. 4, B.E. 2521 (1978), issued under the Ground Water Act B.E. 2520 (1977), published in the Royal Gazette, Vol. 95, Part 66, dated June 27, B.E. 2521 (1978).

For Tha Chin river quality, MWA reported that the raw water quality (Table 6) was as same as surface water class 5 due to the agriculture waste. To produce drinking from Tha Chin river, it needs more process such as put stop log to increase oxygen, add carbon charcoal to eliminate tri-halomethane which is

formed from inorganic substance and chlorine, this substance is harmful for health and cause high cost for drinking water production.

Table 6. Tha Chin River Water Quality Standards (2003)

Parameters	Unit	Oct 02	Nov 02	Dec 02	Jan 03	Feb 03	Mar 03	May 03	Apr 03	Jun 03	Jul 03	Aug 03	Sep 03	Max.	Min.	Ave.	Std.
Date		-	5	-	-	12	-	-	20	-	-	19	-	-	-	-	-
Time		-	11:10	-	-	10.55	-	-	10.36	-	-	11.50	-	-	-	-	-
Temp.	°C	-	25.5	-	-	27.6	-	-	31.3	-	-	31.3	-	31.3	25.5	28.1	-
Colour	Pt-Co	-	-	-	-	-	-	-	13/-	-	-	3/-	-	-	-	-	-
Odour		-	high objectionable	-	-	less objectionable	-	-	less objectionable	-	-	less objectionable	-	objectionable			-
Turbidity	NTU	-	-	-	-	-	-	-	-	-	-	-	-	23.7	16.5	19.3	-
pH		-	7.32	-	-	7.36	-	-	7.32	-	-	7.35	-	7.36	7.32	7.33	5.0-9.0
Total solid	mg/L	-	257	-	-	255	-	-	292	-	-	288	-	292	255	268	-
Hardness	mg/L	-	92	-	-	138	-	-	154	-	-	134	-	154	92	128	-
Chloride	mg/L	-	17	-	-	27	-	-	26	-	-	2	-	27	2	23	-
Sulfate	mg/L	-	20	-	-	1	-	-	32	-	-	18	-	32	1	18	-
Nitrate (as NO ₃)	mg/L	-	0.097	-	-	0.057	-	-	0.114	-	-	0.139	-	0.139	0.057	0.089	5.0
Iron	mg/L	-	0.89	-	-	1.03	-	-	0.59	-	-	0.67	-	1.03	0.59	0.84	-
Fluoride	mg/L	-	0.00	-	-	0.00	-	-	0.00	-	-	0.00	-	0.00	0.00	0.00	-
Manganese	mg/L	-	0.02	-	-	0.13	-	-	0.08	-	-	0.09	-	0.13	0.02	0.08	1.0
Manganese	mg/L	-	6.72	-	-	13.92	-	-	17.28	-	-	12.00	-	17.28	6.72	12.64	-
Mercury	mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.002
Arsenic	mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01
Copper	mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
Zinc	mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Lead	mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05
Chromium	mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05
Cadmium	mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.005
Total Coliform	MPN/100 mL	-	2,400	-	-	2,400	-	-	16,000	-	-	7,000	-	16,000	2,400	6,933	20,000
<i>Escherichia coli</i>	MPN/100 mL	-	900	-	-	1,600	-	-	2,400	-	-	5,000	-	5,000	900	1,633	4,000

Note: Pt-Co = Platinum Cobalt Scale

NTU = Nephelometric Turbidity Unit

Std. = Standard of surface water class 3 as Notification of the Ministry of Industry, No. 4, B.E. 2521 (1978), issued under the Ground Water Act B.E. 2520 (1977), published in the Royal Gazette, Vol. 95, Part 66, dated June 27, B.E. 2521 (1978).

2.3.2 Drinking Water Quality Standard

In assessing the quality of drinking water, the consumer relies completely upon his senses. Water constituents may affect the appearance, smell, or the taste of the water and the consumer will evaluate the quality and the acceptability essentially on these criteria. Water that is highly turbid, highly coloured, or has an objectionable taste will be regarded as dangerous and will be rejected for drinking purposes. However, we can no longer rely entirely upon our senses in the matter of quality judgement. The absence of any adverse sensory effects does not guarantee the safety of water for drinking.

Drinking water standards are regulations that each nation sets to control the level of contaminants in the nation's drinking water (Table 7). These standards includes assessing and protecting drinking water sources; protecting wells and collection systems; making sure water is treated by qualified operators; ensuring the integrity of distribution systems; and making information available to the public on the quality of their drinking water.

As to control water quality, MWA manages water quality at four stages in the process of clean water distribution (Sampling and testing at the water source, water treatment plant and distribution pumps are under the jurisdiction of MWA, but the Ministry of Public Health handles sampling and testing from the faucet in users' homes, with the cooperation of Mahidol University), namely at the water source, the water treatment plant, in conveyance and distribution, and at the faucet in the home. The Thai drinking water standard follows WHO standards (WHO Guideline 1993 for Drinking Water Standard). The quality of clean water treated by MWA was within the allowable Thai drinking water standard values (maximum values). Therefore there is no apparent problem with water quality.

Table 7. Global Drinking Water Quality Standards

Parameters	Units	WHO	TSI	EPA	Canada	EEC	S.Africa	Taiwan
1. Microbiological qualities								
<i>Escherichia coli</i>		ND				0 cfu		
Total Coliform		ND			10 cfu			<1 cfu
<i>Salmonella</i> spp.								
<i>Clostridium perfringens</i>								

Table 7. Global Drinking Water Quality Standards (cont'd)

Parameters	Units	WHO	TSI	EPA	Canada	EEC	S.Africa	Taiwan
<i>Staphylococcus aureus</i>								
<i>Campylobacter jejuni</i>								
<i>Listeria monocytogenes</i>								
<i>Yersinia enterocolitica</i>								
2. Physical qualities								
Colour	TCU	15	20 HU	15		15		15
Odour		agreeable	agreeable					
pH			6.5 - 8.5	6.5 - 8.5	6.5 - 8.5		6 - 9.5	6.5 - 8.5
Turbidity	NTU	5	5 SSC	1	1		0.5	4
3. Chemical qualities								
Hardness	mg/L		100			>60 as Ca	20 - 300	300
Total solids	mg/L	1,000	500	500	500			500
<i>Metals</i>								
Aluminum	mg/L	0.2	0.2	0.2 - 0.5		0.2	0.2	
Arsenic	mg/L	0.01	0.05	0.05	0.025	0.01	0.01	0.05
Barium	mg/L		1	0.7	1		0.7	
Cadmium	mg/L	0.003	0.005	0.005	0.005	0.005	0.01	0.01
Chromium	mg/L	0.05	0.05	0.1	0.05	0.05	0.05	0.05
Copper	mg/L	1	1	1 (AL)	1	2	0.5	1
Iron	mg/L	0.3	0.3	0.3	0.3	0.2	0.2	0.3
Lead	mg/L	0.01	0.05	0.015 (AL)	0.01	0.01	0.05	0.05
Manganese	mg/L	0.1	0.05	0.05	0.05	0.05	0.05	0.05
Mercury	mg/L	0.001	0.002	0.002	0.001	0.001	0.005	0.002
Selenium	mg/L		0.01	0.05	0.01	0.01	0.02	0.01
Silver	mg/L		0.05	0.1	0.05	0.01	0.1	0.05
Zinc	mg/L	3	5	5	5		1	5
<i>Inorganics constituents</i>								
Chloride	mg/L	250	250	250	250	250	250	250
Cyanide	mg/L		0.1					
Fluoride	mg/L	1.5	1.5	4	1.5	1.5	1	0.8
Nitrate (as No ₃ -)	mg/L	50	4	10	10	50	10	10
Sulfate	mg/L	250	250	250	500	250	200	250
<i>Organics constituents</i>								
ABS			0.2					
Phenol			0.001					

Noted: WHO = World Health Organization TSI = Thai Industrial Standards Institute
 EPA = United State Environmental Protection Agency
 Canada = Canadian Drinking Water Quality Guidelines
 EEC = European Economics Community S.Africa = South Africa, Umgeni
 Taiwan = Taiean, Taipei Drinking Water Regulations
 ND = Not detected in 100 mL sample cfu = Colony Forming Unit per 100 mL
 TCU = True Colour Unit
 HU = Hazen Unit SSC = Silica Scale Unit
 NTU = Nephelometric Turbidity Unit ABS = Alkylbenzene Sulfonate

2.3.3 Pipe

A majority of the water distributing pipes used in the Bangkok metropolitan area are made of asbestos (1997 MWA Annual Report). They are used because they are cheap and can be procured from domestic sources. However, they must be replaced with pipes of other materials in view of the environment, and also their inferior durability (Asbestos pipes are vulnerable to impact and have little shear strength. Bangkok has particularly weak soil and under such conditions asbestos pipes can easily lead to water loss, quite apart from their environmental problems.

2.3.3.1 Pipe and Tubing for Drinking Water Services

Ministry of Industry determined, under the provision of the industrial Product Standards Act, B.E. 2511 (1968), Standards for pipe and tubing for drinking water services as follows:

- 1) TIS 17-2532 (1989) Unplasticized Polyvinyl Chloride Pipes for Drinking Water Services which was published in the Government Gazette, Volume 106, Part 153 dated 14th September B.E. 2532 (1989). This standard supersedes TIS 17-2523;
- 2) TIS 910-2532 (1989) Polybutylene (PB) Pressure Pipe and Tubing for Drinking Water Services which was published in the Government Gazette, Volume 107, Part 12, dated 18th January B.E. 2533 (1990);
- 3) TIS 982-2533 (1990) High-Density Polyethylene Pipes for Drinking Water Services which was published in the Government Gazette, Volume 107, Part 127, dated 24th July B.E. 2533 (1990).

These standards specify classes, types and grades, sizes and tolerances, requirements, marking and labeling, sampling and criteria for conformity and testing.

2.3.4 Water Tank

Ministry of Industry determined, under the provision of the industrial Product Standards Act, B.E. 2511 (1968), Standards for pipe and tubing for drinking water services as follows:

1) TIS 238-2520 (1977) Galvanized Mild Steel Water Tanks which was published in the Government Gazette, Volume 95, Part 4 dated 10th January B.E. 2521 (1978);

2) TIS 435-2525 (1982) Reinforced Plastic Water Tanks which was published in the Government Gazette, Volume 99, Part 152 dated 18th October B.E. 2525 (1982);

3) TIS 989-2533 (1990) Stainless Steel Water Tanks which was published in the Government Gazette, Volume 107, Part 159 dated 30th August B.E. 2533 (1990);

4) TIS 1379-2539 (1996) Plastics Water Tanks which was published in the Government Gazette, Volume 113, Part 77 dated 24th September B.E. 2539 (1996).

These standards specify sizes and tolerances, materials, requirements, marking and labeling, sampling and criteria for conformity and testing.

2.3.5 Faucets

Ministry of Industry determined, under the provision of the industrial Product Standards Act, B.E. 2511 (1968), Standards for pipe and tubing for drinking water services as follows:

1) TIS 343-2523 (1980) Water Taps which was published in the Government Gazette, Volume 97, Part 106 dated 10th July B.E. 2523 (1980);

2) TIS 1277-2542 (1999) Faucets for Sinks which was published in the Government Gazette, Volume 116, Part 111 dated 29th December B.E. 2542 (1999). This standard supersedes TIS 1277-2538 (1995);

3) TIS 1278-2542 (1999) Faucets for Wash Basins which was published in the Government Gazette, Volume 116, Part 111 dated 29th December B.E. 2542 (1999). This standard supersedes TIS 1278-2538 (1995).

These standards specify sizes and tolerances, materials, requirements, marking and labeling, sampling and criteria for conformity and testing.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Definitions

Raw Water: water does not make safe by chemical or other treatment or water that is in its natural state; not yet changed, used or made into something else.

Tap Water: water supplied through pipes from MWA water treatment plants to taps/faucets in any schools.

Drinking Water: water that is safe to drink by students from the faucets in any schools.

3.1.2 Collection of samples

Raw and tap water samples were collected from the 4 water treatment plants in Bangkok i.e. Bangkhaen, Mahasawat, Samsen and Thonburi.

Drinking water samples were collected from 54 randomly selected public primary schools. These selected schools were telephone-interviewed to confirm that they are serviced by the 4 water treatment plants and did not pass through any filtration equipment.

Duplicate water samples (4 L each) were collected in sterile glass bottles and was transported in an unrefrigerated container to the Research Center, Ramathibodi Hospital, Mahidol University, Bangkok, where each sample was processed.

3.1.3 Sample size

In this study, results from pilot survey showed there was no *Cryptosporidium* found. Therefore, we calculated the sample size by randomly selecting 25% (54/216) schools from the listed got from the Office of the National Primary Education. This list did not base on the service of the MWA water treatment plants and Bangkhaen treatment plant produced for the most area in Bangkok

Metropolitan that is the reason why sample size of Bangkok is much more than the others.

The sample sizes in this study are as follow:

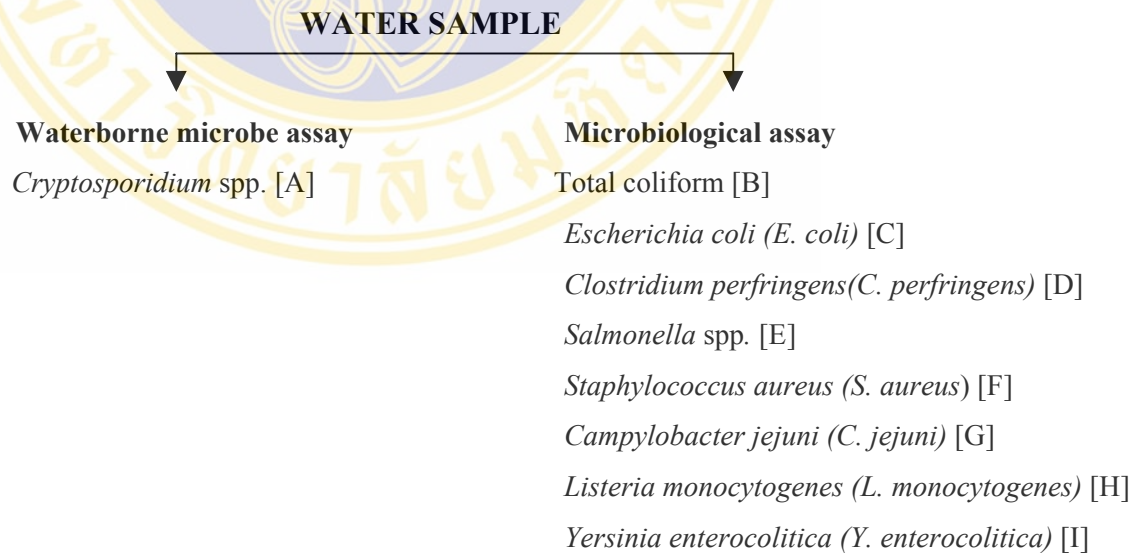
Water treatment plants	No. of schools
Bangkok	34
Mahasarakham	4
Samsen	6
Thonburi	10

However, MWA assured all 4 water treatment plants are set by the same standard which will not be different between plant.

3.2 Methods

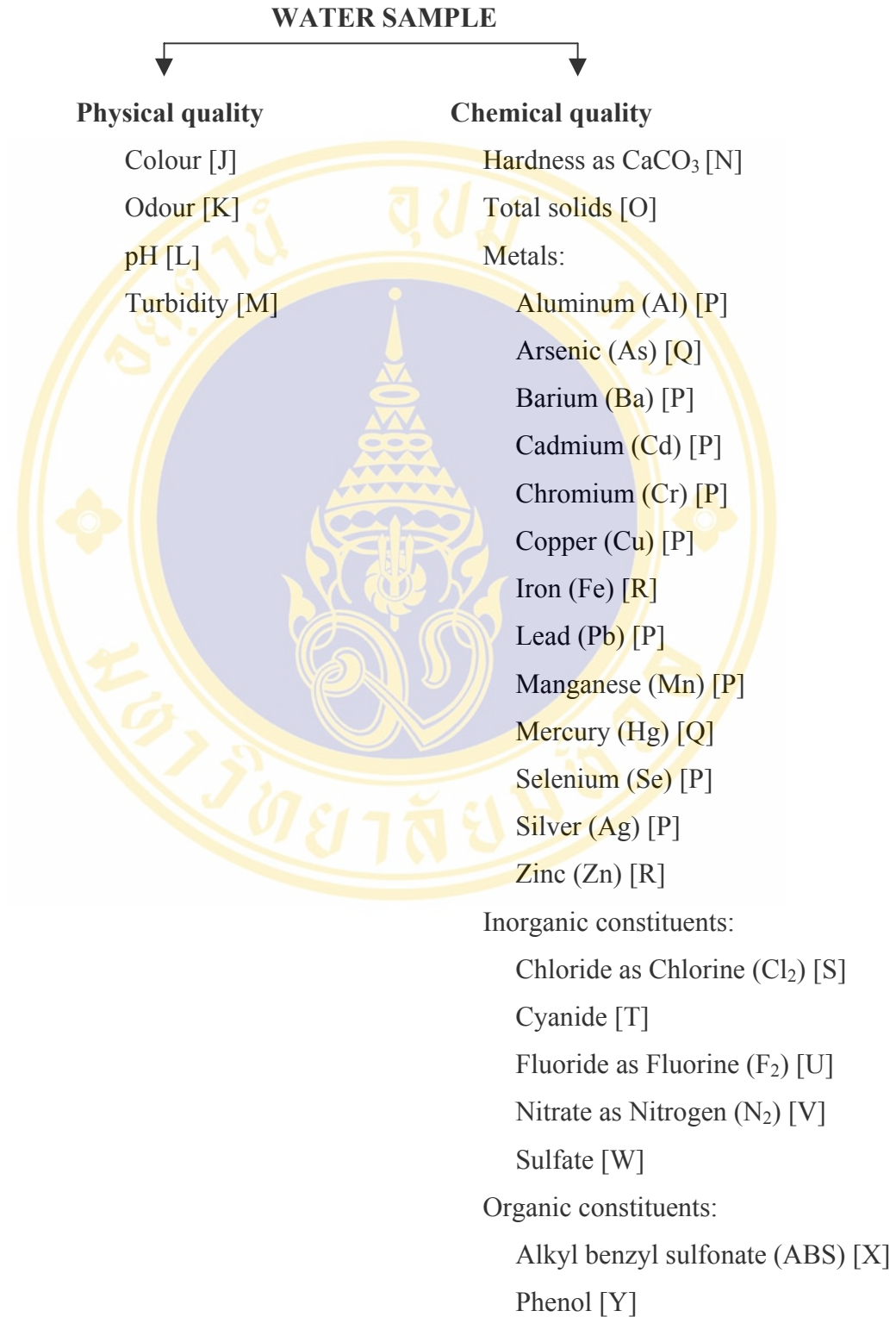
3.2.1 Experimental design

Objective 1. To investigate the waterborne microbe and the microbiological contaminations in drinking water (Appendices A through I):



Note: Appendix is in brackets.

Objective 2. To determine the physical and the chemical qualities of drinking water (Appendices J through Y):



Note: Appendix is in brackets.

3.2.2 Sample preparation

Upon arrival at the Research Center a waterborne microbe assay, a microbiological assay, and tests for the physical qualities of colour, odour, and pH immediately were conducted for each sample. If these analyses were not conducted upon arrival at the laboratory, the sample was preserved by refrigeration at 4°C and analysed within 24 h.

Each water sample was divided into 4 parts and preserved for the analysis by physicochemical assay of each respective part by:

- 1) refrigerating at 4°C for ABS, Cl₂, F₂, sulfate, and total solids;
- 2) adding Sodium hydroxide (NaOH) to pH>12 and refrigerating in the dark at 4°C for cyanide;
- 3) adding Nitric acid (HNO₃) to pH<2 and refrigerating in the dark at 4°C for Ag, Al, As, Ba, Cd, Cr, Cu, Fe, Hg, Pb, Mn, Se, Zn, and hardness; and
- 4) adding Sulfuric acid (H₂SO₄) to pH<2 and refrigerating in the dark at 4°C for N₂ and phenol.

3.2.3 Procedure

The procedural manuals for the waterborne microbe and microbiological assays and for the analyses of the physical and chemical qualities are given in the Appendices A through Y.

The specific procedures followed by the researcher for the waterborne microbe and microbiological assays listed in 3.2.1 for Objective 1 are detailed in Appendices A through I.

The specific procedures followed by the researcher for the physical quality and chemical quality analyses listed in 3.2.1 for Objective 2 are detailed in Appendices J through Y.

3.2.4 Statistical analysis

Data of this study were presented as mean ± SEM (standard error of mean). Nonparametric and Mann-Whitney U were used for comparison of 4 water treatment plants.

CHAPTER IV

RESULTS

4.1 Investigation of the waterborne microbe and the microbiological contaminations in raw, tap, and drinking water samples

4.1.1 Waterborne microbe assay

No *Cryptosporidium* oocyst was found in any of the raw water or tap water samples.

Fifty-four drinking water samples (one from each of the 54 schools serviced by the 4 water treatment plants) were investigated. *Cryptosporidium* oocysts were observed in 11.76% (4/34) of the drinking water samples from the schools serviced by the Bangkhaen water treatment plant with a mean of 1.17 oocysts per litre. However, there was no *Cryptosporidium* oocyst found in any of the water samples from the 20 schools serviced by the 3 other water treatment plants. Therefore, *Cryptosporidium* oocysts were observed in 7.41% (4/54) of the 54 schools with a mean of 0.74 oocyst per litre.

The acid-fast staining technique was used, and observation was made with an electrical microscope. The oocysts appeared as translucent, spherical bodies containing one to four dark granules, and stained red whereas bacteria and yeast cells took up the blue colour of the counterstain. Illustration 1 shows the photomicrographs of *Cryptosporidium* oocysts in the drinking water samples of the 4 schools found to be positive.

4.1.2 Microbiological assays

Raw, tap, and drinking water samples of the 4 water treatment plants in Bangkok were investigated for the contaminations of the following microbiological organisms: total coliforms, *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Yersinia enterocolitica*. The results are displayed in Table 6.

Total coliforms were found in the raw water samples of each of the 4 water treatment plants. *Escherichia coli* were found in the raw water samples of Mahasawat

and Samsen. *Salmonella* spp. were found in the raw water samples of Mahasawat and Thonburi. *Clostridium perfringens* were found in the raw water sample of Samsen. *Yersinia enterocolitica* were found in the raw water samples of Bangkhaen and Samsen. However, *Staphylococcus aureus*, *Campylobacter jejuni*, and *Listeria monocytogenes* were not found in any of the raw water samples.

No microbiological contaminations were found in tap water or drinking water samples from any of the 4 water treatment plants.

4.2 Determination of the physical and the chemical qualities of raw, tap, and drinking water samples

The physical and the chemical qualities of raw, tap, and drinking water samples from the 4 water treatment plants in Bangkok were determined. The results are displayed in Tables 7 and 8 and Figures 2 to 6.

4.2.1 Physical qualities

The values of the following physical qualities were determined: colour, odour, pH, and turbidity. The results are displayed in Table 7 and Figure 2.

Colour

Colour value was 5.00 Pt/Co for the raw water sample from each of the 4 water treatment plants. However, no presence of colour was detected in any of the tap and drinking water samples.

Odour

A slight odour was present in the raw water sample from each of the 4 water treatment plants. However, no presence of odour was detected in any of the tap and drinking water samples.

pH

The pH values of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 7.45-8.05. A significant difference ($p < 0.05$) was found between raw water and drinking water samples from Bangkhaen.

Turbidity

Turbidity values of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 1.45-10.00 NTU with no significant differences.

4.2.2 Chemical qualities

The contents of the following chemical qualities were determined: hardness, total solids, metals, inorganic constituents, and organic constituents. The results are displayed in Table 8 and Figures 3 to 6.

Hardness

Hardness contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 18.15-88.00 mg/L. Significant differences ($p < 0.05$) were found between drinking water samples from Bangkhaen and Samsen, Bangkhaen and Thonburi, and also Samsen and Thonburi. Although Mahasawat (55.75) was not different from Bangkhaen (18.15) but the values was much higher.

Total solids

Total solids contents of the raw, tap, and drinking water samples from the 4 water treatment plants were detected in the range of 160.55-281.00 mg/L. A significant difference ($p < 0.05$) was found between raw water and drinking water samples from Bangkhaen.

Metals

The contents of the following metals were determined: aluminum, arsenic, barium, cadmium, chromium, copper, iron, lead, manganese, mercury, selenium, silver, and zinc.

Aluminum contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.015-0.350 mg/L. A significant difference ($p < 0.05$) was found between raw and drinking water samples from Bangkhaen.

Arsenic contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.333-1.500 $\mu\text{g/L}$. A significant difference ($p < 0.05$) was found between raw water and drinking water samples as well as between tap water and drinking water samples from Bangkhaen.

Barium contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.001-0.070 $\mu\text{g/L}$. A significant difference ($p < 0.05$) was found between raw water and drinking water samples as well as between tap water and drinking water samples from Bangkhaen.

Cadmium contents were not detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants.

Chromium contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.434-1.316 $\mu\text{g/L}$ with no significant differences.

Copper contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.004-0.050 mg/L with no significant differences.

Iron contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.073-0.135 mg/L with no significant differences.

Lead content was not detected in any of the raw and tap water samples from any of the 4 water treatment plants. However, lead content was not detected in the drinking water samples for Samsen and Thonburi, but was detected in the drinking water sample for Bangkhaen (0.765 $\mu\text{g/L}$) and Mahasawat (3.500 $\mu\text{g/L}$) with no significant differences.

Manganese contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.006-0.053 mg/L with no significant differences.

Mercury contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.833-2.500 $\mu\text{g/L}$. A significant difference ($p < 0.05$) was found between raw water and drinking water samples as well as between tap water and drinking water samples from Bangkhaen.

Selenium contents of the raw water samples for the 4 water treatment plants and of the tap and drinking water samples for Bangkhaen, Samsen, and Thonburi were detected in the range of 0.500-5.000 $\mu\text{g/L}$. A significant difference ($p < 0.05$) was found between raw water and drinking water samples as well as between tap water and drinking water samples from Bangkhaen.

Silver contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.006-0.144 $\mu\text{g/L}$ with no significant differences.

Zinc contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.001-0.244 mg/L with a significant difference ($p < 0.05$) between the drinking water samples from Bangkhaen and Samsen.

Inorganic constituents

The contents of the following inorganic constituents were determined: chloride, cyanide, fluoride, nitrate, and sulfate.

Chloride contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 22.75-71.00 mg/L . A significant difference ($p < 0.05$) was found between raw water and drinking water samples as well as between tap water and drinking water samples from Bangkhaen.

Cyanide contents were not detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants.

Fluoride contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 0.05-0.29 mg/L with no significant differences.

Nitrate contents of the raw, tap, and drinking water for the 4 water treatment plants were detected in the range of 1.905-60.500 mg/L . A significant difference ($p < 0.05$) was found between raw water and drinking water samples as well as between tap water and drinking water samples from Bangkhaen.

Sulfate contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 32.25-52.00 mg/L. A significant difference ($p < 0.05$) was found between raw water and drinking water samples from Bangkhaen.

Organic constituents

The contents of the following organic constituents were determined: alkylbenzene sulfonate and phenols.

The alkylbenzene sulfonate contents of the raw, tap, and drinking water samples for the 4 water treatment plants were detected in the range of 5.744-76.50 mg/L. A significant difference ($p < 0.05$) was found between raw water and drinking water samples from Bangkhaen.

Phenols contents were not detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants.

Table 8. Investigation of *Cryptosporidium* oocyst in raw, tap, and drinking water samples.

	Water treatment plants											
	Bangkhaen			Mahasawat			Samsen			Thonburi		
	RW	TW	DW(n=34)	RW	TW	DW(n=4)	RW	TW	DW(n=6)	RW	TW	DW(n=10)
Schools found positive for	0	0	4(11.76)	0	0	0	0	0	0	0	0	0
<i>Cryptosporidium</i> oocysts (%)	0	0	4(11.76)	0	0	0	0	0	0	0	0	0
<i>Cryptosporidium</i> oocysts/L	0	0	1.17	0	0	0	0	0	0	0	0	0

Notes: RW = raw water, TW = tap water, DW = drinking water

RW and DW samples for each water treatment plant were investigated in duplicate

n = number of schools



Illustration 1. Picture of *Cryptosporidium* oocyst (Salaku School), staining by modified acid-fast

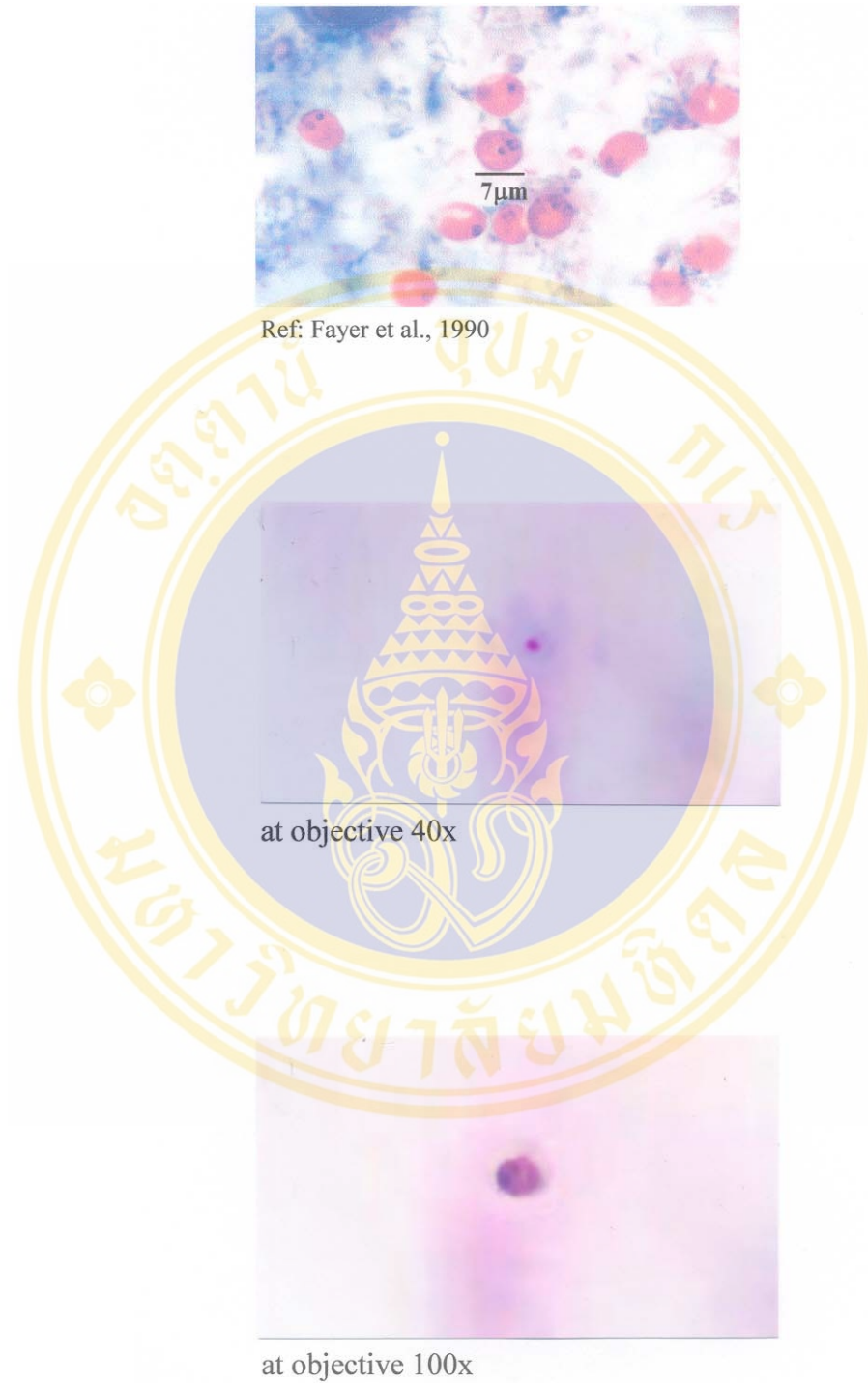


Illustration 2. Picture of *Cryptosporidium* oocyst (Wat Bungbua School), staining by modified acid-fast



Illustration 3. Picture of *Cryptosporidium* oocyst (Nilratupathum School), staining by modified acid-fast



Illustration 4. Picture of *Cryptosporidium* oocyst (Suraokohkhunnane School), staining by modified acid-fast

Table 9. Investigation of microbiological contaminations in raw, tap, and drinking water samples.

Parameters	Water treatment plants											
	Bangkhaen			Mahasawat			Samsen			Thonburi		
	RW	TW	DW(n=34)	RW	TW	DW(n=4)	RW	TW	DW(n=6)	RW	TW	DW(n=10)
Total coliforms	+	-	-	+	-	-	+	-	-	+	-	-
<i>Escherichia coli</i>	-	-	-	+	-	-	+	-	-	-	-	-
<i>Salmonella</i> spp.	-	-	-	+	-	-	-	-	-	+	-	-
<i>Clostridium perfringens</i>	-	-	-	-	-	-	+	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Campylobacter jejuni</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Listeria monocytogenes</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Yersinia enterocolitica</i>	+	-	-	-	-	-	+	-	-	-	-	-

Notes: RW = raw water, TW = tap water, DW = drinking water, (+) = presence, (-) = absence

RW and DW samples for each water treatment plant were investigated in duplicate

n = number of schools

Table 10. Physical qualities of raw, tap, and drinking water samples.

Parameters	Water treatment plants											
	Bangkhaen			Mahasawat			Samsen			Thonburi		
	RW	TW	DW(n=34)	RW	TW	DW(n=4)	RW	TW	DW(n=6)	RW	TW	DW(n=10)
Colour (Pt/Co)	5.00±0.00	0	0	5.00±0.00	0	0	5.00±0.00	0	0	5.00±0.00	0	0
Odour	+	-	-	+	-	-	+	-	-	+	-	-
pH	7.95±0.05	7.55±0.01	7.45±0.02	8.05±0.05	7.75±0.02	7.62±0.06	7.90±0.00	7.62±0.00	7.70±0.04	8.00±0.00	7.76±0.02	7.51±0.05
Turbidity (NTU)	7.50±2.50	1.45±0.05	1.34±0.08	10.00±0.00	1.80±0.10	1.60±0.13	5.00±0.00	1.60±0.10	1.47±0.08	10.00±0.00	1.65±0.05	1.52±0.09

Notes: Results expressed in Mean±SEM

RW = raw water, TW = tap water, DW = drinking water, (+) = presence, (-) = absence

RW and TW samples for each water treatment plant were investigated in duplicate.

n = number of schools

^a = Significantly different (p<0.05) from raw water sample of Bangkhaen

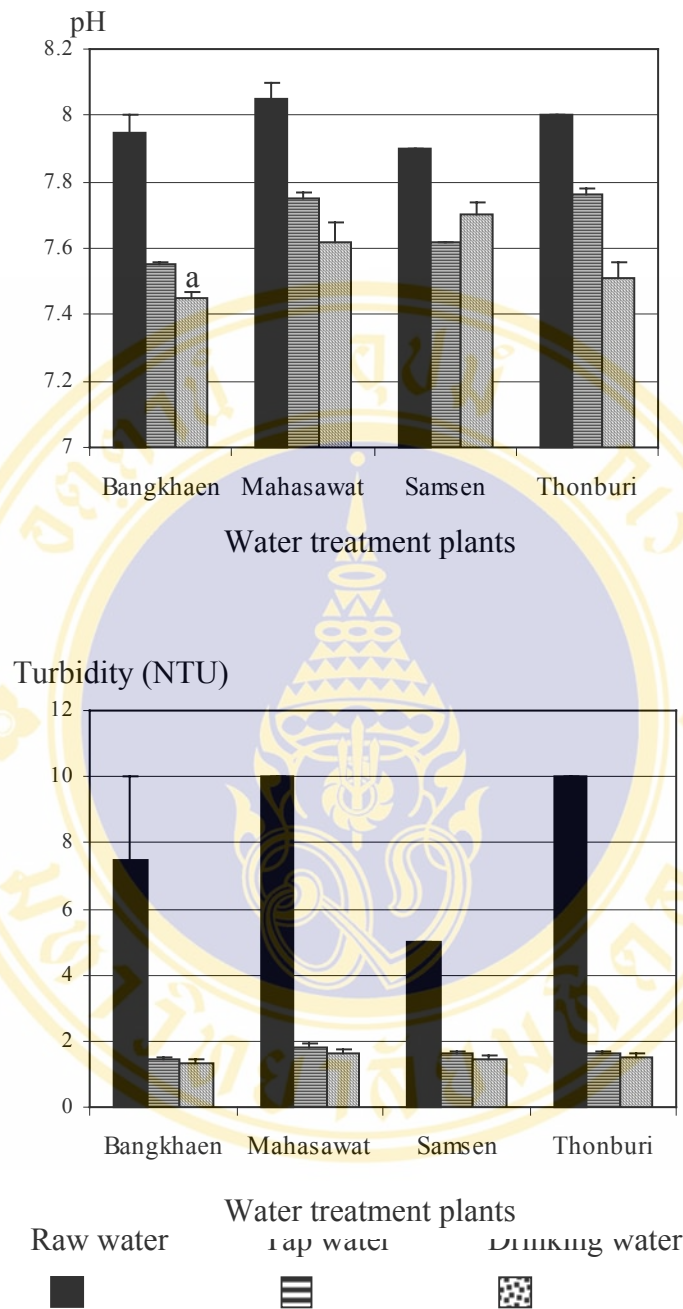


Figure 2. pH and turbidity values of raw, tap, and drinking water samples

Notes: Results expressed in Mean±SEM

^a = Significantly different (p<0.05) from raw water sample of Bangkhaen

Table 11. Chemical qualities of raw, tap, and drinking water samples.

Parameters	Water treatment plants														
	Bangkhaen				Mahasawat				Samsen				Thonburi		
	RW	TW	DW(n=34)	RW	TW	DW(n=4)	RW	TW	DW(n=6)	RW	TW	DW(n=10)	RW	TW	DW(n=10)
Hardness	30.50±2.50	20.50±0.50	18.15±5.01	64.00±3.00	61.50±1.50	55.75±5.14	88.00±2.00	71.00±1.00	63.17±4.67*	58.50±3.50	41.00±1.00	38.70±4.21 [#]	267.00±3.00	211.00±1.00	160.55±7.35
Total solids	281.00±1.00	204.00±4.00	171.72±4.19 ^a	278.00±2.00	209.00±1.00	168.00±17.12	272.50±2.50	203.00±1.00	167.47±13.42	267.00±3.00	211.00±1.00	160.55±7.35			
Metals															
Aluminum	0.154±0.001	0.023±0.001	0.022±0.006 ^a	0.205±0.005	0.026±0.001	0.018±0.002	0.165±0.005	0.021±0.001	0.015±0.001	0.350±0.010	0.035±0.001	0.028±0.014			
Arsenic	1.500±0.500	1.00±0.000	0.618±0.164 ^{a, b}	1.500±0.500	1.00±0.000	0.500±0.289	0.500±0.500	0.350±0.000	0.333±0.211	1.000±0.000	0.550±0.050	0.500±0.224			
Barium	0.040±0.001	0.015±0.002	0.008±0.001 ^{a, b}	0.063±0.003	0.023±0.002	0.014±0.001	0.052±0.002	0.028±0.001	0.010±0.001	0.070±0.001	0.021±0.001	0.009±0.002			
Cadmium	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.			
Chromium	1.025±0.015	0.595±0.004	0.483±0.048	1.100±0.001	0.828±0.001	0.800±0.060	0.900±0.001	0.873±0.001	0.880±0.167	1.316±0.0096	0.509±0.001	0.434±0.114			
Copper	0.050±0.000	0.009±0.000	0.008±0.003	0.045±0.005	0.005±0.002	0.004±0.001	0.035±0.005	0.008±0.002	0.006±0.002	0.040±0.001	0.005±0.001	0.004±0.002			
Iron	0.135±0.005	0.105±0.005	0.097±0.008	0.115±0.005	0.085±0.005	0.073±0.006	0.125±0.005	0.100±0.010	0.088±0.009	0.125±0.005	0.115±0.005	0.106±0.009			
Lead	N.D.	N.D.	0.765±0.414	N.D.	N.D.	3.500±3.500	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.			
Manganese	0.032±0.001	0.013±0.001	0.010±0.003 ^{a, b}	0.022±0.001	0.016±0.002	0.013±0.002	0.053±0.001	0.020±0.001	0.014±0.003	0.023±0.002	0.008±0.002	0.006±0.003			
Mercury	2.000±0.500	1.500±0.000	1.029±0.297 ^{a, b}	1.500±0.000	1.000±0.000	2.000±0.913	1.500±0.500	1.000±0.000	0.833±0.543	2.500±0.500	1.500±0.000	1.100±0.180			
Selenium	3.500±0.500	3.000±0.100	2.941±2.941 ^{a, b}	1.500±0.500	N.D.	N.D.	0.500±0.001	0.500±0.000	0.500±0.224	5.000±1.000	3.500±0.050	2.800±2.149			
Silver	0.055±0.001	0.024±0.001	0.011±0.002	0.039±0.001	0.034±0.002	0.008±0.001	0.097±0.001	0.055±0.001	0.008±0.001	0.146±0.001	0.098±0.001	0.007±0.001			
Zinc	N.D.	N.D.	0.043±0.014	0.003±0.000	0.001±0.001	0.191±0.060	0.002±0.001	0.001±0.001	0.244±0.031*	0.212±0.002	0.150±0.005	0.113±0.045			

Notes: Results expressed in Mean±SEM

RW = raw water, TW = tap water, DW = drinking water, N.D. = not detected, n = number of schools

RW and DW samples for each water treatment plant were investigated in duplicate.

*Significantly different (p<0.05) from drinking water samples of Bangkok

[#]Significantly different (p<0.05) from drinking water samples of Samsen^aSignificantly different (p<0.05) from raw water sample of Bangkok^bSignificantly different (p<0.05) from tap water sample of Bangkok

Table 11. Chemical qualities of raw, tap, and drinking water samples. (cont'd)

Parameters	Water treatment plants											
	Bangkhaen		Mahasawat		Thonburi							
	RW	TW	DW(n=34)	RW	TW	DW(n=4)	RW	TW	DW(n=6)	RW	TW	DW(n=10)
Inorganic Constituents												
Chloride	44.50±0.50	30.50±0.50	23.70±0.52 ^{a, b}	32.50±0.50	27.50±0.50	22.75±1.49	71.00±1.00	59.50±0.50	24.33±0.95	50.50±0.50	64.50±0.50	24.60±1.12
Cyanide	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fluoride	0.100±0.010	0.082±0.002	0.074±0.010	0.110±0.010	0.081±0.001	0.075±0.050	0.200±0.000	0.079±0.001	0.067±0.021	0.290±0.010	0.061±0.001	0.050±0.017
Nitrate	60.50±0.50	4.900±0.100	2.065±0.017 ^{a, b}	41.00±1.00	6.050±0.050	2.065±0.043	33.50±1.50	6.900±0.100	2.100±0.041	14.50±0.50	5.850±0.050	1.905±0.096
Sulfate	52.00±1.00	39.50±0.50	35.09±0.55 ^a	45.00±1.00	35.50±0.50	32.25±0.48	37.50±0.50	38.50±0.50	33.50±0.92	49.50±0.50	36.50±0.50	34.20±0.96
Organic Constituents												
ABS	76.50±0.50	6.500±0.500	5.744±1.177 ^a	52.00±1.00	9.500±0.500	8.275±4.634	32.50±0.50	8.500±0.500	7.767±3.953	68.50±0.50	12.50±0.00	10.31±2.65
Phenols	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Notes: Results expressed in Mean±SEM

RW = raw water, TW = tap water, DW = drinking water, N.D. = not detected, ABS = Alkyl benzylsulfonate

RW and DW samples of each water treatment plant were investigated in duplicate. n = number of schools

^aSignificantly different (p<0.05) from raw water sample of Bangkhaen

^bSignificantly different (p<0.05) from tap water sample of Bangkhaen

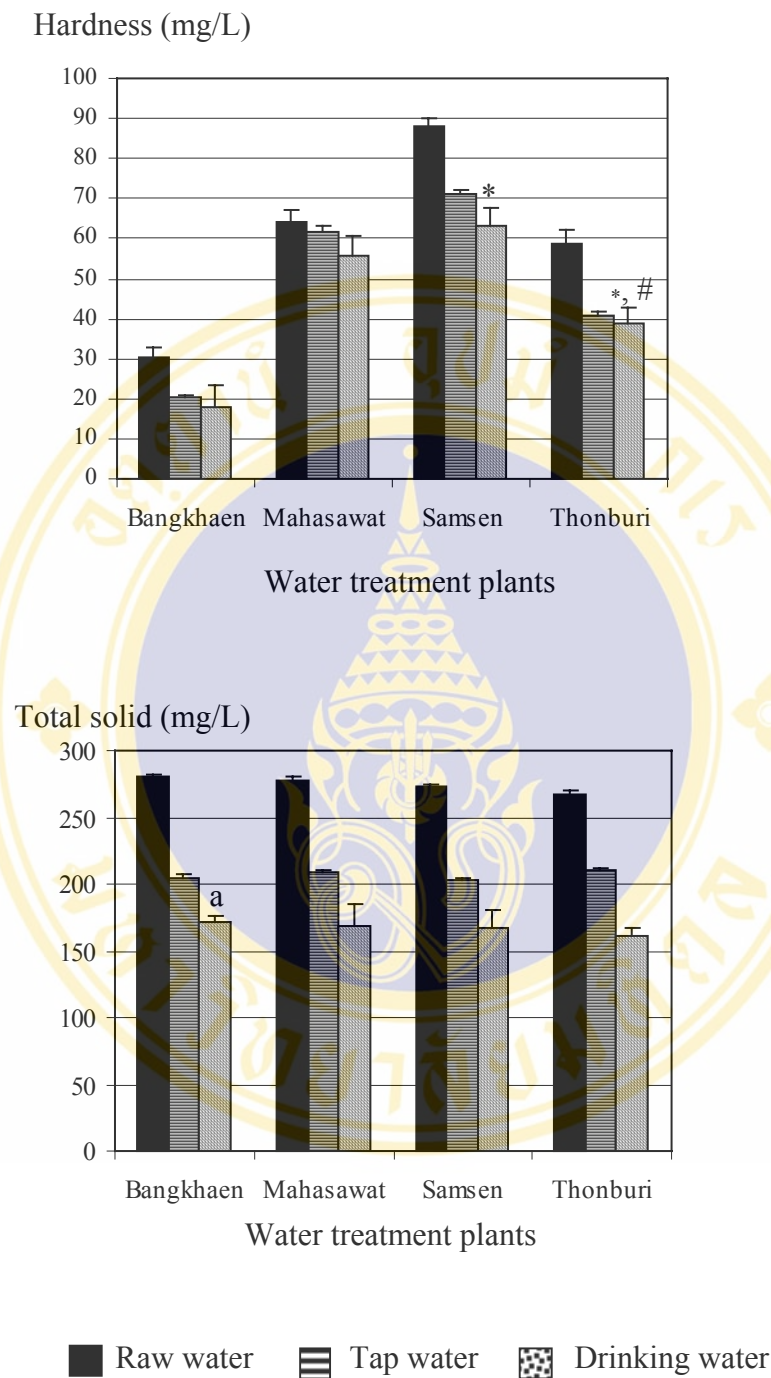


Figure 3. Hardness and total solids contents of raw, tap, and drinking water samples

Notes: Results expressed in Mean±SEM

RW = raw water, TW = tap water, DW = drinking water

*Significantly different (p<0.05) from drinking water samples of Bangkhaen

#Significant different (p<0.05) from drinking water samples of Samsen

^aSignificantly different (p,0.05) from raw water sample of Bangkhaen

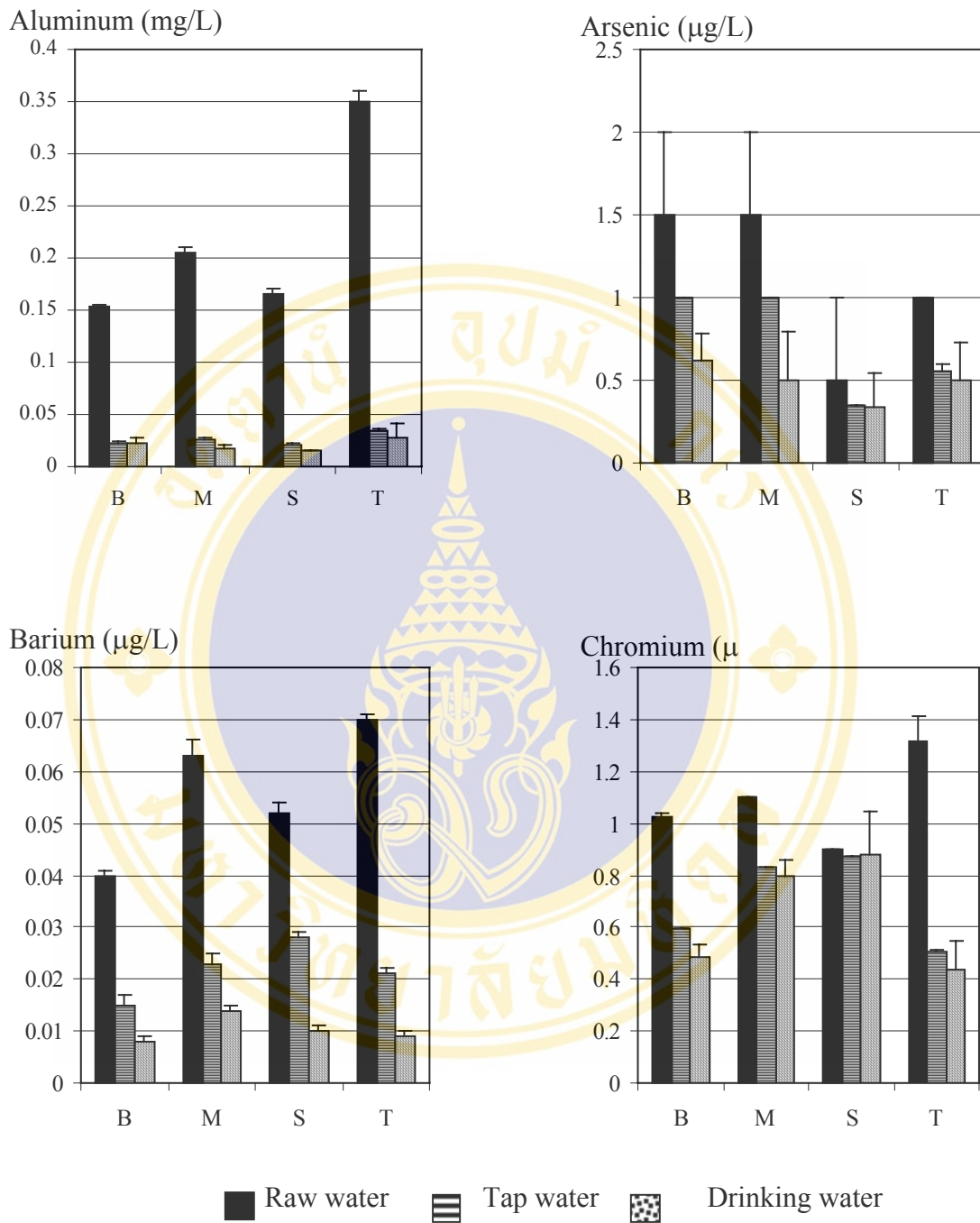


Figure 4. Metal contents of raw, tap, and drinking water samples.

Notes: Results expressed in Mean±SEM

B = Bangkhaen, M = Mahasawat, S = Samsen, T = Thonburi

^aSignificantly different (p<0.05) from raw water sample of Bangkhaen

^bSignificantly different (p<0.05) from tap water sample of Bangkhaen

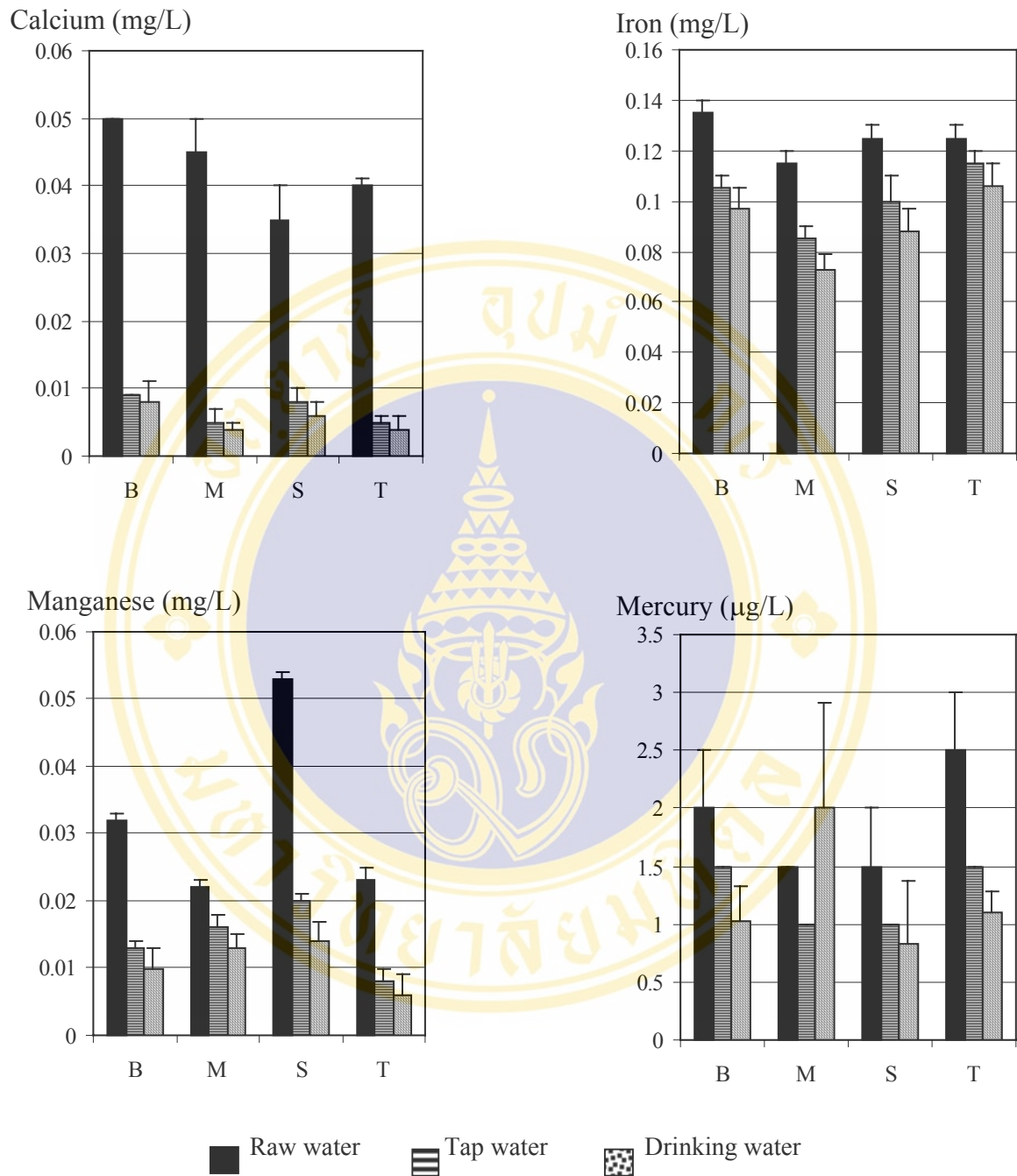


Figure 4. Metal contents of raw, tap, and drinking water samples. (cont'd)

Notes: Results expressed in Mean±SEM

B = Bangkhaen, M = Mahasawat, S = Samsen, T = Thonburi

^aSignificantly different (p<0.05) from raw water sample of Bangkhaen

^bSignificantly different (p<0.05) from tap water sample of Bangkhaen

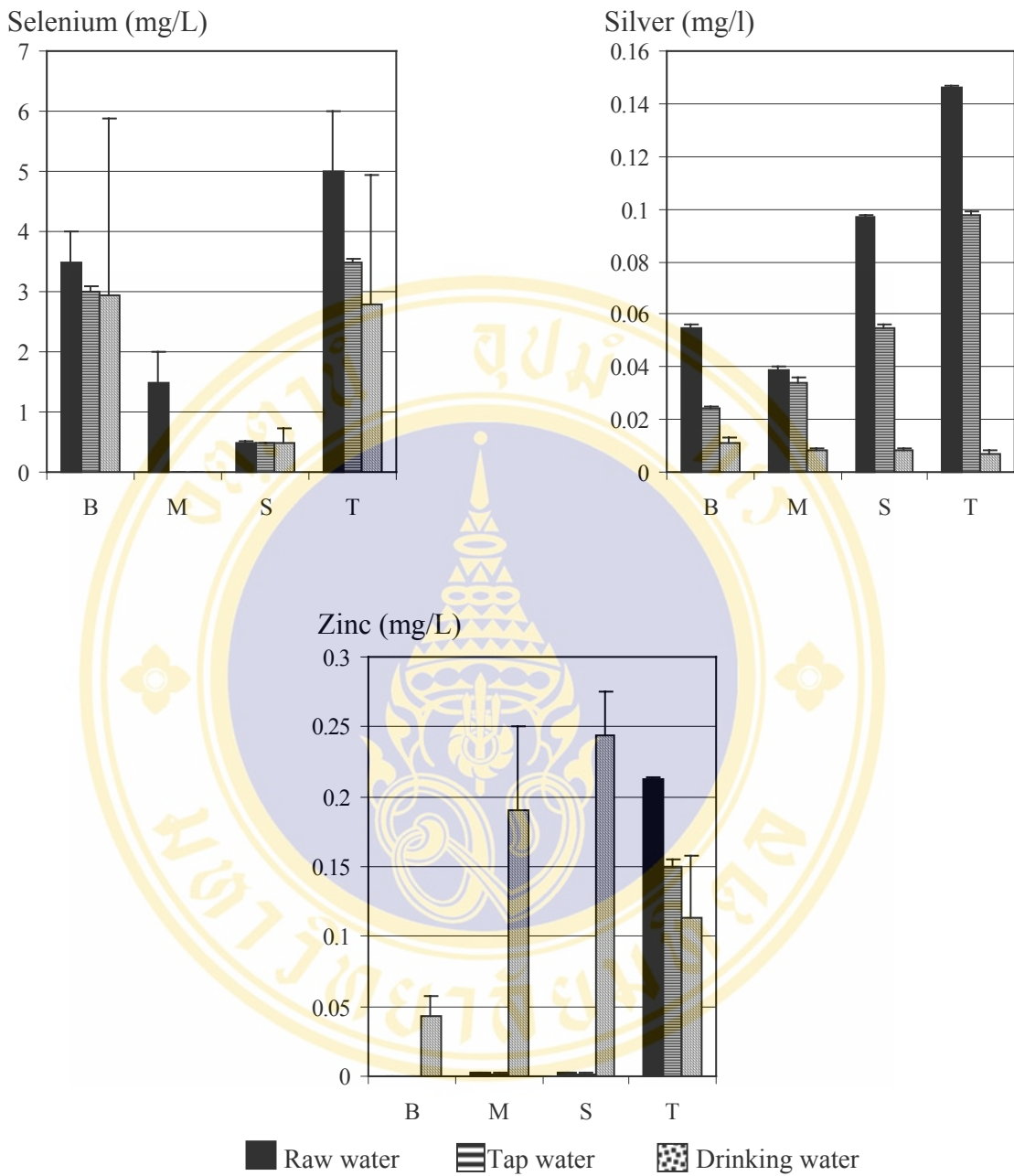


Figure 4. Metal contents of raw, tap, and drinking water samples. (cont'd)

Notes: Results expressed in Mean±SEM

B = Bangkhaen, M = Mahasawat, S = Samsen, T = Thonburi

*Significantly different ($p < 0.05$) from drinking water samples of Bangkhaen

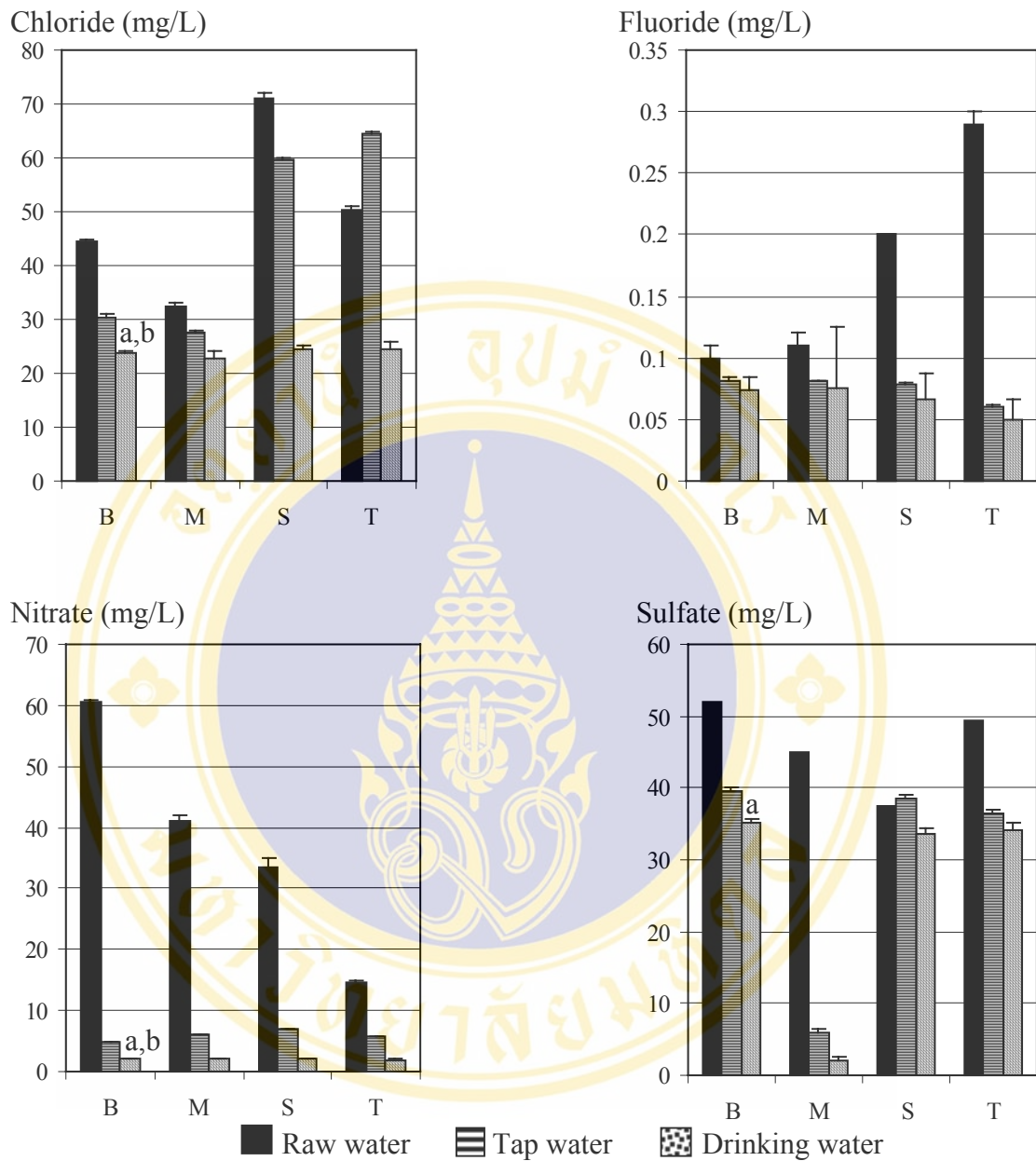


Figure 5. Inorganic constituents contents of raw, tap, and drinking water samples.

Notes: Results expressed in Mean±SEM

B = Bangkhaen, M = Mahasawat, S = Samsen, T = Thonburi

^aSignificantly different ($p < 0.05$) from raw water sample of Bangkhaen

^bSignificantly different ($p < 0.05$) from tap water sample of Bangkhaen

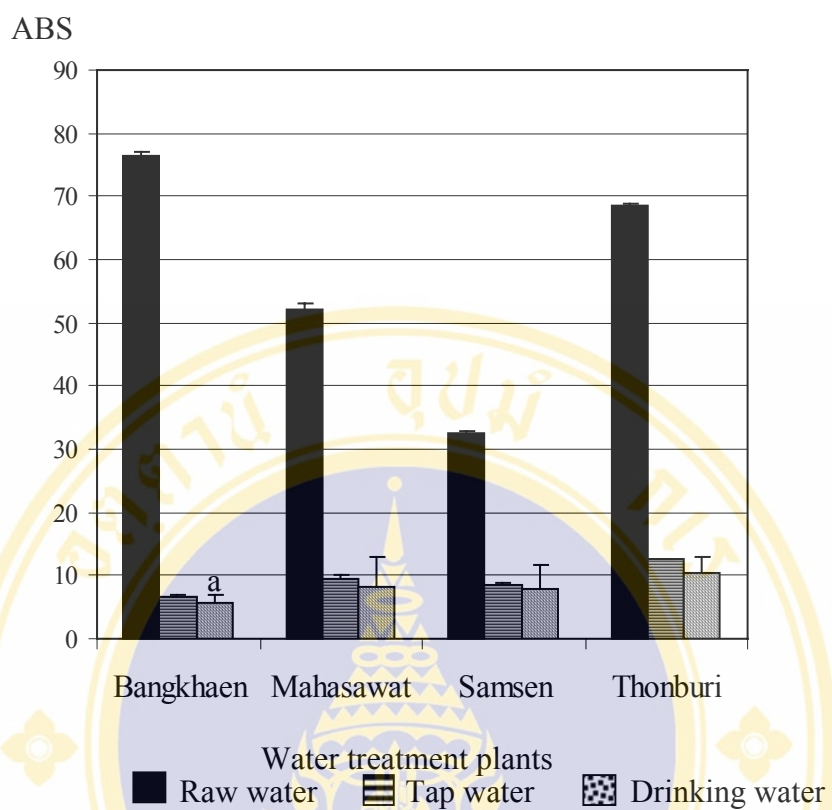


Figure 6. Organic constituents contents of raw, tap, and drinking water samples.

Notes: Results expressed in Mean±SEM

^aSignificantly different ($p < 0.05$) from raw water sample of Bangkhaen

CHAPTER V

DISCUSSION

5.1 Investigation of the waterborne microbe and the microbiological contamination in raw, tap, and drinking water samples.

5.1.1 Waterborne microbe assay

The present study demonstrated that *Cryptosporidium* oocysts were observed in the 4 drinking water samples from the 34 schools serviced by the Bangkhaen Water Treatment Plant, but there was no oocyst found in any of the water samples from the 20 schools serviced by the 3 other water treatment plants; and no oocyst was found in any of the raw water or tap water samples of any of the plants. Bangkhaen, like the other 3 water treatment plants, is well-regulated to insure the production of quality water. All 4 schools' drinking water were passed through unplasticized polyvinyl chloride pipes and were stored in plastics water tanks which are provided by TISI. Therefore, the presence of *Cryptosporidium* oocysts in drinking water samples suggests that the water supply pipelines may have leakages, allowing oocysts from faecal matter to enter the water distribution systems. Another possibility could be that contamination was caused from the children's hands as they turned on a faucet to drink from it.

Early studies indicated that *Cryptosporidium* was the cause of several outbreaks of gastrointestinal illness associated not only with municipal drinking water systems but also with swimming in lakes, and with ground water [Rose et al., 1991; Rose, 1997; Hancock et al., 1998; CDC, 2000]. However, little is known about the survival of *Cryptosporidium* oocysts in terrestrial environments once they are shed by their host [Hansen & Ongerth, 1991; Robertson et al., 1992]. Unfortunately, Viability and infectivity of these oocysts were not determined in this study.

5.1.2 Microbiological assays

The present study demonstrated that total coliforms, *E. coli*, *Salmonella* spp., *Clostridium perfringens* (*C. perfringens*), and *Yersinia enterocolitica* (*Y. enterocolitica*) were found in each of the 4 raw water samples. The presence of these 5 pathogens may be due to a number of different factors (e.g., survival, movement, rain, temperature, presence of cattle, etc.). These bacteria may persist for considerable periods in a natural environment, probably adsorbed in soil particles or elsewhere, thereby increasing their chances of survival. In addition, because *Y. enterocolitica* is able to grow at a low temperature, they may multiply in biofilms in groundwater. Such biofilms may then act as a reservoir for the reproduction of *Y. enterocolitica*. However, no microbiological contamination was found in any of the tap water or drinking water samples. Therefore, this would suggest that Bangkok's water treatment processes are adequate to produce microorganism-free drinking water.

The microbiological quality of drinking water supported by several studies such as *Escherichia coli* (*E. coli*), the *E. coli* group is generally assumed to be related to faecal contamination, but also could imply the presence of microbiological contamination [WHO, 1994]. Monitoring of microbiological water quality has shown that one finds very few cases of waterborne transmission of pathogenic bacteria which are not associated with the detection of coliforms [Lund, 1996]. The direct monitoring of these pathogenic agents is difficult because (1) their concentrations both in source and in the water treatment facility are usually low and strongly fluctuating and (2) analytical procedures are time-consuming and have a low recovery efficiency. The elimination of pathogenic agents from ground water is related also to several other processes such as sedimentation, filtration, and disinfection [MWA, 2002]. In 1976, it was claimed that 25% of all the world's hospital beds were occupied by people with diseases either caused by polluted water or related to water [WHO, 1976]. A study done by Jagals and co-workers in a large socio-economic urban setting with unsatisfactory sanitary facilities and drinking water provisions indicated that the community was exposed to microbiologically-related environmental health risks when consuming water supplied by a public above-ground standpipe system [Jagals et al., 1997]. In South Africa, it has been reported that almost 30% of the population do not

have access to an adequate supply of potable water which implies that most rural communities use raw water from surface or ground for drinking [Venter, 2001].

5.2 Determination of the physical and the chemical qualities of raw, tap, and drinking water samples.

5.2.1 Physical qualities

The results demonstrated that the colour for each of the 4 raw water samples was 5.00 Pt/Co which is lower than the maximum acceptable level (20.00 Pt/Co) for the drinking water standard [TISI, 1978]. This slight presence of colour may have resulted from the presence of natural metallic ions, plankton, and industrial wastes [APHA, 1995]. In Adelaide, South Australia, it has been reported that the colour of 4 water treatment plants (i.e., Hope Valley, Happy Valley, Myponga, and Anstey Hill) were respectively 17.0, 16.0, 67.0, and 4.0 Hasen Units. The colour of Hope Valley and Happy Valley waters likely were similar because these two reservoirs had been supplemented directly with water from the River Murray, a major water source for the southern region of South Australia. The high colour of the Myponga water indicated the natural organic material (NOM) has a more conjugated, aromatic character, and is possibly less degraded than the NOM in the other waters. On the contrary, Anstey Hill Water Treatment Plant shows lower colour because it is received from the River Murray via a 60-kilometre pipeline; and, unlike Hope Valley and Happy Valley, the Anstey Hill water is chlorinated at a pumping station at the river thus maintaining microbiological quality [Cook et al., 2001]. These are all indicative of the reaction to chlorine with the conjugated and aromatic groups of the NOM, leading to the formation of smaller compounds with low colour [Li et al., 1998]. However, in my study no presence of colour was detected in any of the tap and drinking water samples. Therefore, it can be concluded that the water treatment processing removed the colour to make the water aesthetically suitable for drinking.

The slight odour which was present in each of the 4 raw water samples may have originated from municipal and industrial water discharges, from natural sources such as decomposition of vegetable matter, from associated microbial activity, and/or from disinfectants used in the purification process [Bruvold, 1989]. In 1999, the Detroit Water and Sewerage Department (DWSD) experienced periodic odour

events in the raw water supplies. During these episodes, DWSD received complaints from customers throughout the system [Atasi et al., 1999]. A recent study in Australia [Cook et al., 2001] demonstrated that blooms of blue-green algae in reservoirs often produce the musty-earthly taste and odour algal metabolites 2-methylisoberneol (MIB) and geosmin which can be detected by consumers at levels as low as 10 ng/L. However, since the results of the present study showed no presence of odour being detected in any of the tap and drinking water samples, it can be concluded that the odour treatment method used for tap and drinking water in Bangkok is effective.

The results demonstrated that the highest pH value was found in the raw water sample from Mahasawat (8.05) which is lower than the maximum acceptable level (8.50) for drinking water [TISI, 1978]. A pH value which is higher than 8.50 will indicate an impairment at some phase of the water supply because all phases of the water supply (e.g., sedimentation, filtration, disinfection, etc.) are pH-dependent [APHA, 1995].

The results demonstrated that both the Mahasawat and Thonburi contained a maximum turbidity content of 10.00 NTU which is higher than the maximum acceptable level (0.70 NTU) for drinking water [TISI, 1978]. The turbidity may be caused by suspended and colloidal matter, such as clay, silt, finely divided organic and inorganic matter, and plankton, and other microorganisms, etc. [USEPA, 1993]. Nebbache et al. (2001) reported the turbidity values of aquifers were very high in rural areas of France's Brionne Basin during the autumn and winter seasons of 1991-1993. During some rainy episodes they had reached values over 200 NTU, including a high of 1120 NTU on December 19, 1991. They conclude that the production of potable water from most raw water sources should entail the use of a flocculation/coagulation stage to remove turbidity in the form of suspended and colloidal material. Diaz et al. (1999) reported that *Cactus latifaria* and seeds of *Prosopis juliflora* act as natural coagulants. They found that both materials produce comparable turbidity removals and are able to produce a final water turbidity which is close to the required standard of 5.00 NTU, although the initial turbidity may be as high as 100-200 NTU or as low as 30-40 NTU. The turbidity values in the present experiment were found to be lower for tap and drinking water samples than for raw water samples, with drinking water being lower than the maximum acceptable level.

5.2.2 Chemical qualities

Hardness

Hardness is defined as the sum of the calcium and magnesium concentrations [APHA,1995]. The maximum content of hardness (88.00mg/L) was detected in the raw water sample from Samsen which is lower than the maximum acceptable level (100 mg/L) for drinking water [TISI, 1978]. However, the rest of the plants was significant different which suggested that the sources and the treatment of each water treatment plant were different.

Total solids

The results demonstrate the maximum content of total solids (281.00 mg/L) was detected in the raw water sample from Bangkhaen which is lower than the maximum recommended level (500 mg/L) for drinking water [APHA, 1995]. If total solids contents was higher than 500 mg/L, they may affect water quality adversely (e.g., inferior palatability, unfavorable physiological reaction) [Howard, 1933].

Metals

The results demonstrated that the highest aluminum (Al) content was found in raw water sample from Thonburi (0.35 mg/L) which is higher than the maximum acceptable level (0.20 mg/L) for the drinking water standard [TISI, 1978]. The presence of Al may be due to the material of the water tank and faucet in such school. In the literature review of Schintu et al. (2000), they reported that the use of an Al-based coagulants in a water utility may lead to an increased concentrations of the metal in treated water, which will result in problems relating to water quality and supply. However, they investigated aluminum contents of water samples from three drinking water reservoirs in Sardinia, Italy. They found that the treatment of raw water with an Al-based coagulant did not increase the concentration of the metal in the finished water [Schintu et al., 2000]. Also, in 2 other studies, where the initial aluminum concentrations were much lower, aluminum sulfate treatment did not substantially increase the aluminum concentration in finished water [Miller et al., 1987; Lettermann & Driscoll, 1988].

The highest arsenic (As) content (1.50 $\mu\text{g/L}$) was observed in the raw water samples from both Bangkhaen and Mahasawat, but it is lower than the maximum acceptable level (0.05 mg/L) for the drinking water standard [TISI, 1978].

The presence of As may be due to the HDPP or PB pipes. In a review of epidemiological studies carried out in India, Taiwan, Mexico, Argentina, Chile, Japan, England, and Hungary, Das et al. (1996) reported explicit associations between arsenic in groundwater and dermatological diseases and skin cancer. In 2000, it was claimed that of the 125 million inhabitants of Bangladesh, between 35 million and 77 million are at risk of drinking contaminated water [Smith et al., 2000]. Chowdhury et al. (2000) reported that 24.47 % people with arsenical skin lesions in 27 districts of Bangladesh usually drank water containing above 0.30 mg/L of arsenic. In 1987, human health problems associated with arsenic toxicity were first recognised in Ron Phibun District, Nakhon Si Thammarat province, southern Thailand. A preliminary medical survey by the Government of Thailand Ministry of Public Health in 1988 identified approximately 1,000 cases of arsenic-induced skin disorders including 20 cases of skin cancer. In 1994, several potential sources of As pollution are known to exist in Ron Phibun District. These include As rich waste piles derived from mineral separation at former bedrock tin mining sites, disseminated arsenopyrite-rich waste generated by the informal mining sector, As-rich waste generated by two ore-dressing plants near Ron Phibun town, and extensive alluvial placer in deposits. Concentrations of As in groundwater were recorded in the range 2-5115 mg/L (Paijitprapaon et al., 1994). Some researchers have reported that groundwater contamination by arsenic is attributed to either of 2 anthropogenic sources: (1) excessive use of large varieties of pesticide [Bagla & Kaiser, 1996] or (2) oxidation of pyrite by atmospheric oxygen introduced into groundwater due to tube-well pumping [Das et al, 1996]. On the other hand, Nickson et al. (1998) and Acharyya et al. (1999) proposed that reductive dissolution of iron (oxy)-hydroxide is the main source of arsenic in groundwater in Bangladesh.

The highest barium (Ba) content was found in the raw water sample from Thonburi (0.07 µg/L) which is lower than the maximum acceptable level (1.00 mg/L) for the drinking water standard [TISI, 1978]. The presence of Ba may be due to PB or HDPP pipe or tubing. In 1979, it was discovered that the primary sources of naturally occurring barium in drinking water are leaching and eroding from sedimentary rocks into underground aquifers [Kojola et al., 1979]. Barium is known to be a cardiovascular risk factor. However, Wones and colleagues found that barium levels

of 5 and 10 $\mu\text{g/L}$ in drinking water do not appear to cause any cardiovascular risk factors [Fayer et al., 1990].

No cadmium (Cd) contents were detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants. Mohamed & Osman (1998) measured raw Nile River water cadmium contents from 4 treatment plants in Greater Cairo, Egypt. They found that the mean cadmium concentration was $4.15 \pm 0.88 \mu\text{g/L}$. Earlier, it had been reported by Gomaa (1995) that the mean concentration in the Nile was 24 times greater than the mean that would obtain in 1998 and 8 to 10 times greater than internationally-recognized drinking water standards (0.003 mg/L) [TISI, 1978].

The highest chromium (Cr) content was found in the raw water sample for Thonburi ($1.316 \mu\text{g/L}$) which is lower than the maximum acceptable level (0.05 mg/L) for the drinking water standard [TISI, 1978]. Jaleel *et al.* (2001) reported the Cr content in drinking water for different localities of a district east of Karachi, Pakistan. They evaluated the drinking water samples for three sources of water, i.e., piped water, hand pumped water, and water supplied by tanker truck. They found that Cr content was elevated in hand pump water. Another study in 2 districts of Conakry, Republic of Guinea showed that the Cr content in water samples from Lucia-Camara ($31.32 \mu\text{g/L}$) and Matam ($114.85 \mu\text{g/L}$), both of which were high, could have originated from the domestic use of products for the tanning and dyeing of leather since no heavy industries were located in the area [Gélinas et al., 1996]. In 1996, Al-Saleh reported the Cr content in 59 water samples collected from 32 primary schools in Riyadh, Saudi Arabia. He found a mean Cr content of $1.95 \pm 0.79 \mu\text{g/L}$ which is lower than the standard established by both the European Economic Community (EEC) and the WHO [Al-Saleh, 1996].

The highest copper (Cu) content was found in raw water sample from Bangkhaen (0.05 mg/L) which is lower than the maximum acceptable level (1.00 mg/L) for drinking water standard [TISI, 1978]. In 1996, Al-Saleh investigated the contents of trace elements in drinking water coolers collected from primary schools, Riyadh, Saudi Arabia. He reported that the mean Cu content was $18.38 \pm 23.21 \mu\text{g/L}$. Avila-Pérez et al. (1999) measured Cu contents in water samples from 8 sites of the Jose Antonio Alzate Reservoir, Mexico. They reported the mean Cu content was 70 mg/L which is higher than the Mexican acceptable level for irrigation water (0.20

mg/L). However, they concluded that Cu was not a problem because of extremely spiked results in 2 of 40 measurements causing the mean to be skewed.

The highest iron (Fe) content was found in water sample from Bangkhaen (0.135 mg/L) which is lower than the maximum acceptable level (0.300 mg/L) for the drinking water standard [TISI, 1978]. In 1999, Avila-Pérez et al. Measured Fe content in water of the Jose Antonio Alzate Reservoir, Mexico. They concluded that Fe is one of the main metal contamination problem in this area, as the Fe content exceed the Mexico recommded limit [Avila-Pérez P, et al., 1999]. In Riyadh, Saudi Arabia samples from drinking water coolers in 32 schools were collected at a specific time during a typical school day and analyzed for Fe. The results showed that the Fe content was 62.40 µg/L which exceed the guideline limits recommended by WHO (50.00 µg/L) [Al-Saleh IA, 1996].

The highest lead (Pb) content was found in the drinking water sample for Mahasawat (3.50 µg/L) which is lower than the maximum acceptable level (0.05 mg/L) for the drinking water standard [TISI, 1978]. Mohamed & Osman (1998) measured Pb contents in raw Nile River water and in finished drinking water at 4 treatment plants in Greater Cairo, Egypt by collecting monthly samples during the period of September 1993 to August 1994. They reported the mean Pb content in river water was 29.6±8.74 µg/L, while the drinking water, the mean was 9.93±0.50 µg/L. Rajaratnam et al. (2002) measured Pb contents in the drinking water of 95 new houses less than 18 months old in the Sydney metropolitan area. For the first-flush samples, the mean Pb content was above the Australian Drinking Water Guidelines (ADWG) in 60% and above the US EPA Guideline in 81%. For the post-first-flush samples, the mean Pb content was above the ADWG in 24%. In fully flushed water, the mean Pb content was well below the ADWG.

The highest manganese (Mn) content was found in the raw water sample for Samsen (0.053 mg/L) which is higher than the maximum acceptable level (0.050 mg/L) for the drinking water standarard [TISI, 1978]. In 1995, Vieregge et al. Confirmed that long term exposure to Mn in rural well water has no neurological effects [Vieregge et al., 1995]. They conducted the study in rural dwellings located in northern Germany. Group A was exposed to Mn water contents of at least 0.300 mg/L, while group B was exposed to Mn water contents of less than 0.050 mg/L. No

significant difference in any neurological effect was found between two groups. A recent study in Massachusetts showed an elevated Mn content in drinking water [Woolf et al., 2002]. They reported the Mn content was 1.21 mg/ml while the USEPA guideline is less than 0.050 mg/ml.

The highest mercury (Hg) content was found in the raw water sample from Thonburi (0.0025 mg/L) which is higher than the maximum acceptable level (0.002 mg/L) for the drinking water standard [TISI, 1978]. The presence of Hg may be due to the material of HDPP pipe or tubing. In 1995, Iskander & Lyday reported that the West Bouldin site in Austin, Texas showed a Hg content of 30 ng/L which is lower than the maximum acceptable level (0.002 mg/L) allowed by the USEPA drinking water standard [Iskander & Lyday, 1995]. Wyatt and co-workers (1998) measured the Hg content of water supplied to Northern Mexico. They found that the highest mean Hg content (4.00 mg/L) was found in water from the Suaqui Grande reservoirs. The areas that presented a high Hg content were resampled and they found that the highest Hg content (25.00 mg/L) was in the water sample from Guaymas, San José No.1. Another study in Riyadh, Saudi Arabia has shown the Hg content in six first-draw and three 10-min flush household water samples were respectively 2.13 ± 1.38 and 1.53 ± 0.06 $\mu\text{g/L}$ which were higher than the EEC guideline value (1.00 $\mu\text{g/L}$) [Al-Saleh & AL-Doush, 1998].

The highest selenium (Se) content was found in the raw water sample from Thonburi (5.00 $\mu\text{g/L}$) which is lower than the maximum acceptable level (0.01 mg/L) for the drinking water standard [TISI, 1978]. Between 1972 and 1988, a few thousand residents of Reggio Emilia in northern Italy were accidentally exposed to drinking water with unusually high levels of inorganic selenium, range 7-9 $\mu\text{g/L}$, through a local public water supply system [Vincent *et al.*, 2000]. In 1995, Vinceti *et al.* (1995), in another Italian epidemiological study, reported a 7-year mortality for cancer patients who had been exposed to environmental selenium from drinking water. This present study showed that insignificant selenium content was detected in the tap and drinking water samples in all three water treatment plants, but Se was undetected in the Mahasawat plant.

The highest silver (Ag) content was found in raw water sample from Thonburi (0.146 $\mu\text{g/L}$) which is lower than the maximum acceptable level (50 $\mu\text{g/L}$)

for the drinking water standard [TISI, 1978]. USEPA (1980) reported that only 0.1% of the Canadian tap water contained more than 1-5 ng/L. Silver levels in drinking water in USA varied between “non-detectable” and 5 µg/L. The median daily intake of Ag was 7.1 µg [Gibson et al., 1984]. However, the relative contribution of drinking water is usually very low but the daily intake of Ag from drinking water can constitute the major route of oral exposure.

The highest zinc (Zn) content was found in drinking water sample from Samsen (0.244 mg/L) which is lower than the maximum acceptable level (5.00 mg/L) for the drinking water standard [TISI, 1978]. In 1998, Al-Saleh and Al-Doush measured Zn content in the drinking water of 101 households in Riyadh, Saudi Arabia to ascertain the water quality for human consumption. They reported the Zn content were much higher than the EEC guideline limit [Al-Saleh & Al-Doush, 1998]. Iskander et al. (1995) collected water samples at the mouth of each Creek and at one lake site under their investigation. They reported that the highest Zn content was found in the sample from Waller Creek with 22.00 µg/L while the average for all the samples examined was 10.00 µg/L [Iskander FY et al., 1995].

Inorganic constituents

The highest chloride (Cl⁻) content was found in raw water sample from Samsen (71.00 mg/L) which is lower than the maximum acceptable level (250 mg/L) for the drinking water standard [TISI, 1978]. In 1997, it was reported that thirty-five water samples were collected on Mt Etna during August 1994 from 14 springs, 17 water-wells and 4 drainage galleries. Collected samples were quantitatively analyzed at the Istituto Superiore di Sanità, Rome. They concluded that the abundance of Cl⁻ is the result of important interactions among water, volcanic gases, and host rocks. Therefore, the local geological factors should be taken into account when establishing national standards for drinking water quality [Giammanco S, 1997].

No cyanide contents were detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants. A recent study has confirmed the risks of the toxic effects in relation to the prolonged exposure to cyanide [Okolie & Osagie, 1999]. In 2002, Sousa et al. reported the effects of prolonged oral exposure to cyanide in rats. The rats were administered potassium cyanide (KCN) via tap water, at the concentration of which did not alter the palatability. Rats treated with cyanide 9.0

mg KCN/kg/day showed lower weight gain, presence of reabsorption vacuoles in the thyroid follicles, and hydropic degeneration in hepatocytes and epithelial cells of the renal proximal tubules [Sousa et al., 2002].

The highest fluoride content was found in the raw water sample from Samsen (0.29 mg/L) which is lower than the maximum acceptable level (0.70 mg/L) for the drinking water standard [TISI, 1978]. Fluoride may occur naturally in water or it may be added in a controlled amount. If a natural fluoride content approaches 10 mg/L, such water should be defluoridated. Therefore, maintaining an optimal fluoride content is essential in maintaining for the effectiveness and safety of the fluoridation procedure [Harwood, 1969]. Kumar and co-workers investigated the distribution of fluoride from ground water for drinking water in India. They reported high concentrations of fluorides in groundwater ranging from 0.3 to 6 mg/L which increased with alkalinity and decreased with hardness. They also indicated that the presence of weathered fluoride-bearing rocks is the apparent contributing factor influencing the fluoride content in drinking water [Kumar et al., 1991].

The highest nitrate (NO_3) content was found in the raw water sample from Bangkhaen (60.50 mg/L) which is 15 times higher than the maximum acceptable level (4.00 mg/L) for the drinking water standard [TISI, 1978]. The reasons for high nitrate in drinking water probably from the untreated waste water, or leaking sewers and other diffusive sources. The other diffusive sources stem mainly from the excessive application of artificial fertilisers and manure in agriculture [Hell et al., 1998]. Zilliox et al. (1990) and Keeney (1986) reported that the pollution of underground waters by nitrates is sometimes linked to industrial or domestic pollution but is more often correlated to agricultural practices such as the use of nitrogen fertilisers.

The highest sulfate (SO_4^{2-}) content was found in raw water sample from Bangkhaen (52.00 mg/L) which is lower than the maximum acceptable level (250 mg/L) for the drinking water standard [TISI, 1978]. Giammanco et al., (1997) analyzed the major and trace elements in the ground waters of Mount Etna, Italy. They collected 35 ground water samples from wells, springs, and drainage galleries. Two samples from eastern basin (C6-C7) showed a chloride-sulfate alkaline-earth. Isotopic data suggested that sulfate may be dissolved from gypsum in to ground water, derived directly from the dissolution of evaporitic Messinian sediments or that reduced

sulfur compounds, after they are formed from bacterial activity, are rapidly and totally turned into sulfate ions [Giammanco et al., 1997].

Organic constituents

The highest alkylbenzene sulfonate (ABS) content was found in the raw water sample from Bangkhaen (76.50 $\mu\text{g/L}$) which is lower than the maximum acceptable level (0.20 mg/L) for the drinking water standard [TISI, 1978]. A study carried out in 1959 in 32 major cities throughout the United States illustrated that ABS in surface-generated potable water averaged between 15 and 34 mg/L (Jente, 1961). Eichhorn et al. (2001) investigated the ABS content in Laguna de Bay, Philippines in December 1999 and March 2000. They found the ABS contents were 1.1-75.0 and 1.0-66.0 $\mu\text{g/L}$ respectively.

No phenols contents were detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants. Davi & Gnudi (1999) determined the phenolic compounds in Italy's River Po during a 3-year period (1994-1996) at 15 day intervals, and found that 3-Tert-butyl-4-methoxyphenol (BHA) and its isomers were present at the highest content.

CHAPTER VI

CONCLUSION

1. No *Cryptosporidium* oocyst was found in any of the raw water or tap water samples.
2. *Cryptosporidium* oocysts were observed in 11.76% (4/34) of the drinking water samples from the schools serviced by the Bangkhaen water treatment plant.
3. *Cryptosporidium* oocysts were observed in 7.41% (4/54) of the 54 schools with a mean of 0.74 oocyst per litre.
4. The presence of *Cryptosporidium* oocysts in drinking water samples suggests that the water supply pipelines may have leakages, allowing oocysts from faecal matter to enter the water distribution systems or it could be caused from the children's hands as they turned on a faucet to drink from it.
5. Total coliforms, *E. coli*, *Salmonella* spp., *Clostridium perfringens*, and *Yersinia enterocolitica* were found in each of the 4 raw water samples.
6. The presence of total coliforms, *E. coli*, *Salmonella* spp., *Clostridium perfringens*, and *Yersinia enterocolitica* may be due to their survival, movement, rain, temperature, or presence of cattle.
7. No microbiological contaminations were found in tap water or drinking water samples from any of the 4 water treatment plants. This would suggest that Bangkok's water treatment processes are adequate to produce microorganism-free drinking water.
8. The physical and chemical qualities of tap and drinking water samples from the 4 water treatment plants were lower than the maximum acceptable level for the drinking water standard.
9. Cadmium, cyanide and phenols were not detected in the raw, tap, and drinking water samples from any of the 4 water treatment plants.

10. The highest lead (Pb) content was found in drinking water sample from Mahasawat (3.50 $\mu\text{g/L}$) but it is still lower than the maximum acceptable level (50 $\mu\text{g/L}$) for the drinking water standard.
11. The highest zinc (Zn) content was found in drinking water sample from Samsen (0.244 mg/L) but it is still lower than the maximum acceptable level (5.00 mg/l) for the drinking water standard.
12. Because of the 2 schools which are found for highest Pb and Zn are in Mahasawat and Samsen while *Cryptosporidium* oocysts were found in Bangkhaen plant, therefore, there is no correlation between the chemical qualities and waterborne microbe quality.



CHAPTER VII

FURTHER STUDY

To prevent the risk of Cryptosporidiosis by *Cryptosporidium* consumption via drinking water, the research should be emphasized in molecular studies. Therefore, the further studies are as follow;-

1. Investigate water samples in different season
2. Evaluate the correlation of the *Cryptosporidium* oocyst and the surrounding.
3. Identify genotype of *Cryptosporidium* oocyst by using molecular techniques

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APPENDIX A
Waterborne Microbe Assay Analysis for
***Cryptosporidium* spp.**

The following describes the Equipment and Material, Reagents, Procedure, and Staining Process for the Waterborne Microbe Assay Analysis of *Cryptosporidium* Spp.

EQUIPMENT AND MATERIAL

1. 0.45- μ m-pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Sterile plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Sterile 50-ml centrifuge tube
(Corning Costar Corp., Cambridge, MA, USA)
4. 5-speed Cyclo-Mixer
(Becton, Dickinson and Co., Parsippany, NJ, USA)
5. High performance refrigerated centrifuge
(KONTRON Centrikon H-401, Zurich)
6. Culture loop
7. Timer (QT9014-A Citizen, Trading Co., Ltd., Japan)
8. Electrical microscope (BH2 Olympus Co., Ltd. Japan)
9. Microscope slides

REAGENTS (R) (Appendix AA)

1. Deionised distilled water (DDW)
2. Absolute methanol (J.T.Baker, Phillipsburg, NJ, USA)
3. Fuchsin (basic) dye (R1) (BDH Laboratory, England)
4. Tap water
5. Decolorising solution (R3)
6. 6% Methylene blue (R2)

PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.
4. DDW (10 ml) was poured into a centrifuge tube.
5. The filter membrane was removed aseptically from the plastic filter holder and was inserted aseptically into the centrifuge tube containing the DDW.
6. The centrifuge tube was held in a 5-speed Cyclo-Mixer and was vibrated at maximum speed for 30 sec.
7. The filter membrane was removed aseptically from the centrifuge tube.
8. The centrifuge tube was placed in a high performance refrigerated centrifuge and centrifuged at 1,800 x g at 4°C for 10 min.
9. A supernatant (8-9 ml) was withdrawn from the centrifuge tube.
10. The DDW (approximately 1-2 ml) that remained in the centrifuge tube was used for staining.

STAINING PROCESS (modified Kinyoun acid-fast staining method)

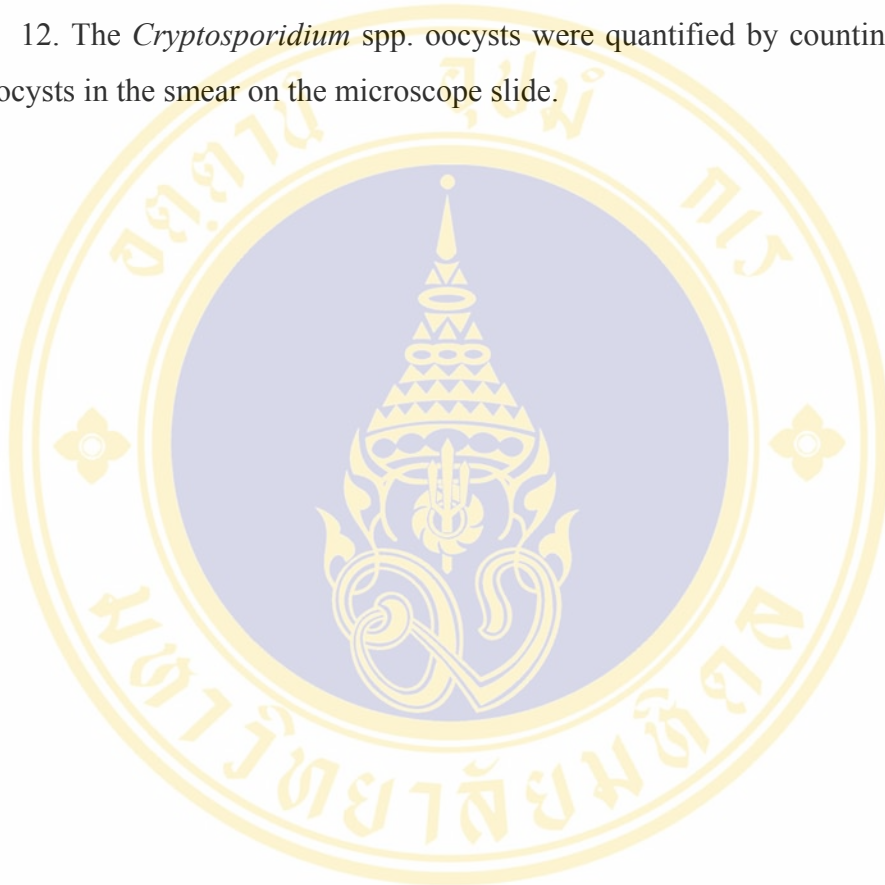
1. A sterile culture loop was used to spread the DDW (1-2 ml) on a microscope slide and the smear was allowed to air dry.
2. The microscope slide was immersed into a jar containing absolute methanol for 5 min.
3. The microscope slide was removed from the absolute methanol and the smear was allowed to air dry.
4. Enough fuchsin (basic) dye was dropped onto the microscope slide so that the smear was completely covered and the smear was allowed to stand for 10 min.
5. The microscope slide was rinsed in a jar containing tap water.
6. A decolorising solution was dropped onto the microscope slide until no more purple dye could be removed from the smear.
7. The microscope slide was rinsed briefly in a jar containing tap water.
8. Enough 6% methylene blue was dropped onto the microscope slide and the smear was allowed to stand for 10 min.

9. The microscope slide was rinsed briefly in a jar containing tap water and allowed to air dry.

10. The smear was examined at 100x magnification.

11. *Cryptosporidium* spp. oocysts were identified by the following criteria: size (4-7 μm), shape (spherical), and red-stain color.

12. The *Cryptosporidium* spp. oocysts were quantified by counting the number of oocysts in the smear on the microscope slide.





APPENDIX B

The Microbiological Assay Analysis for Total Coliform Bacteria

The following describes the Equipment and Material, Medium, and Procedure for the Microbiological Assay Analysis of Total Coliform Bacteria.

EQUIPMENT AND MATERIAL

1. 0.45- μm -pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $35\pm 1^{\circ}\text{C}$

MEDIUM (M) (Appendix BB)

1. Modified fecal coliform (m-FC) agar (M1)

PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.
4. The filter membrane was removed aseptically from the plastic filter holder, and was placed on m-FC agar.
5. The m-FC agar was incubated for 24 ± 2 h at 35°C .
6. A square on the filter membrane that had a blue colony indicated the presence of the total coliform bacteria.
7. The filter membrane was examined by counting the number of squares containing blue colonies.
8. The squares containing blue colonies were quantified and were converted to the most probable number (MPN) and were reported as MPN of total coliform bacteria/100 ml by:

$$MPN = N \log_e \left[\frac{N}{(N - X)} \right]$$

Key: N = total number of squares on the filter membrane

X = number of squares containing blue colonies





APPENDIX C

The Microbiological Assay Analysis for *Escherichia coli* (*E. coli*)

The following describes the Equipment and Material, Reagent, Media, and Procedure for the Microbiological Assay Analysis of *Escherichia coli* (*E. coli*).

EQUIPMENT AND MATERIAL

1. 0.45- μm -pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $35 \pm 1^\circ\text{C}$
4. Covered water bath at $44.5 \pm 0.5^\circ\text{C}$
5. Filter paper
6. Petri dish

REAGENT (R) (Appendix AA)

1. Kovacs' reagent (R5)

MEDIA (M) (Appendix BB)

1. Tryptic soy-magnesium sulfate agar (TSAM) (M2)
2. Tryptone bile agar (TBA) (M3)

PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.
4. The filter membrane was removed aseptically from the plastic filter holder, and was placed on TSAM medium.
5. The TSAM medium was incubated for 4-5 h at 35°C .

6. The filter membrane was transferred aseptically from the TSAM medium to TBA medium.

7. The TBA medium was incubated in a covered water bath for 24 ± 2 h at 44.5°C .

8. Filter paper was placed in a Petri dish and the filter paper was flooded with Kovacs' reagent.

9. The filter membrane was removed aseptically from the TBA medium; and was placed on the filter paper and was allowed to stand for 10-15 min.

10. The filter membrane was transferred from the filter paper back to the TBA medium.

11. A square on the filter membrane that had a pink colony indicated the presence of *E. coli*.

12. The squares containing pink colonies were quantified and were converted to the most probable number (MPN) and were reported as MPN of *E.coli*/100 ml by:

$$MPN = N \log_e \left[\frac{N}{(N - X)} \right]$$

Key: N = total number of squares on the filter membrane

X = number of squares containing pink colonies



APPENDIX D

The Microbiological Assay Analysis for *Clostridium perfringens* (*C. perfringens*)

The following describes the Equipment and Material, Reagents, Media, and Procedure for the Microbiological assay analysis of *Clostridium perfringens* (*C. perfringens*).

EQUIPMENT AND MATERIAL

1. 0.45- μm -pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $35\pm 1^{\circ}\text{C}$
4. Anaerobic jar
5. Sterile inoculating needle

REAGENTS (R) (Appendix AA)

1. Sulfanilic acid (R6)
2. N-(1-naphthyl)ethylenediamine (R7)

MEDIA (M) (Appendix BB)

1. Tryptose sulfite cycloserine (TSC) agar (M4)
2. Motility-nitrate medium (M5)
3. Lactose-gelatin medium (M6)

PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.

4. The filter membrane was removed aseptically from the plastic filter holder and the filter membrane was placed on TSC agar.

5. The TSC agar was incubated in an anaerobic jar for 24 ± 2 h at 35°C .

6. The presence of a black colonies indicated suspected *C. perfringens*.

7. The filter membrane was examined and the number of squares containing black colonies were quantified.

8. A sterile inoculating needle was used to pick an individual black colony from the filter membrane; and was stabbed once in the motility-nitrate medium, followed by two stabbings in the lactose-gelatin medium.

9. The process in Step 8 was applied to one colony from at least 5 individually distinctive black-colony groups.

10. The motility-nitrate media and the lactose-gelatin media were incubated for 24 ± 2 h at 35°C .

11. Examination of the motility-nitrate medium and the lactose-gelatin medium for suspected *C. perfringens*.

Media	Positive results
Motility-nitrate	a) growth along the stab line b) reduction of nitrate to nitrite (violet colour develop) within 5 min after 0.5 ml of sulfanilic acid and 0.2 ml of N-(1-naphthyl) ethylenediamine were added.
Lactose-gelatin medium	a) production of H_2S (g) b) liquefaction of the gelatin after chilled for ≥ 1 h at 5°C

12. If the motility-nitrate medium and the lactose-gelatin medium examination were found to be positive, the presence of *C. perfringens* was reported.



APPENDIX E

The Microbiological Assay Analysis for *Salmonella* spp.

The following describes the Equipment and Material, Reagents, Media, Isolation Procedure, and Serological Test for the Microbiological Assay Analysis of *Salmonella* spp.

EQUIPMENT AND MATERIAL

1. 0.45- μm -pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $35\pm 1^\circ\text{C}$
4. Sterile inoculating loop
5. Sterile pipette
6. Wax pencil
7. Microscope slides

REAGENTS (R) (Appendix AA)

1. Kovacs' reagent (R5)
2. 0.85% saline solution (R8)
3. Salmonella polyvalent somatic O antiserum (R9)

MEDIA (M) (Appendix BB)

1. Tetrathionate (TT) broth (M7)
2. Selenite cystine (SC) broth (M8)
3. Xylose lysine desoxycholate (XLD) agar (M9)
4. Hektoen enteric (HE) agar (M10)
5. Triple sugar iron (TSI) slant (M11)
6. Lysine iron agar (LIA) slant (M12)

ISOLATION PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.
4. The filter membrane was removed aseptically from the plastic filter holder, and then was inserted into the TT broth.
5. The process in Steps 1-4 was repeated, and the filter membrane was inserted into the SC broth.
6. Both the TT broth and the SC broth were incubated for 24 ± 2 h at 35°C .
7. A sterile inoculating loop was used to remove one loop of the TT broth, and then to streak the TT broth on the XLD agar.
8. The process in Step 7 was repeated, and the TT broth was streaked on the HE agar.
9. The process in Steps 7 and 8 were followed for the SC broth being streaked on the XLD agar and the HE agar.
10. The XLD agar and the HE agar were incubated for 24 ± 2 h at 35°C .
11. The XLD agar and the HE agar were examined for the presence of suspected *Salmonella* spp.
12. Examination of the XLD agar and the HE agar for suspected *Salmonella* spp.

Agar	Positive results
XLD	pink or black colony or had become pink colony with a black center
HE	blue, blue-green, or black colony or had become blue, or blue-green colony with a black center

13. A sterile inoculating needle was used to pick individually suspected *Salmonella* spp. colonies from the XLD agar, and inoculated each colony into an individual TSI slant by streaking the slant, and stabbing the butt.

14. For each colony, the inoculating needle without having been flamed was then used to stab twice an LIA butt and to streak an LIA slant.

15. The process in Steps 13 and 14 were repeated for the HE agar.

16. The TSI slants and the LIA slants were incubated for 24 ± 2 h at 35°C .

17. Examination of the TSI slant and the LIA slant for suspected *Salmonella* spp.

Media	Positive results
TSI	yellow butt, blackening
LIA	Purple butt, blackening

18. All colonies suspected of *Salmonella* spp. were submitted for a *Salmonella* spp. identification procedure by use of a serological test.

SEROLOGICAL TEST

1. A wax pencil was used to mark two sections on a microscope slide.

2. 0.85% saline solution was dropped on both sections of a microscope slide.

3. A sterile inoculating needle was used to pick a suspected colony from the TSI slant, and then to emulsify the colony with 0.85% saline solution on both sections of the microscope slide.

4. One drop of *Salmonella* polyvalent somatic O antiserum was added to one section of the microscope slide.

5. The microscope slide was tilted in a back-and-forth motion for one minute, and was observed against a dark background under good illumination.

6. Examination of the serological test for the presence of *Salmonella* spp.

Microscope slide	0.85% saline solution (drop)	Suspected <i>Salmonella</i> spp. (loop)	<i>Salmonella</i> polyvalent somatic O antiserum (drop)	Positive results (agglutination)
I	1	1	1	found
II	1	1	1	Not found

7. If the results of Step 6 was positive, the presence of *Salmonella* spp. colony was reported.



APPENDIX F

The Microbiological Assay Analysis for *Staphylococcus aureus* (*S. aureus*)

The following describes the Equipment and Material, Reagent, Media, Isolation Procedure, and Coagulase Test for the Microbiological Assay Analysis of *Staphylococcus aureus* (*S. aureus*).

EQUIPMENT AND MATERIAL

1. 0.45- μm -pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $35\pm 1^\circ\text{C}$
4. Covered water bath at $44.5 \pm 0.5^\circ\text{C}$
5. Sterile test tubes
6. Sterile inoculating needle

REAGENTS (R) (Appendix AA)

1. Rabbit plasma with EDTA (Merck, Darmstadt, Germany) (R10)

MEDIA (M) (Appendix BB)

1. Baird-Parker agar (M14)
2. Brain Heart infusion (BHI) broth (M15)

PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.

4. The filter membrane was removed aseptically from the plastic filter holder, and was placed on Baird-Parker medium.
5. The Baird-Parker agar was incubated for 48 ± 3 h at 35°C .
6. The Baird-Parker agar was examined for the presence of *S. aureus*.
7. If a colony was black, the presence of *S. aureus* was suspected, and the colony was submitted for a coagulase test.

COAGULASE TEST

1. 0.25 ml BHI broth was dispensed into a sterile test tube.
2. A sterile inoculating needle was used to pick individually suspected colonies from the filter membrane and to inoculate each colony into an individual test tube.
3. The test tubes were incubated in a covered water bath for 2 h at 44.5°C .
4. 0.25 ml Rabbit Plasma with EDTA was dispensed into each test tube.
5. Each test tube was incubated for 4 h at 44.5°C .
6. The test tubes were examined for the presence of *S. aureus*.
7. If a clot formation had occurred and if the clot stayed in place when the test tube was inverted, the presence of *S. aureus* was reported.



APPENDIX G

The Microbiological Assay Analysis for *Campylobacter jejuni* (*C. jejuni*)

The following describes the Equipment and Material, Reagents, Media, Isolation Procedure, Identification Procedure, confirmation Procedure, and biochemical test for the Microbiological Assay Analysis of *Campylobacter jejuni* (*C. jejuni*).

EQUIPMENT AND MATERIAL

1. 0.45- μ m-pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $30\pm 2^{\circ}\text{C}$
4. Incubator at $42\pm 1^{\circ}\text{C}$
5. Anaerobic jar
6. Sterile inoculating loop
7. Wax pencil
8. Microscope slide
9. Sterile Pasteur pipette

REAGENTS (R) (Appendix AA)

1. Cefoperazone (R11)
2. 0.85% saline solution (R8)
3. 30% Hydrogen peroxide (H_2O_2) (R12)
4. Gram stain reagents (R13); counterstain with 0.5% carbol fuchsin (BDH Laboratory, England)

MEDIA (M) (Appendix BB)

1. Hunt enrichment broth (M16)
2. Modified Campy blood-free agar (CCDA) (M17)
3. Abeyta-Hunt blood agar (M18)

PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.
4. The filter membrane was removed aseptically from the plastic filter holder and then inserted into the Hunt enrichment broth.
5. The Hunt enrichment broth was incubated for 3 h at 30°C under carbondioxide (CO₂) condition.
6. 400 µl cefoperazone were dispensed into the Hunt enrichment broth.
7. The Hunt enrichment broth was incubated for 24-48 h at 42°C under CO₂ condition.
8. A sterile inoculating loop was used to remove one loop of the Hunt enrichment broth and then to streak the Hunt enrichment broth on an Abeyta-Hunt blood agar.
9. The process in Step 12 was followed for CCDA agar .
10. The Abeyta-Hunt blood agar and CCDA agar were incubated for 24-48 h at 42°C under CO₂ condition.
11. The Abeyta-Hunt blood agar and the CCDA agar were examined for suspected *C. jejuni*.
12. Examination of the Abeyta-Hunt blood agar and the CCDA agar for suspected *C. jejuni*.

Agar	Positive results
Abeyta-Hunt blood agar	Rainbow sheen colony
CCDA	Round to irregular with smooth edges with a translucent white to spreading film-like transparent growth

IDENTIFICATION PROCEDURE

1. 0.85% saline solution was dropped on a microscope slide.
2. A sterile inoculating needle was used to pick the suspected colony from the agar and then to emulsify the colony with 0.85% saline solution on the microscope slide.
3. An electrical microscope was used to examined the microscope slide.

CONFIRMATION PROCEDURE

1. A sterile Pasteur pipette was used to drop 30% H₂O₂ on the microscope slide.
2. The sterile Pasteur pipette was used to pick the suspected colony from the agar and then to emulsify the colony with 30% H₂O₂ on the microscope slide.

BIOCHEMICAL TEST

1. A Gram stain method with 0.5% carbol fuchsin as counterstain was used to stain the suspected colony.

Examination of identification, confirmation, and biochemical test for the presence of *C.jejuni*.

Procedure/Test	Positive results
Identification	curved, 1-1.5 μm in length, wiggly motility, and usually in chain resembling zigzag shape
Confirmation	bubbles occur on the microscope slide
Biochemical	Gram negative (red stain).



APPENDIX H

The Microbiological Assay Analysis for *Listeria monocytogenes* (*L. monocytogenes*)

The following describes the Equipment and Materials, Reagents, Media, Isolation Procedure, and Identification Procedure for the Microbiological Assay Analysis of *Listeria monocytogenes* (*L. monocytogenes*).

EQUIPMENT AND MATERIALS

1. 0.45- μm -pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $30\pm 1^{\circ}\text{C}$
4. Incubator at $35\pm 1^{\circ}\text{C}$
5. Sterile inoculating loop

REAGENTS (R) (Appendix AA)

1. Acriflavin HCl (R14)
2. Nalidixic acid (Sodium salt) (R15)
3. Cycloheximide (R16)

MEDIUM (M) (Appendix BB)

1. Enrichment broth medium (M19)
2. Oxford medium (OXA) (M20)
3. PALCAM agar (M21)
4. Trypticase soy agar with 0.6% yeast extract (TSAYE) (M22)

PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.

2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.
4. The filter membrane was removed aseptically from the plastic filter holder and then inserted into an EB medium.
5. The EB medium was incubated for 4 h at 30°C.
6. 400 µl of Acriflavin HCl, nalidixic acid, and cycloheximide were added in the EB, and incubation was continued an additional 20 h at 30°C.
7. A sterile inoculating loop was used to remove one loop of EB medium and then to streak on an OXA medium.
8. The process in Step 7 was repeated to streak EB medium on a PALCAM agar.
9. Both the OXA medium and the PALCAM agar were incubated for 24-48 h at 35°C.
10. The OXA medium and the PALCAM agar were examined for the presence of colonies that may be *L. monocytogenes*.
11. Examination of the OXA medium and PALCAM agar for suspected *L. monocytogenes*.

Media	Positive results
OXA	black colony
PALCAM	Sparkling blue or white

IDENTIFICATION PROCEDURE

1. A sterile inoculating needle was used to pick a suspected colony from the OXA medium and then to streak on the TSAYE medium.
2. The process in Step 1 was repeated for the PALCAM agar which then was streaked on the TSAYE medium.
3. The TSAYE media were incubated for 24-48 h at 35°C.
4. The TSAYE media were examined for the presence of *L. monocytogenes*
5. If a colony on the TSAYE media had become blue-gray to blue, *L. monocytogenes* was present and was reported.



APPENDIX I

The Microbiological Assay Analysis for *Yersinia enterocolitica* (*Y. enterocolitica*)

The following describes the Equipment and Materials, Reagent, Media, and Isolation Procedure for the Microbiological Assay Analysis of *Yersinia enterocolitica* (*Y. enterocolitica*).

EQUIPMENT AND MATERIALS

1. 0.45- μm -pore-size cellulose nitrate filter membranes
(Millipore Corp., New Bedford, MA, USA)
2. Plastic filter holder
(Millipore Corp., New Bedford, MA, USA)
3. Incubator at $35\pm 1^\circ\text{C}$
4. Refrigerator at 5°C
5. Sterile inoculating loop
6. Sterile inoculating needle

REAGENTS (R) (Appendix AA)

1. Kovacs' reagent (R5)

MEDIA (M) (Appendix BB)

1. Peptone sorbitol bile broth (PSBB) (M23)
2. MacConkey agar (M24)
3. Celfsulodin-irgasan-novobiocin medium (CIN) (M25)
4. Lysine arginine iron agar (LAIA) (M26)
5. Christensen's urea (CUA) (M27)
6. Bile esculin agar (M28)

ISOLATION PROCEDURE

1. A filter membrane was placed into a sterile plastic filter holder.
2. A water sample (100 ml) was added to the plastic filter holder.
3. A vacuum was created in the plastic filter holder.
4. The filter membrane was removed aseptically from the plastic filter holder and then was inserted into a PSBB medium. .
5. The PSBB medium was incubated for 10 days at 5°C.
6. A sterile inoculating loop was used to remove one loop of the PSBB medium and then to streak on a MacConkey agar.
7. The process in Step 6 was repeated, and the PSBB medium was streaked on a CIN agar.
8. The MacConkey agar and the CIN agar were incubated for 24±1 h at 35°C.
9. The agars were examined for suspected *Y. enterocolotica*.
10. Examination of the MacConkey agar and CIN agar for suspected *Y. enterocolotica*.

Media	Positive results
MacConkey	small, flat, colorless, or pale pink colony
CIN agar	small colony with a red center, had a sharp border, and had a colorless zone

11. A sterile inoculating needle was used to pick suspected colonies from both the MacConkey agar and the CIN agar and to stab the colonies into LAIA slant, CUA slant, and bile esculin slant.

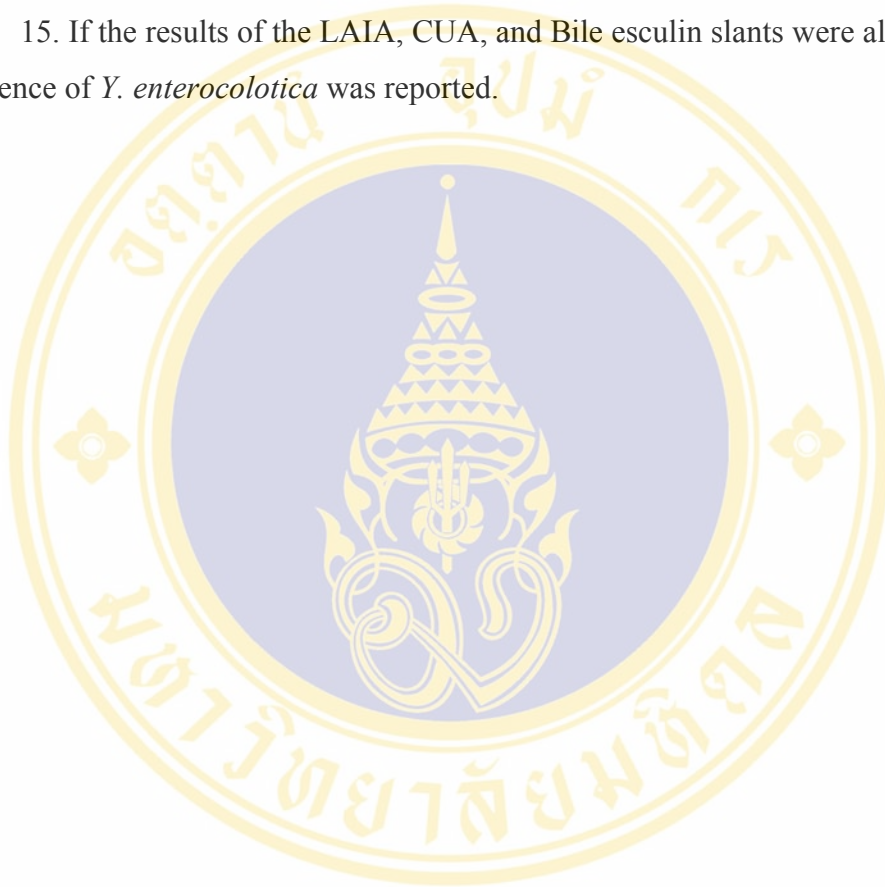
12. The LAIA slant, CUA slant, and bile esculin slant were incubated for 48±2 h at room temperature.

13. The LAIA slant, CUA slant, and bile esculin slant were examined for the presence of *Y. enterocolotica*.

14. Examination of the LAIA, CUA, and Bile esculin slants for the presence of *Y. enterocolotica*.

Media	Positive results
LAIA	not change in colour, no production of H ₂ S (g)
CUA	pink slant, yellow butt
Bile esculin	not change in colour

15. If the results of the LAIA, CUA, and Bile esculin slants were all positive, the presence of *Y. enterocolitica* was reported.





APPENDIX J

DETERMINATION OF THE COLOUR VALUE BY USING THE VISUAL COMPARISON METHOD

PRINCIPLE

Colour was determined by compare the sample colour with known concentration of coloured solutions. The comparison also made with special glass tube. The platinum-cobalt unit of colour provided by 1 mg platinum/L in the form of the chloroplatinate ion. The following describes the Material, Reagents, Procedure, and Calculation used to determine the Colour value by the Visual Comparison Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

MATERIAL

1. Nessler tubes, 50 ml (tall)

REAGENTS

1. Calcium chloride (CaCl_2) solution

300 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were dissolved in deionised distilled water (DDW), and then diluted with DDW to 500 ml.

2. Stock standard solution

1.246 g of potassium chloroplatinate (K_2PtCl_6) and 1.000 g of crystallised cobaltous chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) were dissolved in DDW. 100 ml of concentrated hydrochloric acid (HCl) were added, and then diluted with DDW to 100 ml. This stock standard solution had a colour of 500 Pt-Co units.

3. Colour standard solution

0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml of stock standard solution in nessler tubes were diluted with DDW to 50 ml. These standard solutions had a colour of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 Pt-Co units.

PROCEDURE

1. Turbidity removal

100 ml of the sample and 1.00 ml of CaCl₂ solution were poured into a glass tube and were centrifuged until precipitation occurred. The solution was then compared with DDW in another exact glass tube.

2. Measurement of sample

The sample colour was observed by filling a nessler tube to the 50-ml mark and comparing the colour of the sample with the colour standard solution by looking vertically downward through the tube toward a white surface.

If the colour exceeded 50 Pt-Co units, the sample was diluted with DDW in a known proportion until the colour was ≤ 50 Pt-Co units and thereby within the range of the colour standard.

CALCULATION

Colour units were calculated by the following equation:

$$\text{Colour units} = \frac{A \times 50}{B}$$

Key: A = estimated Pt-Co units of sample

B = sample taken for comparison, ml



APPENDIX K

DETERMINATION OF THE ODOUR BY USING THE SENSORY TEST

PRINCIPLE

Odour was determined by using the sensory testing and a sample temperature of 60°C will permit detection of odour that otherwise might be missed. The following describes the Material, Procedure, and Determination used to determine the Odour by the Sensory Test Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

MATERIAL

1. Erlenmeyer flask, 500 ml

PROCEDURE

250 ml of the sample were very slowly swirled in an erlenmeyer flask at room temperature, and then slowly sniffed for odour.

If odour was present but was slight, the flask was heated to 60°C and again sniffed.

DETERMINATION

	Odour	Report
Sample	sensed	found
Sample	not sensed	not found



APPENDIX L

DETERMINATION OF THE pH VALUE BY USING THE ELECTROMETRIC METHOD

PRINCIPLE

pH value was determined potentiometrically by using a glass electrode. The electromotive force (emf) produced in the glass electrode system varied linearly with pH value. The following describes the Equipment, Reagents, and Procedure used to determine the pH value by the Electrometric Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT

1. Glass-electrode pH meter
(Model Digiphase, Cole Parmer, Santa Ana, CA, USA)

REAGENTS

1. Standard buffer solution, pH 4.01 at 25°C
(Orion Research Inc., Beverly, MA, USA)
2. Standard buffer solution, pH 7.00 at 25°C
(Orion Research Inc., Beverly, MA, USA)

PROCEDURE

1. Calibration

Electrodes were removed from the storage solution, rinsed, blotted dry with a soft tissue, and placed in a pH 4.01 buffer solution; and the isopotential point was set. The electrodes were removed from the pH 4.01 buffer solution, rinsed thoroughly with distilled water, blotted dry with a soft tissue, and immersed in a pH 7.00 buffer solution; and the isopotential point

was set. The electrodes were removed from the pH 7.00 buffer solution, rinsed thoroughly with distilled water, and blotted dry with a soft tissue.

2. Measurement of sample

The electrodes were immersed in the sample for 1 min, and a pH value was read directly from the meter.





APPENDIX M

DETERMINATION OF THE TURBIDITY VALUE BY USING THE NEPHELOMETRIC METHOD

PRINCIPLE

Turbidity was determined by comparing the intensity of light scattered by the sample with the intensity of light scattered by a working standard solution under the same conditions. The higher the intensity of scattered light, the higher the turbidity value. The following describes the Equipment and Material, Reagents, Procedure, and Interpretation of Results used to determine the Turbidity value by the Nephelometric Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Nephelometer (Model 131S, Orion Research Inc., Beverly, MA, USA)
2. Sample cells

REAGENTS

1. Solution I

1.000 g of hydrazine sulfate $[(\text{NH}_2)_2 \cdot \text{H}_2\text{SO}_4]$ was dissolved with deionised distilled water (DDW) and diluted in a volumetric flask to 1000 ml.

2. Solution II

10.000 g of hexamethylenetetramine $[(\text{CH}_2)_6\text{N}_4]$ were dissolved with DDW and diluted in a volumetric flask to 100 ml.

3. Stock standard suspension

5.0 ml of Solution I and 5.0 ml of Solution II were mixed in a flask, and allowed to stand for 24 h at $25 \pm 3^\circ\text{C}$. This resulted in a 4000-NTU stock standard suspension. The stock suspension was transferred to a UV-light-blocking bottle for storage, and would remain stable for up to 1 year if properly stored.

4. Working standard solution

The stock standard solution was used to prepare the working standard solutions.

PROCEDURE

1. Calibration

At least 1 working standard solution in nephelometer range was used to check the stable readings of all sensitivity ranges to be used.

2. Measurement of sample

Sample was gently agitated. The air bubbles were allowed to disappear, and the sample was poured sample into sample cell. Turbidity was read directly from instrument display.

INTERPRETATION OF RESULTS

Turbidity was reported as followed:

Turbidity Range (NTU)	Report to the nearest NTU
0-1	0.05
1-10	0.10
10-40	1.00
40-100	5.00
100-400	10.00
400-1000	50.00
>1000	100.00



APPENDIX N

DETERMINATION OF THE HARDNESS CONTENT BY CALCULATION

PRINCIPLE

Hardness was defined as the sum of the calcium (Ca) and magnesium (Mg) contents, both expressed as calcium carbonate (CaCO₃). The mg of hardness as CaCO₃ was calculated by using the following equation:

$$\text{mg hardness as CaCO}_3/\text{L} = 2.497 [\text{Ca, mg/L}] + 4.118 [\text{Mg, mg/L}]$$

The following describes the Equipment, Reagents, and Procedure used to determine the Calcium and Magnesium contents for Hardness as CaCO₃ in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT

1. Flame atomic absorption spectrophotometer
(GBC Scientific Equipment Pty Ltd, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 µl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.
3. 4 N nitric acid (HNO₃)
276.967 ml of concentrated HNO₃ were pipetted into a 1000 ml volumetric flask, and then diluted with DDW to 1000 ml.

4. 5% lanthanum (La₂O₃) solution

11.730 g of La₂O₃ were dissolved with 50 ml of concentrated hydrochloric acid (HCl), mixed until the solution cleared, and then in a volumetric flask were diluted with DDW to 200 ml .

5. Working standard solution

Concentration (mg/ml)	Internal standard (ml)	4 N HNO ₃ (ml)	5% La ₂ O ₃ (ml)	DDW (ml)	Total volume (ml)
0.100	0.1	0.5	1.0	3.4	5
0.200	0.2	0.5	1.0	3.3	5
0.300	0.3	0.5	1.0	3.2	5
0.400	0.4	0.5	1.0	3.1	5

6. Blank solution

10 ml of 4 N HNO₃ and 20 ml of 5% Lanthanum solution were pipetted into a 100 ml volumetric flask, and then diluted with DDW to 100 ml.

PROCEDURE

The calcium content was determined by using a flame atomic absorption spectrophotometer under the following conditions:

Lamp current	5.00 mA
Wavelength	422.70 nm
Slit width	0.50 nm
Flame type	Air-Acetylene

A standard curve from the working standard solution was plotted. The calcium content was determined by comparing the absorbance of the sample with the standard curve.

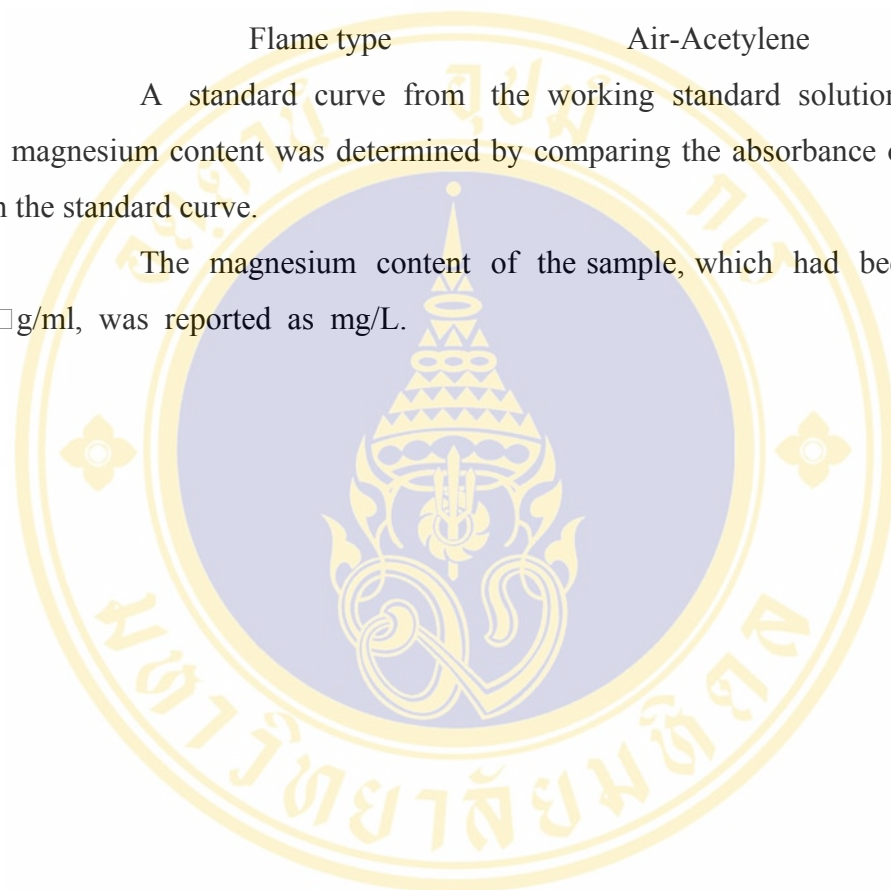
The calcium content of the sample, which had been expressed in □g/ml, was reported as mg/L.

The magnesium content was determined by using a flame atomic absorption spectrophotometer under the following conditions:

Lamp current	3.00 mA
Wavelength	285.20 nm
Slit width	0.50 nm
Flame type	Air-Acetylene

A standard curve from the working standard solution was plotted. The magnesium content was determined by comparing the absorbance of the sample with the standard curve.

The magnesium content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.





APPENDIX O

DETERMINATION OF THE TOTAL SOLIDS CONTENT BY DRYING AT 103-105⁰C.

PRINCIPLE

A well-mixed sample was evaporated in a weighed porcelain dish and dried to constant weight in an oven at 103-105⁰C. The increase in weight over that of the empty dish represented the total solids content. The following describes the Equipment and Material, Procedure, and Calculation used to determine the Total Solids Content by drying at 103-105⁰C in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Porcelain dish, 90 ml diameter
2. Drying oven at 103-105⁰C
3. Desiccator
4. Analytical balance
5. Steam bath

PROCEDURE

1. Preparation of porcelain dish

A porcelain dish was heated for 1 h at 103-105⁰C and cooled and stored in a desiccator.

The porcelain dish was weighed immediately before being used.

2. Sample analysis

100 ml of the sample were pipetted into the porcelain dish, and then evaporated to dryness on a steam bath.

The evaporated sample was dried for at least 1 h at 103-105⁰C.

The porcelain dish was cooled in the desiccator and then weighed.

The cycle of drying, cooling, desiccating, and weighing was repeated until a constant weight of the porcelain dish was obtained, or until the weight change was less than 0.1 mg.

CALCULATION

Total solids were calculated by the following equation:

$$\text{mg total solids/L} = \frac{(A - B) \times 1000}{C}$$

Key: A = weight of dried residue + dish, mg

B = weight of dish, mg

C = sample volume, ml



APPENDIX P

DETERMINATION OF THE ALUMINUM, BARIUM, CADMIUM, CHROMIUM, COPPER, LEAD, MANGANESE, SELENIUM, AND SILVER CONTENTS BY USING THE GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRIC METHOD

PRINCIPLE

A sample was dispensed into the graphite tube and was heated in 3 stages. First, a low current heated the tube to dry the sample. Second, organic matter and matrix components were destroyed and were volatilised at an intermediate temperature. Finally, a high current heated the tube to incandescence and, in an inert atmosphere, atomised the element being determined. The resultant ground-state atomic vapour absorbed monochromatic radiation from the source and photoelectric detector measured the intensity of transmitted radiation. The inverse of the transmittance was related logarithmically to the absorbance, which was directly proportioned to the number density of vapoured ground-state atoms. The following describes the Equipment and Material, Reagents, and Procedure used to determine the Aluminum (Al), Barium (Ba), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Manganese (Mn), Selenium (Se), and Silver (Ag) Contents by the Graphite Furnace Atomic Absorption Spectrophotometric Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, Victoria, Australia)

REAGENTS

Reagents for the determination of the Al, Ag, Ba, Cd, Mn, Pb, and Se contents.

1. Stock standard solution (Merck, Darmstadt, Germany)

2. Internal standard solution

25 μ l of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.

3. Working standard solution

Concentration (μ g/ml)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

Reagents for the determination of Cr and Cu contents.

1. Stock standard solution (Merck, Darmstadt, Germany)

2. Internal standard solution

25 μ l of stock standard solution were diluted with DDW to 25 ml (Intermediate standard solution).

500 μ l of intermediate standard solution were diluted with DDW to 5 ml. (Internal standard solution).

3. Working standard solution

Concentration (μ g/ml)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The aluminum content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	10.0 mA
Wavelength	396.2 nm
Slit width	0.5 nm
Gas type	Nitrogen

The barium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	20.0 mA
Wavelength	553.6 nm
Slit width	0.5 nm
Gas type	Nitrogen

The cadmium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	4.0 mA
Wavelength	228.8 nm
Slit width	0.5 nm
Gas type	Nitrogen

The chromium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	7.0 mA
Wavelength	357.9 nm
Slit width	0.2 nm
Gas type	Nitrogen

The copper content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	4.0 mA
Wavelength	327.4 nm
Slit width	0.5 nm
Gas type	Nitrogen

The lead content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	5.0 mA
Wavelength	283.3 nm
Slit width	0.5 nm
Gas type	Nitrogen

The manganese content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	5.0 mA
Wavelength	279.5 nm
Slit width	0.2 nm
Gas type	Nitrogen

The selenium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	10.0 mA
Wavelength	196.0 nm
Slit width	1.0 nm
Gas type	Nitrogen

The silver content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	4.0 mA
Wavelength	328.1 nm
Slit width	0.5 nm
Gas type	Nitrogen

Standard curves were plotted from the working standard solutions. The Al, Ag, Ba, Cd, Cr, Cu, Mn, Pb, and Se contents were determined by comparing the absorbance of the sample with the standard curve.

The Al, Ag, Ba, Cd, Mn, Pb, and Se contents of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

The Cr and Cu contents of the sample, which had been expressed in ng/ml, was reported as mg/L.

A standard curve was plotted from the working standard solution. The chromium content was determined by comparing the absorbance of the sample with the standard curve.

A standard curve was plotted from the working standard solution. The copper content was determined by comparing the absorbance of the sample with the standard curve.

A standard curve was plotted from the working standard solution. The lead content was determined by comparing the absorbance of the sample with the standard curve.

The lead content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

A standard curve was plotted from the working standard solution. The manganese content was determined by comparing the absorbance of the sample with the standard curve.

The manganese content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

A standard curve was plotted from the working standard solution. The selenium content was determined by comparing the absorbance of the sample with the standard curve.

The selenium content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

A standard curve was plotted from the working standard solution. The silver content was determined by comparing the absorbance of the sample with the standard curve.

The silver content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

DETERMINATION OF BARIUM

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution

25 μl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.

3. Working standard solution

Concentration ($\mu\text{g/ml}$)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The barium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	20.0 mA
Wavelength	553.6 nm
Slit width	0.5 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The barium content was determined by comparing the absorbance of the sample with the standard curve.

The barium content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

DETERMINATION OF CADMIUM

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 μl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.

3. Working standard solution

Concentration ($\mu\text{g/ml}$)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The cadmium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	4.0 mA
Wavelength	228.8 nm
Slit width	0.5 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The cadmium content was determined by comparing the absorbance of the sample with the standard curve.

The cadmium content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

DETERMINATION OF CHROMIUM

EQUIPMENT AND MATERIAL

- Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)

2. Internal standard solution

25 μ l of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml. (Internal standard solution I).

500 μ l of internal standard solution I were diluted with DDW to 5 ml. (Internal standard solution II).

3. Working standard solution

Concentration (ng/ml)	Internal Standard II (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
10.00	0.100	0.400	0.5	1.00
20.00	0.200	0.300	0.5	1.00
30.00	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The chromium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	7.0 mA
Wavelength	357.9 nm
Slit width	0.2 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The chromium content was determined by comparing the absorbance of the sample with the standard curve.

The chromium content of the sample, which had been expressed in ng/ml, was reported as mg/L.

DETERMINATION OF COPPER

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 µl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml. (Internal standard solution I).
500 µl of internal standard solution I were diluted with DDW to 5 ml. (Internal standard solution II).
3. Working standard solution

Concentration (µg/ml)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
30.00	0.03	0.47	0.5	1.00
50.00	0.05	0.45	0.5	1.00
70.00	0.07	0.43	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The copper content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	4.0 mA
Wavelength	327.4 nm
Slit width	0.5 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The copper content was determined by comparing the absorbance of the sample with the standard curve.

The copper content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L .

DETERMINATION OF LEAD

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 μl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.
3. Working standard solution

Concentration ($\mu\text{g/ml}$)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The lead content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	5.0 mA
Wavelength	283.3 nm
Slit width	0.5 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The lead content was determined by comparing the absorbance of the sample with the standard curve.

The lead content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

DETERMINATION OF MANGANESE

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 μl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.

3. Working standard solution

Concentration ($\mu\text{g/ml}$)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The manganese content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	5.0 mA
Wavelength	279.5 nm
Slit width	0.2 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The manganese content was determined by comparing the absorbance of the sample with the standard curve.

The manganese content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

DETERMINATION OF SELENIUM

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer

(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)

2. Internal standard solution

25 μ l of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.

3. Working standard solution

Concentration (μ g/ml)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The selenium content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	10.0 mA
Wavelength	196.0 nm
Slit width	1.0 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The selenium content was determined by comparing the absorbance of the sample with the standard curve.

The selenium content of the sample, which had been expressed in μ g/ml, was reported as mg/L.

DETERMINATION OF SILVER

EQUIPMENT AND MATERIAL

1. Graphite furnace atomic absorption spectrophotometer
(Varian SpectrAA 300/400 Zeeman, Varian Techtron Pty. Limited, Mulgrave, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 μ l of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.
3. Working standard solution

Concentration (μ g/ml)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

PROCEDURE

The silver content was determined by using a graphite furnace atomic absorption spectrophotometer under the following conditions:

Lamp current	4.0 mA
Wavelength	328.1 nm
Slit width	0.5 nm
Gas type	Nitrogen

The standard curve from the working standard solution was plotted. The silver content was determined by comparing the absorbance of the sample with the standard curve.

The silver content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L .





APPENDIX Q

DETERMINATION OF THE ARSENIC AND MERCURY CONTENTS BY USING THE VAPOR GENERATION METHOD

PRINCIPLE

Arsenic (As) and mercury (Hg) were converted to their volatile hydrides by sodium borohydride reagent in acid solution. The hydrides were purged continuously by the flame of an atomic absorption spectrophotometer and were converted to the gas-phase atoms. The following describes the Equipment and Material, Reagents, and Procedure used to determine the Arsenic and Mercury Contents by the Vapor Generation Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Atomic absorption spectrophotometer hydride system
(GBC Scientific Equipment Pty Ltd, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution

25 μ l of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.

3. Working standard solution

Concentration (μ g/ml)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

5. Concentrated hydrochloric acid (HCl)

6. Potassium iodide (KI) (Mallinckrodt Inc., Paris)

PROCEDURE

Both standard solution and sample were acidified with concentrated HCl to give a 20% v/v solution for each. KI was added to each to give an approximately 200 mg/L solutions which allowed to stand for 1 h.

The arsenic content was determined by using an atomic absorption spectrophotometer hydride system under the following conditions:

Lamp current	8.0 mA
Wavelength	193.7 nm
Slit width	1.0 nm
Flame type	Air

A standard curve was plotted from the working standard solution. The arsenic content was determined by comparing the absorbance of the sample with the standard curve.

The arsenic content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

The mercury content was determined by using an atomic absorption spectrophotometer hydride system under the following conditions:

Lamp current	4.0 mA
Wavelength	253.7 nm
Slit width	1.0 nm
Flame type	No flame

A standard curve was plotted from the working standard solution. The mercury content was determined by comparing the absorbance of the sample with the standard curve.

The mercury content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L .

DETERMINATION OF MERCURY

EQUIPMENT AND MATERIAL

1. Atomic absorption spectrophotometer hydride system
(GBC Scientific Equipment Pty Ltd, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution

25 μl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.

3. Working standard solution

Concentration ($\mu\text{g/ml}$)	Internal Standard (ml)	DDW (ml)	Sample (ml)	Total volume (ml)
0.100	0.100	0.400	0.5	1.00
0.200	0.200	0.300	0.5	1.00
0.300	0.300	0.200	0.5	1.00

4. Blank solution

DDW was used as a blank solution.

5. Concentrated hydrochloric acid (HCl)

PROCEDURE

Both standard solution and sample were acidified with concentrated HCl to give a 20% v/v solution. KI was added to give an approximately 200 mg/L solution and allowed to stand for 1 h.

The mercury content was determined by using an atomic absorption spectrophotometer hydride system under the following conditions:

Lamp current	4.0 mA
Wavelength	253.7 nm
Slit width	1.0 nm
Flame type	No flame

A standard curve was plotted from the working standard solution. The mercury content was determined by comparing the absorbance of the sample with the standard curve.

The mercury content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.



APPENDIX R
DETERMINATION OF THE IRON AND ZINC CONTENTS
BY THE FLAME ATOMIC ABSORPTION
SPECTROPHOTOMETRIC METHOD

PRINCIPLE

A sample was aspirated into a flame and atomised. A light beam was directed through the flame, into a monochromator, and onto a detector that measured the amount of light absorbed by the atomised element in the flame. The amount of energy at the characteristic wavelength absorbed in the flame was proportional to the concentration of the element in the sample. The following describes the Equipment, Reagents, and Procedure used to determine the Iron (Fe) and Zinc (Zn) Contents by the Flame Atomic Absorption Spectrophotometric Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT

1. Flame atomic absorption spectrophotometer
(Avanta Σ , GBC Scientific Equipment Pty Ltd, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 μ l of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.
3. 4 N nitric acid (HNO₃)
276.967 ml of concentrated HNO₃ were pipetted into a 1000 ml volumetric flask, and diluted with DDW to 1000 ml.

4. 5% lanthanum (La₂O₃) solution

11.730 g of La₂O₃ were dissolved with 50 ml of concentrated hydrochloric acid (HCl), mixed until the solution cleared, and then diluted in a volumetric flask with DDW to 200 ml.

5. Working standard solution

Concentration (mg/ml)	Internal standard (ml)	4 N HNO ₃ (ml)	5% La ₂ O ₃ (ml)	DDW (ml)	Total volume (ml)
0.300	0.3	0.5	1.0	3.2	5
0.500	0.5	0.5	1.0	3.0	5
0.700	0.7	0.5	1.0	2.8	5
1.000	1.0	0.5	1.0	2.5	5
1.300	1.3	0.5	1.0	2.2	5
1.500	1.5	0.5	1.0	2.0	5

6. Blank solution

10 ml of 4 N HNO₃ and 20 ml of 5% La₂O₃ solution were pipetted into a 100 ml volumetric flask, and then diluted with DDW to 100 ml.

PROCEDURE

The iron content was determined by using a flame atomic absorption spectrophotometer under the following conditions:

Lamp current	3.00 mA
Wavelength	372.00 nm
Slit width	0.20 nm
Flame type	Air-Acetylene

A standard curve was plotted from the working standard solution. The iron content was determined by comparing the absorbance of the sample with the standard curve.

The iron content of the sample, which had been expressed in □g/ml, was reported as mg/L.

The zinc content was determined by using a flame atomic absorption spectrophotometer under the following conditions:

Lamp current	5.00 mA
Wavelength	213.90 nm
Slit width	0.50 nm
Flame type	Air-Acetylene

A standard curve was plotted from the working standard solution. The zinc content was determined by comparing the absorbance of the sample with the standard curve.

The zinc content of the sample, which had been expressed in $\mu\text{g/ml}$, was reported as mg/L.

DETERMINATION OF ZINC

EQUIPMENT

1. Flame atomic absorption spectrophotometer
(Avanta Σ GBC Scientific Equipment Pty Ltd, VIC, AUS)

REAGENTS

1. Stock standard solution (Merck, Darmstadt, Germany)
2. Internal standard solution
25 μl of stock standard solution were diluted with deionised distilled water (DDW) to 25 ml.
3. 4 N nitric acid (HNO_3)
276.967 ml of concentrated HNO_3 were pipetted into a 1000 ml volumetric flask, and diluted with DDW to 1000 ml .
4. 5% lanthanum solution

11.73 g of Lanthanum oxide (calcium free) were dissolved with 50 ml of concentrated hydrochloric acid, mixed until the solution cleared, and then diluted in a volumetric flask with DDW to 200 ml.

5. Working standard solution

Concentration (mg/ml)	Internal standard				Total volume (ml)
	standard (ml)	4 N HNO ₃ (ml)	5% Lanthanum (ml)	DDW (ml)	
0.300	0.3	0.5	1.0	3.2	5
0.500	0.5	0.5	1.0	3.0	5
0.700	0.7	0.5	1.0	2.8	5
1.000	1.0	0.5	1.0	2.5	5
1.300	1.3	0.5	1.0	2.2	5
1.500	1.5	0.5	1.0	2.0	5

6. Blank solution

10 ml of 4 N HNO₃ and 20 ml of 5% lanthanum solution were pipetted into a 100 ml volumetric flask, and then diluted with DDW to 100 ml.

PROCEDURE

The zinc content was determined by using a flame atomic absorption spectrophotometer under the following conditions:

Lamp current	5.00 mA
Wavelength	213.90 nm
Slit width	0.50 nm
Flame type	Air-Acetylene

The standard curve was plotted from the working standard solution. The zinc content was determined by comparing the absorbance of the sample with the standard curve.

The zinc content of the sample, which had been expressed in µg/ml, was reported as mg/L.



APPENDIX S

DETERMINATION OF THE CHLORIDE CONTENT BY USING THE ARGENTOMETRIC METHOD

PRINCIPLE

Chloride, in a form of chloride (Cl^-) ion, is one of the major inorganic anions in water. In a neutral or slightly alkaline solution, potassium chromate (K_2CrO_4) can indicate the end point of the silver nitrate (AgNO_3) of Cl^- . Silver chloride (AgCl) was precipitated quantitatively before red silver chromate (Ag_2CrO_4) was formed. The following describes the Material, Reagents, Procedure, and Calculation used to determine the Chloride (Cl^-) Content as Chlorine (Cl) Content by the Argentometric Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

MATERIAL

1. Erlenmeyer flask, 250 ml
2. Buret, 50 ml

REAGENTS

1. 0.0141 N standard sodium chloride solution (NaCl)
0.8241 g of NaCl (dried at 140°C) were dissolved with deionised distilled water (DDW), and diluted to 1000 ml ($1.00 \text{ ml} = 0.500 \text{ mg Cl}^-$).
2. Potassium chromate indicator solution (K_2CrO_4)
50 g of K_2CrO_4 were dissolved in a small amount of DDW. AgNO_3 was added until a definite red precipitate was formed, let stand 12 h, then filtered, and diluted with DDW to 1000 ml.
3. 0.0141 N standard silver nitrate solution (AgNO_3)

2.395 g of AgNO_3 were dissolved with DDW and diluted to 1000 ml, standardised against 0.0141 N NaCl to a pinkish yellow end point as indicated by the K_2CrO_4 indicator, and then stored in a brown bottle (1.00 ml = 0.500 mg Cl^-).

4. Blank solution

DDW was used as a blank solution.

5. Special reagents for removal of interference

a) Aluminum hydroxide suspension [$\text{Al}(\text{OH})_3$]

125 g of aluminum ammonium sulfate [$\text{AlNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$] were dissolved with 1000 ml of DDW, and then warmed to 60°C . 55 ml of concentrated ammonium hydroxide (NH_4OH) slowly were added while stirring, let stand about 1 h, and then transferred to a large bottle. The precipitate was washed by successive additions of DDW by mixing and decanting until free of chloride.

When freshly prepared, the suspension occupied a volume of approximately 1000 ml.

b) Phenolphthalein ($\text{C}_{20}\text{H}_{14}\text{O}_4$) indicator solution

0.500 g of $\text{C}_{20}\text{H}_{14}\text{O}_4$ were dissolved with 50 ml of 95% ethyl alcohol ($\text{C}_2\text{H}_5\text{OH}$), and diluted with 50 ml of DDW. 0.02 N sodium hydroxide (NaOH) then was added until a definite pink colour was formed.

c) 1 N Sodium hydroxide (NaOH)

40 g of NaOH were dissolved with DDW and diluted to 1000 ml.

d) 1 N Sulfuric acid (H_2SO_4)

28 ml of concentrated sulfuric acid slowly were poured into DDW while being stirred and diluted to 1000 ml.

e) 30% Hydrogen peroxide (H_2O_2) (Merck, Darmstadt, Germany)

PROCEDURE

1. Sample preparation

100 ml of sample were used for titration.

If the sample used was highly coloured, 3 ml of $\text{Al}(\text{OH})_3$ suspension were added, mixed, let settle, and then filtered.

If sulfide, sulfite, or thiosulfate was present, NaOH was added until the sample became alkali as indicated by the phenolphthalein indicator. 1 ml of H₂O₂ was added and stirred. The sample was then neutralised by adding H₂SO₄.

2. Titration

The pH of the sample was adjusted to 7-10 with H₂SO₄ (in lieu of NaOH).

1 ml of K₂CrO₄ indicator solution was added into the sample and titrated with standard AgNO₃ to a pinkish yellow end point.

CALCULATION

mg Cl⁻/L was calculated by using the following equation:

$$\text{mg Cl}^{-} / \text{L} = \frac{(A - B) \times N \times 35450}{\text{sample volume, ml}}$$

Key: A = ml AgNO₃ for sample,

B = ml AgNO₃ for blank,

N = normality of AgNO₃



APPENDIX T

DETERMINATION OF THE CYANIDE CONTENT BY USING THE ION-SELECTIVE ELECTRODE METHOD

PRINCIPLE

Cyanide can be determined potentiometrically as the cyanide ion, CN^- , by using the ion-selective electrode in combination with a reference electrode and a pH meter. The following describes the Equipment and Material, Reagents, and Procedure used to determine the Cyanide (CN^-) Content by the Ion-Selective Electrode Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Benchtop ISE/pH meter
(Model 710A, Orion Research Inc., Beverly, MA, USA)
2. Cyanide-ion-selective electrode
(Solid-State, Orion Research Inc., Beverly, MA, USA)
3. Magnetic stirrer (Model MAG-O, Gerhardt, Germany)

REAGENTS

1. Standard sodium chloride (NaCl) solution
0.824 g of NaCl (dried at 140°C) was dissolved with deionised distilled water (DDW) and diluted to 1000 ml.
2. Potassium chromate (K_2CrO_4) indicator solution
50 g of K_2CrO_4 were dissolved in a small amount of DDW. AgNO_3 was added until a definite red precipitate was formed, let stand 12 h, filtered, and diluted with DDW to 1000 ml.

3. Standard silver nitrate (AgNO_3) solution

3.270 g of AgNO_3 were dissolved with DDW and diluted to 1000 ml. This solution was standardised against the standard NaCl solution to a pinkish yellow end point which was indicated by the K_2CrO_4 indicator.

4. Stock standard solution

1.600 g of sodium hydroxide (NaOH) and 2.510 g of potassium cyanide (KCN) were dissolved with 1000 ml of DDW. 25 ml of stock standard solution were standardised against standard silver nitrate (AgNO_3) solution to a change in colour from a canary yellow to a salmon hue (1.00 ml = 1.00 mg CN^-).

5. Sodium hydroxide (NaOH) dilution solution

1.600 g of NaOH were dissolved with DDW and diluted to 1000 ml.

6. Internal standard solution

25 ml of stock standard cyanide solution were diluted with the NaOH dilution solution to 1000 ml. (Intermediate standard solution)

10 ml of intermediate standard solution were diluted with NaOH dilution solution to 1000 ml [1.00 ml = 2.50 μg CN^- (Internal standard solution)]. Prepared fresh daily and kept in a glass-stoppered bottle.

7. Working standard solution

0, 5.00, 25.00, 50.00, and 500.00 ml of the internal standard solution were diluted with NaOH dilution solution to 500 ml. 100 ml of each of these working standard solution was transferred into a 250-ml beaker prerinced with a small amount of the working standard solution being measured.

PROCEDURE

1. Calibration

Cyanide-ion-selective electrode was immersed in each of the 5 working standard solutions while being mixed on a magnetic stirrer at 25°C.

After equilibrium was reached (5-10 min), CN^- concentration obtained directly from Benchtop ISE/pH meter.

2. Measurement of sample

100 ml of sample were poured into a 250-ml beaker. Cyanide-ion-selective electrode was immersed in the sample while being mixed on a magnetic stirrer at the same stirring rate used for calibration.

After equilibrium was reached (5-10 min), CN^- concentration obtained directly from Benchtop ISE/pH meter.

CALCULATION

CN^- concentration was calculated by the following equation:

$$\text{mg CN}^- / \text{L} = \mu\text{g CN}^- / \text{ml from meter} \times \frac{100}{x} \times \frac{250}{y}$$

Key : x = volume of absorption solution, ml

y = volume of original sample, ml



APPENDIX U

DETERMINATION OF THE FLUORIDE CONTENT BY USING THE ION-SELECTIVE ELECTRODE METHOD

PRINCIPLE

Fluoride can be determined potentiometrically as the fluoride ion, F^- , by using the ion-selective electrode in combination with a reference electrode and a pH meter. The fluoride electrode measured the ion activity of fluoride rather than concentration. Adding fluoride buffer, the electrode measured concentration. The following describes the Equipment and Material, Reagents, and Procedure used to determine the Fluoride (F^-) Content as Fluorine (F) Content by the Ion-Selective Electrode Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Benchtop ISE/pH meter
(Model 710A, Orion Research Inc., Beverly, MA, USA)
2. Fluoride-ion-selective electrode
(Solid-State, Orion Research Inc., Beverly, MA, USA)
3. Magnetic stirrer (Model MAG-O, Gerhardt, Germany)

REAGENTS

1. Stock standard solution
0.221 g of anhydrous sodium fluoride (NaF) were dissolved with DDW and diluted to 1000 ml (1.00 ml = 100 $\mu\text{g } F^-$).
2. Internal standard solution
100 ml of stock standard solution were diluted with DDW to 1000 ml (1.00 ml = 10.0 $\mu\text{g } F^-$).

3. Working standard solution

0, 0.50, 1.00, and 2.00 ml of internal standard solution were diluted with DDW and diluted to 500 ml. 25 ml of each of these standard solutions was transferred into 100-ml beaker prerinced with a small amount of working standard solution being tested, and then 30 ml of fluoride buffer were added

4. Fluoride buffer (Orion Research Inc., Beverly, MA, USA)

PROCEDURE

1. Calibration

Fluoride-ion-selective electrode was immersed in each of the 4 working standard solutions while being mixed on a magnetic stirrer at 25°C.

After equilibrium was reached (5-10 min), F⁻ concentration obtained directly from Benchtop ISE/pH meter.

2. Measurement of sample

25 ml of sample were pipetted by using volumetric pipette into 100-ml beakers prerinced with a small amount of working standard solution being tested, and then 30 ml of fluoride buffer were added

Fluoride-ion-selective electrode was immersed in the sample while being mixed on a magnetic stirrer at the same stirring rate used for calibration.

After equilibrium was reached (5-10 min), F⁻ concentration obtained directly from Benchtop ISE/pH meter.

CALCULATION

Fluoride concentration was calculated by the following equation:

$$\text{mg F}^- / \text{L} = \frac{\mu\text{g F}^-}{\text{ml sample}}$$



APPENDIX V

**THE DETERMINATION OF THE CHEMICAL QUALITY OF
NITRATE (NO₃⁻) AS NITROGEN (N) BY USING THE
ULTRAVIOLET SPECTROPHOTOMETRIC METHOD**

The following describes the Equipment and Material, Reagents, and Procedure used to determine the Chemical Quality of Nitrate as Nitrogen (NO₃⁻-N) by the Ultraviolet Spectrophotometric Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Spectrophotometer, for use at 220 nm, providing a light path 2.5-10 cm (Spectronic 2000, Bausch&Lomb Analytical Systems Division, NY, USA)

REAGENTS

1. Stock nitrate solution

Potassium nitrate (KNO₃) was dried in an oven for 24 h at 105°C. 0.7218 g of KNO₃ was dissolved with DDW and diluted to 1000 ml (1.00 ml = 100 µg NO₃⁻-N). This solution was preserved with 2 ml of CHCl₃/L and remained stable for at least 6 months.

2. Standard nitrate solution

100 ml of stock nitrate solution were diluted with DDW to 1000 ml (1.00 ml = 10.0 µg NO₃⁻-N). This solution was preserved with 2 ml of CHCl₃/L and remained stable for at least 6 months.

3. 1 N hydrochloric acid (HCl)

36.5 ml of concentrated HCl were diluted with DDW to 1000 ml.

PROCEDURE

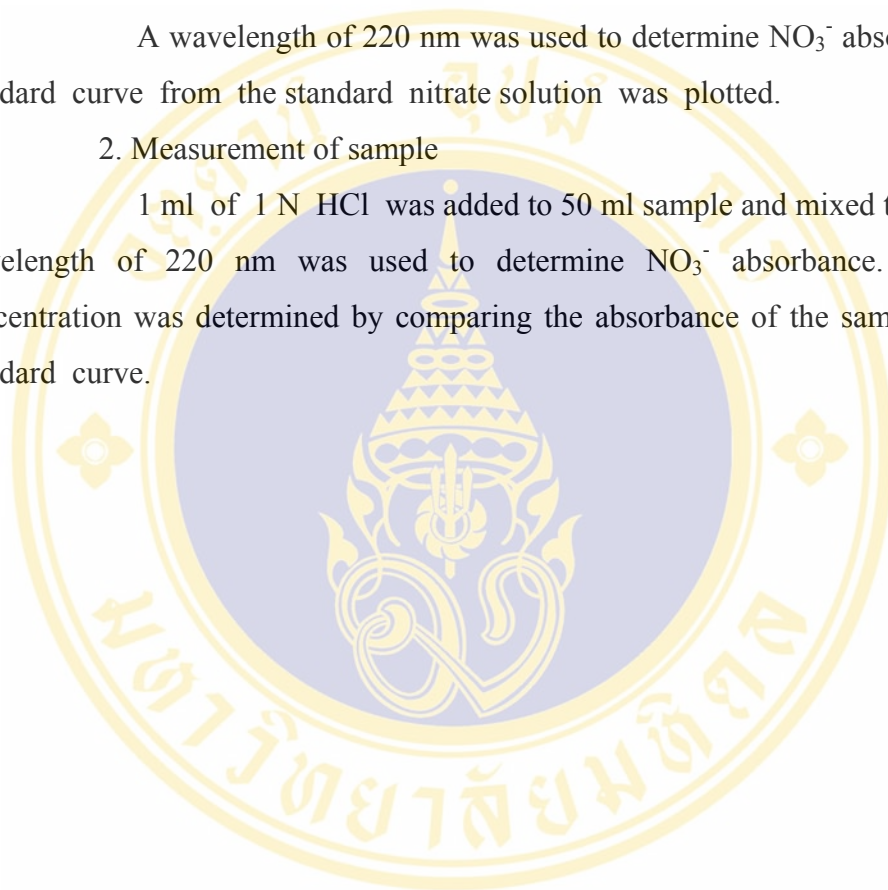
1. Preparation of standard curve

0, 1.00, 2.00, 4.00, 7.00,....., 37.0 ml of standard nitrate solution were diluted with DDW to 50 ml. 1 ml of 1 N HCl was added to these 50 ml standard nitrate solution and mixed thoroughly.

A wavelength of 220 nm was used to determine NO_3^- absorbance. The standard curve from the standard nitrate solution was plotted.

2. Measurement of sample

1 ml of 1 N HCl was added to 50 ml sample and mixed thoroughly. A wavelength of 220 nm was used to determine NO_3^- absorbance. The NO_3^- concentration was determined by comparing the absorbance of the sample with the standard curve.





APPENDIX W

THE DETERMINATION OF THE CHEMICAL QUALITY OF SULFATE BY TURBIDIMETRIC METHOD

The following describes the Equipment and Material, Reagents, Procedure, and Calculation used to determine the Chemical Quality of Sulfate by the Turbidimetric Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. Magnetic stirrer (Model MAG-O, Gerhardt, Germany)
2. Spectrophotometer, for use at 420 nm, providing a light path 2.5-10 cm (Spectronic 2000, Bausch&Lomb Analytical Systems Division, NY, USA)
3. Measuring spoon

REAGENTS

1. Buffer solution

30 g of magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), 5.000 g of sodium acetate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$), 1.000 g of potassium nitrate (KNO_3), and 20 ml of acetic acid (99% CH_3COOH) were dissolved with 500 ml of deionised distilled water (DDW) and diluted to 1000 ml.

2. Barium Chloride (BaCl_2), crystal, 20-30 mesh (Merck, Darmstadt, Germany)

3. Standard sulfate solution

0.1479 g of anhydrous sodium sulfate (Na_2SO_4) was dissolved with DDW and diluted to 1000 ml ($1.00 \text{ ml} = 100 \mu\text{g SO}_4^{2-}$).

PROCEDURE

1. Preparation of standard curve

SO₄²⁻ standards were prepared at 5-mg/L intervals in a 0-40 mg/L range.

Turbidity was measured at a wavelength of 420 nm. The standard curve from the standard sulfate solution was plotted.

Reliability of standard curve was checked by measuring a standard solution with every 3-4 samples.

2. Measurement of sample

a) Formation of barium sulfate turbidity

100 ml of sample was poured into a 250-ml erlenmeyer flask. 20 ml of buffer solution were added and mixed on a magnetic stirrer. A spoonful of BaCl₂ was added during stirring at constant speed and timing immediately was begun for 60±2 sec.

b) Measurement of barium sulfate turbidity

Sample was stirred for 5±0.5 min, and then turbidity was measured at a wavelength of 420 nm. The SO₄²⁻ concentration was determined by comparing the absorbance of the sample with the standard curve.

Sample colour and turbidity were corrected by running blank to which BaCl₂ had not been added.

CALCULATION

Sulfate concentration was calculated by the following equation:

$$\text{mg SO}_4^{2-} / \text{L} = \frac{A \times 1000}{\text{ml sample}}$$

Key: A = mg SO₄²⁻ estimated from standard curve



APPENDIX X
DETERMINATION OF THE ALKYL BENZYL SULFONATE
CONTENT BY USING METHYLENE BLUE ACTIVE
SUBSTANCES METHOD

PRINCIPLE

Alkyl benzyl sulfonate (ABS) was determined by 3 successive extractions from acid aqueous medium containing excess methylene blue into chloroform, followed by an aqueous backwash and measurement of the blue colour in the chloroform by spectrophotometer at 652 nm. The following describes the Equipment and Material, Reagents, Procedure, and Calculation used to determine the ABS Content by the Methylene Blue Active Substances (MBAS) Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. UV/Visible spectrophotometer, for use at 652 nm.
(Novaspec, LKB Biochrom Ltd., Cambridge, England)
2. Separatory funnels, 500 ml, with inert TFE stopcocks

REAGENTS

1. 1 N sodium hydroxide (NaOH)
40 g of NaOH were dissolved with deionised distilled water (DDW) and diluted to 1000 ml.
2. Phenolphthalein (C₂₀H₁₄O₄) indicator solution
5.000 g of C₂₀H₁₄O₄ were dissolved with 500 ml of 95% ethyl alcohol (C₂H₅OH) and diluted with 500 ml of DDW. 0.02 N of sodium hydroxide (NaOH) was then added until a pink colour was developed.

3. 1 N sulfuric acid (H₂SO₄)

28 ml of concentrated H₂SO₄ were poured into DDW and diluted to 1000 ml.

4. Chloroform (CHCl₃) (Merck, Darmstadt, Germany)

5. Methylene blue (C₁₆H₁₈ClN₃S) reagent

0.100 g of C₁₆H₁₈ClN₃S•3H₂O was dissolved with 100 ml DDW and 30 ml were transferred to a 100-ml flask. 500 ml DDW, 6.8 ml of concentrated H₂SO₄, and 50 g of monosodium dihydrogen phosphate monohydrate (NaH₂PO₄•H₂O) were added, shook until dissolved, and diluted to 1000 ml.

6. Wash solution

500 ml DDW were poured into 1000-ml volumetric flask, and 6.8 ml of concentrated H₂SO₄ were slowly added. 50 g of NaH₂PO₄•H₂O were added, shook until dissolved, and diluted to 1000 ml.

7. Stock standard solution

0.100 g of sodium dioctylsulfosuccinate was dissolved with DDW and diluted to 1000 ml (1.00 ml = 100 µg ABS).

8. Working standard solution

10 ml of stock standard solution were diluted with DDW to 1000 ml (1.00 ml = 10.0 µg ABS).

9. Blank solution

CHCl₃ was used as a blank solution.

PROCEDURE

1. Extraction and colour development

a) 1 N NaOH was dropped into the sample and the alkalinity was measured by phenolphthalein indicator. 1 N H₂SO₄ was dropped into the sample until any pink colour disappeared. Sample was then poured into a separatory funnel.

b) 10 ml of CHCl₃ and 25 ml of methylene blue were added. The funnel was shaken vigorously for 30 sec and allowed to stand for phase separation. Because excessive agitation may cause emulsion formation, and some samples required a longer period of phase separation than others.

c) CHCl_3 layer was drawn off into a second separatory funnel and a delivery tube of first separatory funnel was rinsed with a small amount of CHCl_3 . The extraction was repeated 2 additional times by using 10 ml CHCl_3 each time. If blue colour in water phase became faint or disappeared, 25 ml of methylene blue was added.

d) All CHCl_3 extracts were combined in the second separatory funnel. 50 ml of wash solution were added. Separatory funnel was shaken vigorously for 30 sec and allowed to stand. Emulsion did not form at this stage. CHCl_3 layer was drawn off through a funnel containing a plug of glass wool into a 100-ml volumetric flask. Wash solution was extracted 2 additional times by using 10 ml CHCl_3 each time and adding each extraction to a flask through a glass wool. Glass wool and funnel were rinsed with CHCl_3 . Washings were collected in volumetric flask, diluted with CHCl_3 to 100 ml and mixed well.

2. Preparation of standard curve

0.00, 1.00, 3.00, 5.00, 7.00, 9.00, 11.00, 13.00, 15.00, and 20.00 ml of working standard solution was pipetted into the separatory funnels. DDW were added to make the total volume 100 ml in each separatory funnel and treated as described in Step 1. A wavelength of 652 nm was used to measure the absorbance of the working standard solutions against a blank of CHCl_3 . The standard curve from the working standard solutions was constructed by plotting absorbance of the working standard solutions against ABS concentration of the working standard solutions.

3. Measurement of sample

A wavelength of 652 nm was used to measure the absorbance of the sample. The ABS concentration was obtained by comparing the absorbance of the sample with the standard curve.

CALCULATION

mg ABS/L was calculated by using the following equation:

$$\text{mg ABS/L} = \frac{A \times 1000}{\text{sample volume, ml}}$$

Key: A = mg ABS estimated from standard curve





APPENDIX Y

DETERMINATION OF THE PHENOL CONTENT BY USING THE CHLOROFORM EXTRACTION METHOD

PRINCIPLE

Phenol reacted with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a coloured antipyrine dye. This dye was extracted from an aqueous solution with CHCl_3 and the absorbance is measured at 460 nm. The following describes the Equipment and Material, Reagents, and Procedure used to determine the Phenol Content by the Chloroform Extraction Method in accordance with the Standard Methods for the Examination of Water and Wastewater.

EQUIPMENT AND MATERIAL

1. UV/Visible spectrophotometer, for use at 460 nm.
(Novaspec, LKB Biochrom Ltd., Cambridge, England)
2. Glass electrode pH meter (Cole Parmer, Santa Ana, CA, USA)

REAGENTS

1. Ammonium chloride solution (NH_4Cl)
50.000 g of NH_4Cl were dissolved with DDW, and diluted to 1000 ml.
2. Concentrated ammonium hydroxide (NH_4OH)
(Merck, Darmstadt, Germany)
3. Aminoantipyrine ($\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}$) solution
2.000 g of $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}$ were dissolved with DDW, and diluted to 100 ml.

Prepared fresh daily.

4. Potassium ferricyanide solution ($\text{K}_3\text{Fe}(\text{CN})_6$)

8.000 g of $\text{K}_3\text{Fe}(\text{CN})_6$ were dissolved with DDW and diluted to 100 ml.

Prepared fresh weekly.

5. Chloroform (CHCl_3) (Merck, Darmstadt, Germany)

6. 0.1 N bromate-bromide solution

2.784 g of anhydrous potassium bromate (KBrO_3) and 10 g of potassium bromide (KBr) crystal were dissolved with DDW and diluted to 1000 ml.

7. Concentrated hydrochloric acid (HCl) (Merck, Darmstadt, Germany)

8. Potassium iodide (KI) (Mallinckrodt Inc., Paris)

9. 10% sulfuric acid (H_2SO_4) solution

10 ml of concentrated H_2SO_4 were diluted with DDW to 1000 ml.

10. 0.025 N potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution

$\text{K}_2\text{Cr}_2\text{O}_7$ was dried for 2 h at 103°C . 1.226 g of $\text{K}_2\text{Cr}_2\text{O}_7$ was dissolved with DDW and diluted to 1000 ml.

11. Starch solution indicator

5.000 g of soluble starch were dissolved with DDW to form suspension. The suspension was poured into the 800 ml being heated DDW, stirred, and diluted to 1000 ml. The suspension was allowed to boil for 2-3 min, and stood overnight for precipitation. The upper cleared portion of the solution was preserved with 1.25 g of salicylic acid before being used.

12. 0.025 N standard sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution

6.205 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ was dissolved with freshly boiled and cooled DDW, diluted to 1000 ml, preserved by adding 0.400 g of NaOH , and standardised as follows:

2.000 g of KI were dissolved in an erlenmeyer flask with 100-150 ml DDW. 10 ml of sulfuric acid and 20 ml of $\text{K}_2\text{Cr}_2\text{O}_7$ were added, kept in the dark for 5 min, and diluted to 400 ml. $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ was used to titrate iodine, which was indicated by starch solution indicator. The end point was straw colour.

13. Stock standard solution

1.000 g of phenol was dissolved in freshly boiled and cooled DDW, diluted to 1000 ml, and standardised as follows:

50 ml of stock phenol solution and 10 ml of 0.1 N bromate-bromide solution were added to 100 ml of DDW. 5 ml of concentrated HCl were added, and the solution was gently swirled. If brown colour of free bromine did not persist, 10-ml

portions of bromate-bromide solution was added until brown colour persisted. The flask was kept corked and the solution was allowed to stand for 10 min. 1.000g of KI then was added. Usually four 10-ml portions of bromate-bromide solution were required if the stock phenol solution contained 1000 mg phenol/L. The stock standard solution was standardised with 0.025 M $\text{Na}_2\text{S}_2\text{O}_3$ solution which was indicated by starch solution indicator.

Blank solution was prepared exactly the same manner, using DDW and 10 ml of bromate-bromide solution. Both blank solution and sample were titrated with 0.025 M $\text{Na}_2\text{S}_2\text{O}_3$, which was indicated by starch solution indicator.

Concentration of phenol solution was calculated by using the following equation:

$$\text{mg phenol/L} = 7.842 [(A \times B) - C]$$

Key: A = ml thiosulfate for blank

B = ml bromate-bromide solution used for sample, divided by 10

C = ml thiosulfate used for sample

14. Internal standard solution

1.00 ml of stock standard solution was diluted with freshly boiled and cooled DDW to 100 ml (1.00 ml = 10.0 μg phenol) (Intermediate standard solution).

50 ml of intermediate standard solution was diluted with freshly boiled and cooled DDW to 500 ml (1.00 ml = 1.0 μg phenol). Prepared within 2 h of use (Internal standard solution).

15. Working standard solution

0, 5.00, 10.00, 20.00, 30.00, 40.00, and 50.00 ml of the internal standard solution were diluted with DDW to 500 ml.

16. Blank solution

DDW was used as a blank solution.

PROCEDURE

1. Extraction

a) 10 ml of NH_4Cl were added to sample. Concentrated NH_4OH was added to the solution in order to adjust pH to 10.0 ± 0.2 and was transferred to a 1-L separatory funnel.

b) 3 ml of $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}$ were added, and solution was mixed well. 3 ml of $\text{K}_3\text{Fe}(\text{CN})_6$ solution were added, mixed well, and allowed to stand for 3 min. The solution was clear and light yellow.

c) 25 ml of CHCl_3 immediately were used to extract phenol from the solution. The solution was shaken 10 times, allowing CHCl_3 to settle, and then was shaken again 10 times, allowing CHCl_3 again to settle.

d) CHCl_3 extract was filtered through filter paper. Extracts were collected for absorbance measurements.

2. Preparation of standard curve

Working standard solutions were extracted as described in Step 1. A wavelength of 460 nm was used to measure the absorbance of the working standard solution against a blank solution. The standard curve was constructed from the working standard solutions by plotting absorbance of the working standard solutions against phenol concentration of the working standard solution.

3. Measurement of sample

A wavelength of 460 nm was used to measure the absorbance of the sample. The phenol concentration was obtained by comparing the absorbance of the sample with the standard curve.

CALCULATION

mg phenol/L was calculated by using the following equation:

$$\text{mg phenol/L} = \frac{A}{B}$$

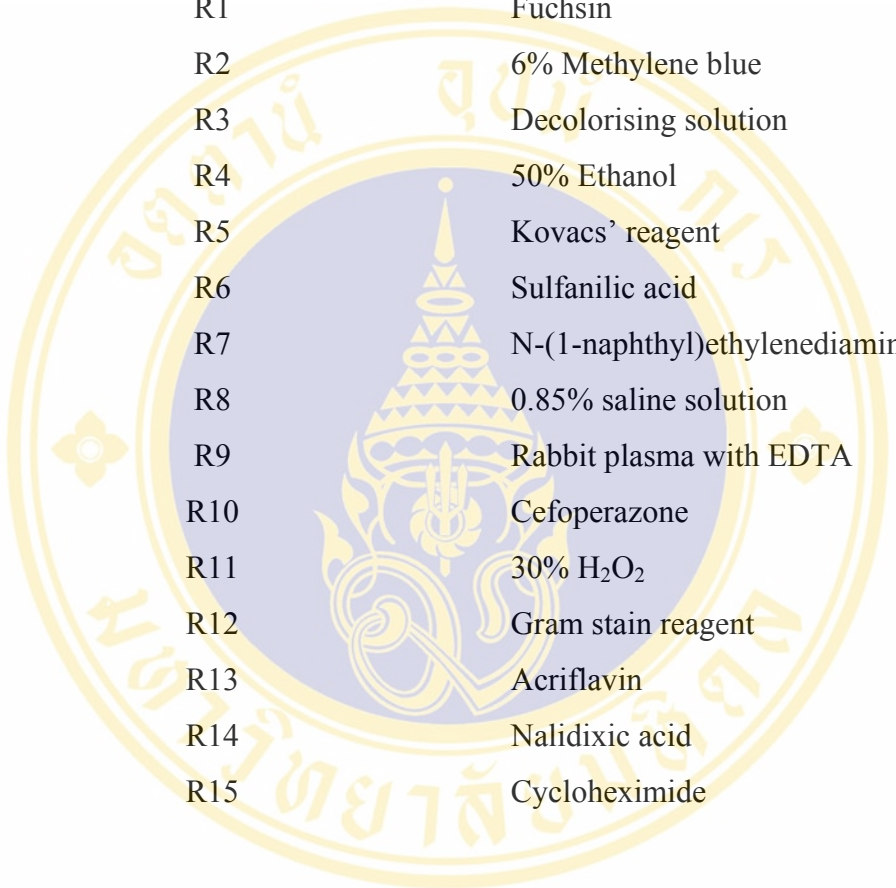
Key: A = μg phenol in sample from calibration curve

B = ml sample



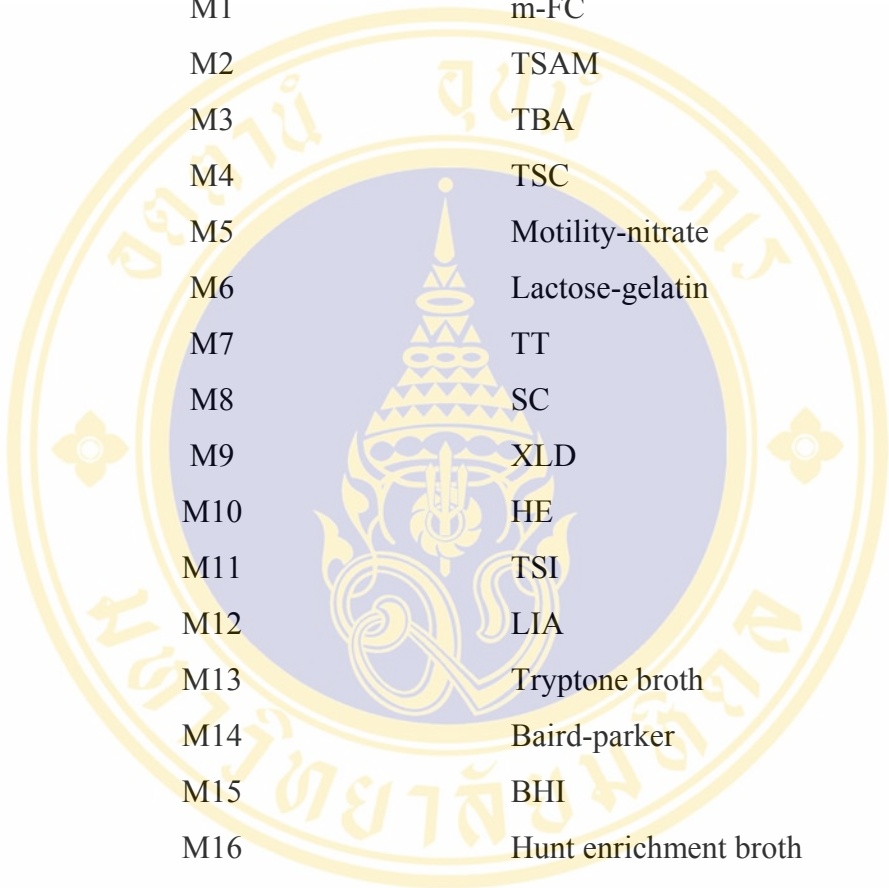
APPENDIX AA

REAGENTS



R1	Fuchsin
R2	6% Methylene blue
R3	Decolorising solution
R4	50% Ethanol
R5	Kovacs' reagent
R6	Sulfanilic acid
R7	N-(1-naphthyl)ethylenediamine reagent
R8	0.85% saline solution
R9	Rabbit plasma with EDTA
R10	Cefoperazone
R11	30% H ₂ O ₂
R12	Gram stain reagent
R13	Acriflavin
R14	Nalidixic acid
R15	Cycloheximide



APPENDIX BB**MEDIA**

M1	m-FC
M2	TSAM
M3	TBA
M4	TSC
M5	Motility-nitrate
M6	Lactose-gelatin
M7	TT
M8	SC
M9	XLD
M10	HE
M11	TSI
M12	LIA
M13	Tryptone broth
M14	Baird-parker
M15	BHI
M16	Hunt enrichment broth
M17	CCDA
M18	Abeyta-Hunt
M19	EB
M20	OXA
M21	PALCAM
M22	TSAYE
M23	PSBB
M24	MacKonkey
M25	CIN
M26	LAIA
M27	CUA

M28

Bile esculin



BIOGRAPHY

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