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A STUDY OF THE BACTERIOLOGICAL QUALITY OF BOTTLED AND TAP WATER IN CEBU CITY, PHILIPPINES

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Abstract

A study was conducted in the mega urban centre of Cebu City, Philippines between July and October 2013, with the aim of assessing the bacteriological quality of bottled and tap drinking waters. The study was performed during the warm, rainy season, when prevalence of diarrhoea linked to bacterial contamination of water supply is usually at its highest level in tropical countries. The bacteriological tests performed on the water samples were: isolation of *E. coli*, thermotolerant coliforms, total coliforms, and heterotrophic plate counts. In addition, sanitation programs applied by bottled water manufacturers and the local water system supplier were surveyed by means of a questionnaire. The study focussed on bottled water brands with the greatest combined market share in the study area, and the household taps surveyed were located in villages with the greatest number of local water supply concessionaires.

The results indicated the presence of bacteria in both bottled and tap water samples. A considerable number of bottled water samples from one brand were positive for heterotrophic plate count bacteria. On the other hand, positive tap water samples collected from one household were positive for *E. coli*, thermotolerant coliforms, total coliforms, and the HPC bacteria. Multivariable Poisson regression modelling indicated a significant variability in heterotrophic bacterial counts between production batches of bottled water. For the tap water samples, statistical analysis was not indicated because all contaminated samples belonged to a single household tap and were collected on one sampling occasion.

In regard to sanitation programs, the municipal water supplier and all except one bottled water manufacturer reported the application of Good Manufacturing Practice and Quality Control programs in the production plant. Interestingly, the only bottled water manufacturer which did not respond to the survey questionnaire manufactured the only brand consistently showing positive bacterial counts and failing the Philippine regulatory standards. It is concluded that a number of factors associated with bacterial contamination in the study area require close monitoring by bottled water manufacturers, water suppliers, and health authorities.

Preface

‘Because water is life,

Water quality reflects the quality of life’

- From the Author

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I wish to thank the following individuals and organizations for making this study a success:

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Dedication

I dedicate this study to my mom who passed away while I am writing this thesis

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List of Abbreviations

ABWA	Asia Middle-East Bottled Water Association
ABWI	Australasian Bottled Water Institute
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
APHA	American Public Health Association
AWWA	American Water Works Association
BAM	Bacteriological Analytical Manual
BGLB	Brilliant Green Lactose Broth
CAC	Codex Alimentarius Commission or Codex
CC	Total Coliform Count
CCP	Critical Control Point
CFR	Code of Federal Regulations
CFU	Colony-forming units
CPDO	Cebu City Planning and Development Office
DOST7-RSTL	Department of Science and Technology - Regional Office VII – Regional Standards and Testing Laboratory

EC	European Council
ECC	*Escherichia coli* count or *E. coli* count
EC medium	*E. coli* medium
EEC	European Economic Community
EFSA	European Food Safety Authority
EMB	Eosin Methylene Blue
EPA (US)	Environmental Protection Agency (United States)
ESR (NZ)	Institute of Environmental Science and Research (New Zealand)
EU	European Union
FDA (US)	Food and Drug Administration (United States)
FEFO	First-to-Expire, First-Out Policy
FGD	Focus Group Discussion
FSMS	Food Safety Management System
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
HPC	Heterotrophic plate count

IBWA	International Bottled Water Association
IMViC	Indole, Methyl Red, Voges-Proskauer, Citrate
ISO/IEC	International Organization for Standardization / International Electrotechnical Commission
LTB	Lauryl Tryptose Broth
MCWD	Metro Cebu Water District
MPN	Most probable number
MR	Methyl Red
MTFT	Multiple-Tube Fermentation Technique
NSF (US)	National Sanitation Foundation (United States)
OECD	Organization for Economic Cooperation and Development
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PET	Polyethylene Terepththalate
Phil. DOH	Philippine Department of Health
Phil. FDA	Philippine Food and Drug Administration
PNS	Philippine National Standards
PRP	Prerequisite Programs

PVC	Polyvinyl Chloride
QMS	Quality Management System
SMEWW	Standard Methods for the Examination of Water and Wastewater
SOP	Standard Operating Procedure
SSOP	Sanitation Standard Operating Procedure
TTCC	Thermotolerant Coliform Count
UV	Ultraviolet radiation
VBNC	Viable but non-culturable
VP	Voges-Proskauer
WEF	Water Environment Federation
WHO	World Health Organization

Glossary of Terms

AIC	the 'Akaike Information Criterion' used as index of the relative quality of a statistical model for a given set of data which provides a measure for model selection; the lower the AIC, the better is the fit of the model for a given data set
Allochthonous	microorganisms which are not normally found in the source water and, hence, only introduced via contamination
Autochthonous	microorganisms that occur naturally in the source water
Bacteria	single-celled prokaryotic living microorganisms comprised of either beneficial, spoilage, or pathogenic (disease-causing) members
Batch	a specific quantity of a product intended to have uniform quality and character, within specified limits, and produced under the same manufacturing cycle and order
Biofilms	microbial communities resulting from aggregation of bacterial cells that are attached to surfaces and enclosed in their self-produced exopolysaccharide matrix
Bottle	a vessel made of plastic or glass used to contain a water sample
Bottled water	commercially available drinking water delivered in various bottle packaging formats

Brand	the name of a product uniquely identified and commercially manufactured by a particular company
Concessionaire	a person or business who is given the right to operate, sell, or use something on property or resource (e.g. water) owned by someone else
Effervescence	producing or emitting small bubbles of gas in a liquid
Household	one or more persons living in the same dwelling place or house
Indicator organism	microorganism which fulfil a set of criteria that indicates the presence of other organisms especially pathogens
Lot	a collection of units of a product or a specific identified portion of a batch supposedly produced under identical manufacturing conditions from which a sample is to be drawn
Oligotrophic	environments that have low nutrient concentration
Pathogen	microorganism that causes a disease
Poisson distribution	statistical probability distribution of counts that randomly occur in a given interval of space or time
Potable	drinking water that is safe and wholesome for human consumption

Prototrophic	No specific nutritional requirements but mostly inorganic compounds
Psychrotrophic	Adapted to lower temperatures of 25 - 30°C
Residual chlorine	the amount of chlorine remaining in the water after a given contact time of oxidizing organic matter
Sample unit	the individual portion or container (individual bottles) of water taken as part of the sample and from which one or more analytical units is drawn for analysis
Sample	total number of individual sample units (bottles) drawn from a lot which will be tested in accordance with a specific sampling plan and method(s)
Sampling occasion	the period of time where all samples from all water types are collected
Sampling	the activity of collecting samples from the field based on the sampling plan and schedule
Store	a commercial establishment selling goods (e.g. bottled water) to the public
Tap water	drinking water delivered through valves, spouts, or faucets usually at the end of a municipal pipe distribution system such as in households

Turbidity	the haziness or cloudiness of a liquid caused by high amounts of individual particles
Unit of sampling	the volume of water taken as required for a particular sample unit (e.g. 500ml of tap water or 500ml packaged bottled water brand)
Village	the smallest administrative division in the Philippines, also known as ‘Barangay’

CHAPTER 1 - INTRODUCTION

1.1 Research background

1.1.1 Importance of water

Water is a very essential natural resource in the world (Bates, 2000) and life cannot exist without it. It is a critical requirement in the maintenance of metabolic functions and homeostasis [the ability to maintain stable body conditions] in living cells. The human body is composed of about 60% water by weight in adult males, 50% in females, and 70% in new born infants (Svagzdiene, Lau, & Page, 2010). The human dietary requirement for water is estimated to be approximately two litres per day for an average adult (EFSA, 2010).

The regular intake of adequate amounts of water is essential in the maintenance of good health and well-being (EFSA, 2010). However, the most important attribute of drinking water that has to be assured and maintained is its safety and quality (Codex, 2001) to ensure that it is safe for human consumption. This means that drinking water must not contain harmful contaminants, such as disease-causing microorganisms (pathogens), toxic substances, physical and chemical residues, as well as undesirable organoleptic properties like odour, colour, and taste (Codex, 2001).

Waterborne illnesses can occur as a result of water supply contamination, especially from microbiological hazards. In 2004, a waterborne illness outbreak affected 1,450 residents and visitors in South Bass Island, Ohio (Fong et al., 2007). This was reportedly caused by run-off and/or leaching of microbiological contaminants from a wastewater treatment plant to the nearby lake and groundwater in the area (Fong et al., 2007). In a fatal waterborne disease

outbreak occurring in Walkerton, Ontario, Canada, in May, 2000, 2,300 persons became seriously ill and seven died because of water contamination with the bacterial pathogens *Escherichia coli* 0157:H7 and *Campylobacter jejuni* (Carter, Rice, Buchberger, & Lee, 2000). Another incident was recorded in April, 2001, in which treatment failure of a chlorinated and filtered water supply led to 1,900 cases of human diseases caused by the protozoan *Cryptosporidium parvum* in North Battleford, Saskatchewan, Canada. In Gideon, Missouri, in the United States of America, 600 cases of salmonellosis with seven deaths were reported in December, 1993. These are just a few examples of outbreaks of human illnesses acquired through contaminated drinking water registered in developed countries (Carter et al., 2000). How much worse can the situation be in developing nations such as the Philippines, where water supply technology and sanitation are still unsatisfactory? In order to control the occurrence of waterborne diseases, the assurance of microbial safety should be based on the use of multiple barrier systems that include source protection, monitoring, and treatment schemes together with sustainable water safety management plans. Most recently, several intensive monitoring systems have been employed to understand the level of water quality and the appropriate treatments to be applied (Green & Kane, 2014; Kumpel & Nelson, 2014).

The United Nations (UN) General Assembly has declared the period 2005 to 2015 as the International Decade for Action, 'Water for Life' (WHO, 2012). The UN also recently announced that "safe and clean drinking water is a human right essential to the full enjoyment of life and all other human rights" (WHO, 2012). Hence, the provision of safe drinking water is a paramount issue of health and development at the local, national, and international levels. Moreover,

development interventions and initiatives to enhance access to safe drinking water are some of the strategies for poverty alleviation and reflect the level of a country's national economic development.

1.1.2 Common and alternative ways of delivering drinking water in the Philippines

There are a number of ways to deliver drinking water to consumers in residential urban areas (Alingasa, 2010; AllAboutWater.org, 2004; Barrell, Hunter, & Nichols, 2000; Bates, 2000; DOH, 2007; Doria, 2006; Fantin, Masoni, & Scalbi, 2011; Landu & Brent, 2006; Senior & Dege, 2005). Water delivery to consumers in urban centres comes in many forms, from conventional unprocessed pipes to the more rigorously processed bottled water types (Fantin et al., 2011; Gleick & Cooley, 2009; Senior & Dege, 2005). The main delivery mechanisms are distribution from main local water district utilities, through transport tankers and by the use of bottles. Water can also be treated (processed) or untreated (unprocessed).

Drinking water is treated whenever the source cannot be guaranteed to be safe for human consumption (APHA, AWWA, & WEF, 2012d). However, treatment of the water is not necessary if the source water quality and manner of extraction can assure its safety and quality. Both treated and untreated water can be delivered to consumers through bottles, transport vehicles, and municipal taps (Francisco, 2014; WHO, 2012). Treated water, such as many of the available bottled water brands and municipal water supplies, is defined as drinking water that undergoes various standard processing steps involving one or a combination of the following physical and chemical treatments: filtration, ozonation, reverse osmosis, distillation, chlorination (Edberg, 2005; Senior & Dege, 2005), and other

more rigorous disinfection techniques, all aimed at producing a highly safe product. Conversely, untreated water, which is a characteristic of natural mineral waters and spring waters in the world, is water which undergoes little processing or only basic disinfection treatments (APHA et al., 2012d; DOH, 1993; Senior & Dege, 2005). Bottled water has gained international popularity in recent decades, particularly because of the inherent convenience and quality it provides to consumers. In addition, bottled water plays an important role as an imported product in some parts of the world, such as in Middle Eastern countries, where a normal underground water supply is scarce (Ahmad & Bajahlan, 2009) or, in the case of many developing countries, where the quality of the municipal water supply is dubious (Foote, 2011).

1.1.3 The world's growing bottled water industry

The demand for safe and high quality drinking water by the world's growing population has dramatically increased (Herath, Abayasekara, Chandrajith, & Adikaram, 2012; Warburton et al., 1998). This is particularly true for bottled water, which has gained enormous popularity and acceptance in the last ten years (Rodwan, 2011; Senior & Dege, 2005; Svagzdiene et al., 2010), because of its inherent convenience, safety, and quality, despite its higher price. International studies reveal that water consumers are prompted to choose bottled water because of the assumption of it being safer and of better quality than municipal tap water sources (Kassenga, 2007; Nunes & Fuzihara, 2011; Raj, 2005; Varga, 2011). The main consequence of this perception, as mentioned, is the increase in the consumption of processed and bottled water (Raj, 2005). In Sri Lanka, for instance, both the consumption of bottled water and the number of new brands introduced to the market have increased significantly (Herath et al.,

2012). According to the International Bottled Water Association (Rodwan, 2011), bottled water consumption worldwide increased by 7.6% between 2002 - 2007.

In 2002, the USA was the largest market for bottled water in the world, with a consumption of around 24 billion litres a year, much greater than other big bottled water consuming countries, such as Mexico, China, and Italy, that consumed about 14 billion, 13 billion, and 10 billion litres, respectively (Finlayson, 2005; Rodwan, 2011). The increase in bottled water sales in the USA is a result of it being the world's largest and most developed economy. In addition, its market is more integrated because of greater domestic penetration in homes, and well adopted by Americans compared to its closest bottled water industry competitors in European countries. Most importantly, in the USA there is big competition between the biggest global bottled water and beverage manufacturers and brands, such as Nestle Waters North America, Danone, PepsiCo/Aquafina, and Coca-Cola/Dasani (Finlayson, 2005). Hence, the majority of the global market for bottled water is in North America and Europe (Rodwan, 2011). However, the most rapidly growing markets are in the Asia Pacific regions (Table 1-1), particularly China, which has the highest annual growth rates among the global markets, estimated at 13%. However, among the emerging global markets, Thailand and Indonesia showed the highest annual growth rates, at 16.9% and 11.8%, respectively (Rodwan, 2011). Asian nations are among the greatest consumers of bottled water based on total volume but despite having some of the most populous countries, Asian countries do not have the highest per capita intake levels (Finlayson, 2005). Mexico still has the highest per person intake levels, but the per capita consumption in other countries, such as Hong Kong and Thailand, is growing. Because of the increasing demand in line with population

growth, bottled water consumption is expected to quickly grow throughout the world in the coming years. Figure 1-1 shows the regional growth of bottled water for the period 1997 to 2003 based on Zenith's Global Bottled Water Market Report (2003, as cited in Senior & Dege, 2005). Asia and Australasia combined showed the biggest growth in consumption after West Europe. However, Asia and Australasia had the lowest per capita consumption, just slightly ahead of Africa (Finlayson, 2005; Rodwan, 2011).

Table 1-1 Global Bottled Water Market: Consumption and Compound Annual Growth Rates for the Period 2006 – 2011.

Rank in 2011	Countries	Consumption (Millions of gallons)		Compound annual growth rates
		2006	2011	2006/2011
1	United States	8,255.00	9,107.30	2.00%
2	China	4,163.30	7,686.40	13.00%
3	Mexico	5,359.90	7,520.70	7.00%
4	Brazil	3,301.60	4,500.90	6.40%
5	Indonesia	2,155.90	3,760.60	11.80%
6	Thailand	1,426.20	3,118.80	16.90%
7	Italy	3,115.50	3,034.70	-0.50%
8	Germany	2,808.90	2,954.20	1.00%
9	France	2,285.30	2,291.00	0.00%
10	Spain	1,524.00	1,514.60	-0.10%
	Top 10 Subtotal	34,395.60	45,489.30	5.80%
	All Others	12,606.80	15,880.70	4.70%
	WORLD TOTAL	***47,002.40***	***61,370.00***	***5.50%***

Source: Rodwan, J., Beverage Marketing Corporation, 2012, page 17.

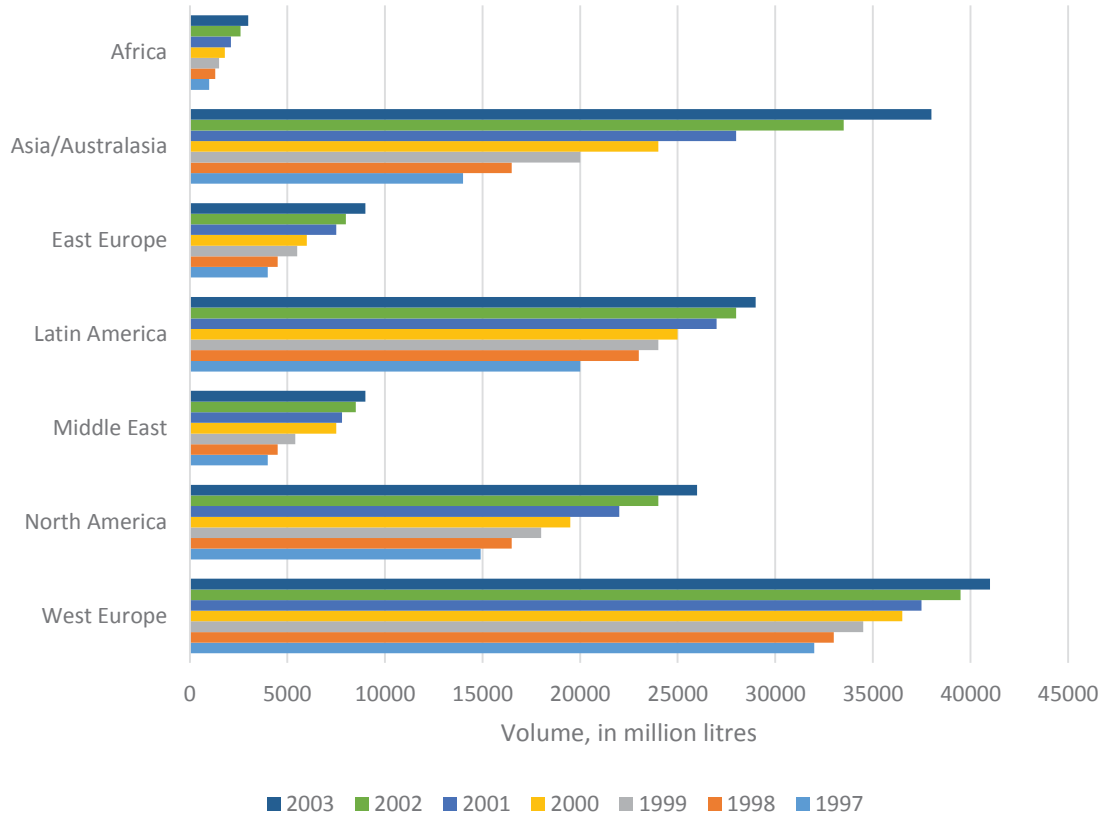

Figure 1-1 Growth of bottled water consumption in different regions of the world from 1997 to 2003. Source: Finlayson, D., 2005, page 7.

In the Philippines, people tend to consume bottled water because of its perceived high safety and quality compared with the municipal tap water supply (Francisco, 2014; Smith, 1999; Soriano-Pasumbal & Ong-Lim, 2005). The existence of poor environmental conditions and inadequate management of water supply utilities, highlighted by numerous incidences of waterborne diseases in some parts of the country, have resulted in a considerable portion of the population shifting to bottled and purified water as their main source of drinking water (Francisco, 2014; McGlynn, 2011). The increase in purified water consumption is highlighted by the popularity of water refilling stations in the Philippines since the 1990s. Their total sales just in Metro Manila, the country's capital, was USD 320 million in 2010 and is still growing (Francisco, 2014). Bottled water sold by large bottling

manufacturers and water from refilling stations sold in refillable containers by small companies cost approximately one hundred times more than municipal tap water. A recent study by Francisco (2014) in Metro Cebu analysing the reasons for the consumption of bottled water revealed similar reasons that, despite its higher cost, people still purchase bottled water because of its perceived better quality and safety.

1.1.4 The municipal water supply

Municipal water systems are basic utility services provided to the public by local governments in most countries and localities (Francisco, 2014; Liang et al., 2006; McKenzie & Ray, 2005; WHO, 2012). However, many households have embraced the use of refillable water cooler bottles, such as home and office delivery (HOD) containers (Senior & Dege, 2005; Zamberlan da Silva et al., 2008). Besides safety concerns, the chemical treatments, such as chlorination, applied by many local water districts and the effect of pipe materials on the organoleptic quality of municipal waters are disliked by consumers. Importantly, there are also growing concerns about the human health effects of chlorination by products such as trihalomethanes (THMs) present in treated municipal drinking waters (Ahmad & Bajahlan, 2009; Rosenfeldt, Baeza, & Knappe, 2009). Studies have implicated these substances possess carcinogenic potential to humans when present in excessive levels in drinking water (Edberg & Allen, 2004; Rosenfeldt et al., 2009) . On the other hand, an ineffectively chlorinated municipal water supply can lead to the growth of disease-causing microorganisms (Carter et al., 2000; Payment et al., 1997). Taken together, these factors may pose a public health risk, in the absence of sound water safety management practices in municipal water systems. The same study by

Francisco (2014) conducted in Metro Cebu, Philippines, showed that public distrust of the safety and quality of the municipal water supply is the main reason for the population's preference for bottled water as the primary drinking water source (Francisco, 2014).

1.1.5 Overview of human health and environmental issues associated with delivering drinking water

The maintenance of good water quality is one of the most pressing issues in environmental and public health today (Kassenga, 2007; Kouadio, Ekra, Atindehou, Nanou, & Monnet, 1998). The quality of drinking water has decreased dramatically due to environmental contamination, pollution from industrial and agricultural development, and resource over-extraction [due to changes in hydrological dynamics leading to contamination] (Perk, 2006; Tulchinsky et al., 2000). These are also aggravated by the increasing demand due to the growing population and urbanisation as well as the lack of sustainable resource management programs (Besic, Obradovic, Pasalic, & Zilic, 2011; Robles et al., 2011). Most countries rely on groundwater as their main source of potable drinking water (Kumpel & Nelson, 2014; Zamberlan da Silva et al., 2008). However, this source is exposed to large amounts of pollutants, whether from chemical or microbiological sources. Pollution originating from extensive agriculture has led to the contamination of subsurface water with fertilisers and pesticides (Perk, 2006). Similarly, industrial and mining activities have resulted in the intrusion of harmful chemicals to the groundwater and surface water environments. Improper waste management and the failure of sewage systems have contributed to the contamination of water bodies with disease-causing microorganisms (McGlynn, 2011). The safety and quality of the drinking water

supply has, therefore, been significantly compromised by pollution and the alterations in the natural hydrogeological dynamics of water systems.

1.1.6 Microbiological water quality problems in the world, with particular reference to the Philippines

Water is often associated with some degree of microbiological contamination. According to the WHO (2002), most of the diarrhoeal diseases in the world [88%] are attributable to unsafe water, unhygienic practices, and poor sanitation. Studies show that hundreds of millions of people worldwide are using highly contaminated water supplies (Liang et al., 2006; Mac Kenzie et al., 1994; Moe, Sobsey, Samsa, & Mesolo, 1991; Payment et al., 1997). In 2003 – 2004, a total of 30 waterborne disease outbreaks associated with drinking water were reported in the United States alone, with 68% of cases represented by gastroenteritis (Liang et al., 2006). The aetiologic agents identified include bacterial, viral, and parasitic pathogens, as well as toxins and chemical poisonings. In Milwaukee, Wisconsin, USA, an estimated 400,000 cases of cryptosporidiosis from a filtered, chlorinated surface water supply occurred during the period March–April 1993 (Mac Kenzie et al., 1994).

In the Philippines, outbreaks of waterborne and foodborne diseases ranked first among all disease outbreaks in the decade between 1988 and 1998 (DOH, 2011). In 2005, acute diarrhoea outbreaks linked to poor sanitation ranked as the third cause of morbidity in the country (DOH, 2005). In Cebu City, which is the second largest industrial-urban and commercial centre in the Philippines, epidemiological studies were conducted to investigate the link between diarrhoeal diseases and the presence of waterborne indicator microorganisms (Moe et al., 1991). According to the city's health department, 1,443 diarrhoea cases with four deaths

were recorded from January to August, 2010 alone (Wikihealth, 2011). This figure rose to 3,586 total diarrhoeal cases, with 23 deaths, in 2012. From these cases, 3,212 were caused by acute gastroenteritis, 365 from amoebiasis, eight from paralytic shellfish poisoning (PSP), and one from cholera. Based on recent statistics from the same department, a total of 2,255 diarrhoeal cases with 20 deaths were recorded for the period January to October 2013 (Ygonia, 2013). These cases may have been caused by either unsafe water, or food consumption. Nonetheless, since water is an essential raw material in food preparation and processing, the likelihood that the cause is waterborne could not be ignored. Despite these cases of waterborne illnesses in the area, studies to investigate the aetiological agents are rare. Further, no comprehensive bacteriological studies combining an investigation of both deep well tap water and bottled water have been conducted, despite the above mentioned epidemiological data (DOH, 2005; Moe et al., 1991; Wikihealth, 2011) and the growing consumption of bottled water in the city (Alingasa, 2010).

Although, as said above, bottled water consumption has increased in the world and in the Philippines due to its perceived better quality and consumer convenience, studies show that many bottled water brands in many countries fail to meet the required microbiological quality criteria and are not safe for human consumption (Hasell & Capill, 2000; Kassenga, 2007; Moniruzzaman, Akter, Islam, & Mia, 2011; Raj, 2005; Svagzdiene et al., 2010; Varga, 2011).

1.1.7 Water quality monitoring and prevention of waterborne illnesses

Health problems associated with the consumption of contaminated drinking water may include gastro-intestinal illnesses with vomiting, diarrhoea, and nausea,

depending on the type of pathogen and health condition of the person (ESR, 2011). Worst case symptoms, such as bloody diarrhoea, sepsis, renal failure, and even death, may occur to susceptible and immune-compromised individuals infected with certain pathogens (USFDA, 1998). Therefore, national and local studies and regular monitoring of drinking water for the presence of pathogens and/or indicator organisms are needed.

In addition to regular monitoring and end-product testing for microbiological hazards in drinking water, several researchers have emphasised the importance of implementing good manufacturing practices [GMP] and Hazard Analysis and Critical Control Points [HACCP] programs in the production system (Dzwolak, 2014; Edberg & Allen, 2004; Green & Kane, 2014; Jagals & Jagals, 2004; Nunes & Fuzihara, 2011). GMP is one of the prerequisite programs of HACCP; hence, its development and implementation should be completed prior to the application of a HACCP system (Codex, 2001). Furthermore, studies show that consistent compliance with national and international standards by bottled water manufacturers and water service providers requires more stringent regulatory requirements and increased monitoring by health authorities (Krewski et al., 2004; Tulchinsky et al., 2000; Varga, 2011). In the Philippines, the Food and Drug Administration (FDA) (formerly known as the Bureau of Food and Drugs) of the Department of Health [BFAD-DOH], has issued regulatory guidelines for the implementation of GMPs and mandatory compliance by food manufacturers, including bottled water processors, to ensure good water quality (DOH, 2004, 2007).

1.2 Objectives of the study

1.2.1 The broad objectives of the study were

a) To review the literature on the types and sources of drinking water, their bacteriological quality, and impact on public health;

b) To assess the bacteriological quality of bottled and tap drinking water available in Cebu City, Philippines

1.2.2 The following specific aims were formulated:

a) To obtain baseline data on the bacteriological quality of bottled and tap water in Cebu City, Philippines

b) To assess the sources of variation in the bacteriological results using multivariable statistical approaches

c) To assess the compliance with the Philippine regulatory requirements

d) To identify possible associations between the sanitation programs applied by the water suppliers and the bacteriological results

CHAPTER 2 - LITERATURE REVIEW

2.1 Types of drinking water based on delivery and treatment methods

Drinking water can be classified based on the type of delivery to the consumers, and whether it is treated, or untreated. Drinking water can be delivered to consumers through municipal taps via distribution pipes, delivery vehicles, and the use of bottles. In addition, drinking water can be categorized as treated (synonym: processed) or untreated (unprocessed) (APHA et al., 2012d).

Treated water is defined as drinking water that undergoes various standard processing steps, including one, or a combination of the following physical and chemical treatments: filtration, ozonation, reverse osmosis, distillation (Percival, Walker, & Hunter, 2000; Robertson & Edberg, 1997; Senior & Dege, 2005), and other more rigorous disinfection techniques aimed at inactivating pathogens and producing a highly safe product (Edberg, 2005). Treated water includes many bottled water brands and municipal tap water systems delivered by local water districts. Municipal drinking water systems usually apply chlorination to the water as the main treatment method. Conversely, untreated water, which include many natural mineral waters and spring waters (including bottled or directly derived from these sources but delivered by other delivery methods), refers to drinking water that does not undergo any of the above-mentioned treatments, whilst maintaining the original quality present at the source (Finlayson, 2005).

The treatment of drinking water is required due to the existence of undesirable physical, chemical, and microbiological traits or constituents which are harmful to public health (Edberg, 2005; Percival et al., 2000). Technically, when none of

these factors occur, there should be no need to apply any treatment. Thus, legislation on bottled water in European countries prescribes the requirements for drinking water types that do not require treatment. For instance, a European Economic Community (EEC) directive on 'natural mineral waters' states that bottled natural mineral water should come from a protected source, possess consistent mineral composition, be naturally wholesome and safe, bottled in an unaltered state, and microbiological, chemical, and physical qualities be representative of the quality at its source (EuropeanCouncil, 1998). Hence, to ensure these characteristics are maintained, the treatment of 'natural mineral water' in Europe is illegal (Dege, 2005). In the United States municipal tap waters taken from subterranean sources and groundwaters are not required to be treated, provided that these water sources are pathogen free as proven by sanitary and hydrogeological surveys and monitoring (Dege, 2005). Nonetheless, not all areas in the world are naturally provided with good quality water sources. Also, the growing demand for drinking water consequent to the growing human population is putting enormous pressure on providing good quality water sources, with the reported increasing use of water sources of dubious bacteriological quality (Sakai, Kataoka, & Fukushi, 2013; WHO, 2002).

Constant maintenance of the bacteriological safety of drinking water is challenging and resource-demanding, due to the possibility of pollution at the source and during abstraction at any time, inadequate source monitoring, and hydrological limitations of watersheds (WHO, 2002). Maintaining safety and quality is especially difficult in highly urbanised and industrialised areas where contamination from industrial and domestic wastes is prevalent and water safety management plans are lacking. In addition, some areas are naturally scarce in

water supplies, and continued over extraction of the water can lead to intrusion of undesirable microbiological and chemical contaminants to the groundwater (Kumpel & Nelson, 2014; McGlynn, 2011). One of the major microbiological contaminants are the pathogenic bacteria, such as the pathogenic strains of *E. coli*, *Campylobacter*, *Salmonella*, and *Shigella*; parasites, such as *Cryptosporidium parvum* and *Giardia lamblia*; and pathogenic viruses (El-Taweel & Shaban, 2001; Leclerc & Moreau, 2002; Odonkor & Ampofo, 2013). These microorganisms can potentially cause disease when present in considerable viable levels in drinking water used by consumers. Therefore, necessary treatments are applied to the drinking water supply, specifically on municipal tap water systems and bottled water brands, to ensure the removal or inactivation of harmful microorganisms and other undesirable contaminants. Normally, drinking water that undergoes effective treatments can have an improved microbiological quality and safety (Percival et al., 2000; Senior & Dege, 2005; ServSafe, 2006).

There are a number of water treatment methods available to produce a drinking water free of pathogens. In general these involve the application of sequential multiple barriers aimed at inactivating different kinds of pathogens (Edberg, 2005; Percival, 2000). The most powerful disinfection treatments for water include: (1) filtration; (2) reverse osmosis (RO); (3) distillation; (4) ozonation; (5) chlorination; and (6) UV radiation (APHA, AWWA, & WEF, 2012; Edberg, 2005; Percival, 2000; Senior & Dege, 2005). Each method possesses unique strengths and limitations for each target group of microorganism (Table 2-1) (Edberg, 2005). Filtration is one of the most commonly used microbiological treatment method for drinking water. This method employs filters, screens, and granular material or membranes to trap particulate material, including microorganisms. The size of

the particles accumulating in the filter is usually between 0.001 and 100 μm diameter, and the drop in water pressure is an important monitoring parameter to check the efficiency of the method (APHA, AWWA, & WEF, 2012; Senior & Dege, 2005). Filtration is particularly effective for the removal of protozoan parasites such as *Cryptosporidium* and *Giardia lamblia* (Edberg, 2005).

Table 2-1 Relative Effectiveness of Water Treatment Types on Pathogen Groups

Pathogen Group	**Treatment effectiveness**					
	Filtration	Reverse Osmosis	Distillation	Ozonation	Chlorination	UV Radiation
Bacteria	Low	Good	High	Good	High	Good
Protozoa	High	High	High	Fair	Fair	Good
Viruses	Low	Good	High	Good	Good	Good

Source: Adapted from Edberg (2005), Percival et al. (2000), and Senior and Dege (2005).

Another treatment method is known as reverse osmosis and is commonly applied to alter the water's mineral content, but also results in the removal of pathogens. Reverse osmosis is a membrane process similar, in principle, to filtration; however, RO employs a controlled diffusion mechanism using pumps that deliver the required pressure and flow velocity across the membranes (Edberg, 2005; Senior & Dege, 2005). Proper maintenance of the membranes is one of the challenges of this water treatment method because of its usual spiral, winding configuration, resulting in cleaning difficulty. Hence, inadequate maintenance can lead to bacterial build-up in the membranes that serves as intrusion points for contamination. Nonetheless, the implementation of effective preventive

maintenance and monitoring systems can help prevent these problems (Edberg, 2005; Senior & Dege, 2005).

Distillation is a water treatment process where water is boiled and the resulting hot vapours are cooled, condensed and collected (APHA, AWWA, & WEF, 2012). Proper operation of this process normally results in the production of sterile, hence pathogen-free water. However, once the water passes through the pipes after leaving the still, it may again acquire microflora in the absence of proper equipment maintenance (Edberg, 2005). Ozonation is the treatment of water with a chemical oxidant known as ozone. Ozone is a high-energy, short-acting, and powerful disinfecting agent (Edberg, 2005; van der Walt, 2002). It is mostly employed in the bottled water industry because of its strong oxidizing capacity that damages cell membranes of bacteria resulting in bacterial cell death whilst also oxidising nuisance minerals specifically dissolved manganese and iron present in the water. Ozone is usually effective against viruses and bacteria, but not much on parasites as, for instance, *Cryptosporidium parvum* cysts (Edberg, 2005; van der Walt, 2002). Another chemical oxidant widely applied in drinking water treatments is chlorine, the agent used in the disinfection process known as chlorination. It is one of the most economical water treatment chemicals. Similar to other oxidation treatments, this process results in the destruction of microbial cells in the water (Senior & Dege, 2005). However, chlorination also produces undesirable chemical by-products, such as trihalomethanes (THMs), after reacting with natural organic contaminants in the water (Rosenfeldt et al., 2009). Excessive chlorine residuals, which are harmful to human health, can also occur in the water. Thus, subsequent processes, such as the adsorption with activated carbon, are applied to neutralize or remove these contaminants, in addition to

proper process application and quality control (Senior & Dege, 2005). Chlorination methods, either in aqueous hypochlorite or chlorine dioxide gas forms or in combination, are mostly applied in municipal tap water systems (Carter et al., 2000; Percival et al., 2000) due to chlorine's effective disinfection residual present in the water distribution pipes after the treatment process. The disinfecting potential of a disinfectant is related to its activity concentration and the contact time with a pathogen. Compared with ozone, chlorine has a higher disinfection power because it is low energy and slow acting, and not being easily dissipated characteristics (Edberg, 2005). Thus, chlorine produces a higher and effective disinfection residual throughout the municipal piped distribution system (Carter et al., 2000; Percival et al., 2000). Another water treatment aimed at inactivating microorganisms is ultraviolet (UV) radiation. A microbicidal activity is achieved through the action of the radiation energy at around a 260 nm wavelength on a microbial cell, causing the destruction of nucleic acid bases adenine and thymine and eventually resulting in bacterial cell death (Edberg, 2005). The advantage of using UV treatments in water is the absence of chemical by-products after the process.

Whilst each water treatment method possesses unique advantages, the limitations of their anti-microbial activity and in providing pathogen-free water should be acknowledged Table 2-1. Hence, the combined application of more than one treatment can compensate for each method's limited disinfection capacity and improve the quality of the final water product (Edberg, 2005; Montemayor et al., 2008; Senior & Dege, 2005; van der Walt, 2002; Venczel, Likirdopulos, Robinson, & Sobsey, 2004; Wang, Pryor, Edwards, Falkinham, & Pruden, 2013). Moreover, the effectiveness of each treatment system or

treatment combination is also influenced by the quality of the source water (Percival et al., 2000). In addition, the above-mentioned water treatment processes, whilst purposely aimed at removing or inactivating microbiological contaminants, are simultaneously also used to remove other undesirable chemical and physical contaminants to ensure the safety and quality of drinking water for human consumption (Senior & Dege, 2005). To ensure the quality and safety of treated bottled and municipal tap waters, the consistent implementation of a multiple barrier system, including the protection of the water source, source monitoring, effective disinfection treatment methods, and good sanitation and manufacturing programs, must be adopted.

2.2 Municipal tap water and bottled water brand categories in the study area

In residential and commercial urban areas, the dominant sources of drinking water supply usually originate from municipal taps provided by local water districts as well as commercial bottled water brands. The municipal drinking water system is a basic public service utility normally administered by local government, semi-government, or government-controlled authorities and companies (Francisco, 2014). On the other hand, bottled water is usually marketed by private manufacturers (Rodwan, 2011). In the Philippines, while there is growing apprehension about the reliability of municipal water supplies leading to the increasing use of bottled water, strong collective efforts by local water districts are still needed to ensure the provision of safe and high quality drinking water to consumers (Husayan, 2013). As required by law in the Philippines and other countries, municipal water districts must provide consistently good quality drinking water to the public (CPDO, 2008; DOH, 2007;

WHO, 2012). Nonetheless, consumers make the final choice as to which drinking water type they use. This decision is, however, influenced by environmental and personal factors, such as convenience, lifestyle, health risk information, personal values, and economic considerations.

In most countries, drinking water from the municipal supply and bottled types are normally treated drinking waters (Kumpel & Nelson, 2014; WHO, 2012). One major reason for treatment is to disinfect the water to ensure it is free from pathogens hence, safe for human consumption. A variety of water disinfection methods are used, ranging from conventional chlorination as mostly applied in municipal taps, to a combination of filtration, reverse osmosis, distillation and ozonation, and other treatments for many of the bottled water brands.

Municipal drinking water supplies are usually tapped from groundwater or surface water sources (Carter et al., 2000; Francisco, 2014). Other sources include springs, rainwater, desalinated seawater, and others (Dege, 2005; Shehane, 2003). These sources should undergo disinfection treatments when the quality is dubious and cannot be guaranteed. Treated municipal drinking waters are mostly delivered in distribution pipe utility networks. Consequently, these are accessed by consumers in household taps for domestic use or in business establishments for commercial purposes. Likewise, drinking water is also delivered to consumers through the use of bottles.

Bottled drinking water in various brands is also often taken from groundwater sources similar to municipal tap waters. The major difference comes in the mode of delivery to consumers. Bottled waters are packaged and sold in various packaging formats, while municipal taps are distributed continuously through a

network of pipes leading to household and domestic taps or faucets. Therefore, although tap and bottled water may have similar sources, their life cycles differ.

The categories of bottled waters vary greatly between countries and are largely influenced by national and local regulations (DOH, 1993; Senior & Dege, 2005). In some countries, bottled waters are classified into various categories based on particular attributes or criteria (Dege, 2005; Rodwan, 2011). The commonly used attributes are: (1) the type of water used; (2) source of the water; (3) treatment type; and (4) packaging formats (Dege, 2005). Based on water type, bottled water can be carbonated or still. The water source, as defined by legal criteria, can be grouped as natural mineral water, spring water, and 'other' waters (DOH, 1993; EuropeanCouncil, 1998). Waters classified under 'other' have been further subcategorised into treated well water, remineralized water, and purified mains water. Bottled water can also be categorised based on the packaging material used: glass or plastic packaging. The glass packaging used may be of returnable or non-returnable types. Likewise, plastic packaging for bottled water can be made of polyethylene terephthalate (PET) in either returnable or non-returnable formats, multi-layered PET, polyvinyl chloride (PVC), polyethylene, and returnable polycarbonate bottles (Dege, 2005). Whilst there are differences in categorizing bottled waters, there could be similarities and differences in the naming and definition of different bottled water types among countries (Codex, 2001; Dege, 2005). Nevertheless, it is important to understand some of the most conventional types of bottled water as defined by IBWA (2011) and widely used in the bottled water market to provide consumers with better informed choice on which types to purchase (Table 2-2).

Table 2-2 Major Types of Bottled Water

Bottled water type	Description and characteristics*
Mineral water	Natural water which contains not less than 250 parts per million of total dissolved solids. This water is distinct from other types because of its constant level and proportions of minerals and trace elements at the point of emergence from the source and cannot be remineralized.
Spring water	Water extracted from an underground formation from which water flows naturally to the earth's surface. This must be collected only at the spring or through a borehole tapping the underground formation feeding the spring. The water must have all the physical properties before treatment and of the same quality and composition as the water that naturally flows to the surface of the earth.
Purified water	Water that is produced by deionization, reverse osmosis, distillation, or other suitable processes while meeting the definition of purified water in the United States Pharmacopoeia. The product can be labelled as "distilled water" if produced by distillation, "deionized water" if processed by deionization, or "reverse osmosis water" if produced by reverse osmosis.
Well water	Water that is from a holed bored, drilled, or constructed in the ground, which taps the water from an aquifer.
Artesian water/ Artesian well water	Water from a well that taps a confined aquifer (a water-bearing underground layer of rock or sand) in which the water level stands at some height above the top of the aquifer.
Sparkling bottled water	Water which, after treatment and possible replacement of CO2, contains the same amount of CO2 that it contained as it emerged from the source and can be labelled as sparkling mineral water.

*as defined by IBWA, 2011.

Source: International Bottled Water Association, 2011, page 1.

2.3 Water quality standards and regulations

The bacteriological quality of drinking water refers to the level of occurrence of microorganisms in the final product, and is an index determining whether the water is safe for human consumption (USFDA, 1998; WHO, 2012). Further, the

bacteriological quality of drinking water is defined by parameters which separate acceptability [safe] from unacceptability [unsafe] (Codex, 2001), based on the measurement of multiple indicator species of microorganisms present in a water sample. If the bacteriological testing results is within the required standard limits, then the water is said to be of good bacteriological quality for human consumption (APHA et al., 2012d).

In many countries, the regulatory requirements for bottled and municipal waters are different because of the significant differences between the two water types. These two water types are also different from the microbiological standpoint (DOH, 1993, 2007; EuropeanCouncil, 1998; USFDA, 2013). In the United States, the bottled water industry is regulated by the Food and Drug Administration (FDA) (Senior & Dege, 2005; USFDA, 2013). Since the 1970s, the agency, in collaboration with the International Bottled Water Association (IBWA), has developed codes of practice and standards for the manufacturing of bottled water (Rodwan, 2011). Nevertheless, three levels of regulation are implemented in the USA for bottled water: the Federal (FDA), the State, and at the Industry level, through IBWA and FDA. Based on FDA's 'Final Rule on a Standard of Identity for Bottled Waters' and the Code of Federal Regulations (CFR) Part 165.110, bottled waters are classified into mineral water, natural spring water, artesian water, sparkling water, purified water, and water for infant use (USFDA, 2013). On the other hand, tap waters supplied through public and private treatment and distribution systems are regulated by the Environmental Protection Agency (EPA) (Senior & Dege, 2005). Both water regulations provide requirements for the microbiological limits in each drinking water type.

In Europe, bottled waters are categorized into spring waters, natural mineral waters, and 'other' drinking waters (Dege, 2005; EuropeanCouncil, 1998). The regulations for natural mineral waters are described in the Natural Mineral Waters Directive 80/777/EEC, whilst spring waters and other drinking waters are regulated by the Drinking Water Directive 80/778/EEC (EuropeanCouncil, 1980). Both directives were promulgated in 1980. However, in 1996, spring waters were incorporated into the natural mineral waters directive due to a new Directive (96/70/EC; (EuropeanCouncil, 1998). On the other hand, the Directive 80/778/EEC was repealed in December, 1998, by a new one known as Council Directive 98/83/EC that specifies the general quality of water for human consumption. These directives apply for all European Union (EU) member countries, that is, they must be incorporated into the existing national regulations. Nonetheless, some member states still apply their own standards, justifying it by the state of the market, cultural differences and historical practice (Dege, 2005; Senior & Dege, 2005).

In Australia and New Zealand, regulations governing bottled waters are established in the Australia New Zealand Food Standards Code (Reasoner, 2004). This standard regulates labelling, residues and contaminants, and microbiological limits. The industry is also self-regulated through the Australasian Bottled Water Institute (ABWI) (Dege, 2005). The ABWI has developed standards of quality incorporated into a Model Code which is applied by bottled water manufacturers for certification by third-party audits.

There are differences in each country's bottled water regulation, hence, there is no single standard to categorise bottled water across countries. Currently, there

are numerous bodies at the international and national levels, each one dealing with different aspects of bottled water regulation, therefore, we will only discuss those prominently involved with the industry. One of the international bodies is the Codex Alimentarius Commission (Codex, 2001), also known as Codex, founded in the early 1960s by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Codex has attempted to develop a worldwide standard for food, including bottled waters, with the goal of promoting equivalence in definitions and requirements in order to facilitate harmonization and enhance international trade. This standard has been adopted in some developing countries where food laws are inadequate, or lacking (Codex, 2001; Senior & Dege, 2005). Codex has produced numerous standards and guidelines aimed at ensuring the safety and quality of food and water. Given the urgency to provide standards for all bottled waters, regardless of the source and type, the Codex developed two separate standards: one for bottled natural mineral waters and another for bottled waters other than natural mineral waters.

In Asia, the bottled water market is comprised of over a thousand manufacturers across 40 countries (Dege, 2005). Similar to other global regions, the industry is also becoming harmonised in the application of water quality standards through membership with the Asia Bottled Water Association (ABWA), later known as the Asia and Middle East Bottled Water Association (Dege, 2005). The association has created a Model Code based on the Codex Standard for Bottled/Packaged Drinking Water other than natural mineral waters (Dege, 2005). All bottled waters must be safe for human consumption. Hence, various water treatments have been recognised and approved for those where safety of the source and processing cannot be assured, to ensure bottled water products meet minimum

microbiological standards of safety. The various drinking water microbiological standards in some Asian countries, in comparison with the WHO, IBWA, and ABWA, are presented in Table 2-3.

In the Philippines, bottled waters are regulated by the Philippine Food and Drug Administration or FDA (DOH, 1993), whilst municipal tap waters are under the joint jurisdiction of the Department of Health and the local government (CPDO, 2008; DOH, 2007). Growth of the bottled water market in the country started in the 1990s (Smith, 1999) and included the introduction of popular bottled brands from domestic and international companies. Likewise, water refilling stations in bigger bottles also became popular during the same decade (Soriano-Pasumbal & Ong-Lim, 2005). Like the other standards, bottled waters are also considered food in the Philippines, and its requirements and regulation is placed under the Philippine FDA. Separate drinking water standards have been established for bottled waters and municipal tap waters (Table 2-3). Due to frequent reports of waterborne illnesses and inadequate regulation in the Philippines, the drinking water standards have been reviewed and harmonised with the WHO and USA FDA requirements.

Table 2-3 Comparison of Microbiological Standards for Drinking Water from WHO, IBWA, ABWA, and Some Standards from Regulations Applicable in Some Asian Countries, Including the Philippines.

Parameter	WHO	IBWA	ABWA	China	Indonesia	Philippines (bottled water)	Philippines (tap water)
***E. coli* or thermotolerant coliforms**	0/100 ml	0/100 ml	0/100 ml	3 MPN	0/100 ml	0 / 100 ml	<1.1 MPN/100ml
Total coliforms	-	1/100 ml No validated total coliform detectable in a 100 ml sample as substantiated by resampling	-	-	1/100ml	1 MPN/100 ml, but must not be *E. coli*	<1.1 MPN/100ml
Colony counts at 37°C	-	-	-	-	10/ml	10^5 cfu/ml	<500

Source: adopted from DOH, 1993, 2007; and Dege, 2005

2.4 Sanitation programs and quality control

The establishment of basic sanitation programs such as good manufacturing practices (GMPs), sanitation standard operating procedures (SSOPs), good commercial practice (GCP), as well as food safety management tools such as Hazard Analysis and Critical Control Points (HACCP), and ISO 22000: Food Safety Management Systems (FSMS) can help maintain and improve the bacteriological quality of bottled water (Codex, 2001, 2003; Edberg & Allen, 2004; Green & Kane, 2014; ISO, 2005; Kokkinakis, Fragkiadakis, & Kokkinaki, 2008). GMP, SSOP, and GCP are minimum requirement to operate a bottled water manufacturing business in the Philippines (DOH, 1993, 2004), as well as other countries (Liang et al., 2006; Senior & Dege, 2005; Sun, Liu, Cui, & Liu, 2013; Zamberlan da Silva et al., 2008). On the other hand, HACCP and FSMS are

systems that are voluntarily applied in the food and beverage industries but require the prior implementation of the basic sanitation programs (e.g. GMP).

GMPs are basic activities and conditions that include documented policies and procedures designed to create a hygienic environment and sanitary conditions in a food processing facility (Codex, 2003; DOH, 2004; Senior & Dege, 2004). On the other hand, HACCP is a management tool that analyses hazards [including pathogens] in a product and develops control measures to prevent, eliminate, or reduce them to safe levels (Codex, 2001, 2003; Senior & Dege, 2004; ServSafe, 2006).

Many studies highlighted the integration of good manufacturing practices (GMP) and sanitation standard operating procedures (SSOP) in the processing of food and bottled drinking water. Marzano et al. (2011) emphasized the refinement of hygiene practices in their microbial study of bottled spring waters in Italy, that found high levels of *Pseudomonas aeruginosa* contamination. Nonetheless, their study did not indicate a serious public health risk because other tests, most notably *E. coli* and coliforms, were negative. A study of bottled waters in New Zealand indicated that the manufacturing plants did not implement GMP, or the implemented programs were ineffective, as some bottled brands contained significant levels of coliforms and yeasts and moulds (Svagzdiene et al., 2010).

A food safety management system (FSMS) is a management tool which combines the principles of HACCP system and basic sanitary programs such as GMPs and implemented within the framework of system management and interactive communication, to ensure the safety of a food product. Mossel and Struijk (2004) demonstrated the application of a HACCP system using marker

organisms in ensuring the integrity and safety of bottled and piped water. HACCP focusses on Critical Control Points (CCPs) or steps in the production process where potential hazards are likely to occur that must be controlled (Codex, 2009; Wallace, Holyoak, Powell, & Dykes, 2014). Nevertheless, a HACCP certified manufacturer may not immediately control all potential hazards, especially when critical process steps are missed during a HACCP study, as indicated by Kokkinakis et al. (2008). In that study, the plant which had been implementing a HACCP-based system failed to include the water source as a CCP, and the source was eventually found contaminated with 83% coliforms and 13% *E. coli* in the samples, suggesting poor quality water at the source. While further treatments such as filtration can eliminate or reduce the hazards, it is not good hygienic practice to harvest water from a highly contaminated source, especially when effective multiple barrier systems are not in place (Senior & Dege, 2005). GHPs, GMPs, and SSOPs are prerequisite programs of a HACCP system.

2.5 Impact of water quality on public health

There have been reported cases of non-compliance with the microbiological requisites for drinking water during monitoring in some countries (Hunter, Hartemann, & Zmirou-Navier, 2009). In New Zealand, for instance, approximately 251,000 [6%] people were supplied with drinking water that failed the bacteriological quality criteria for the period July 2009 to June 2010 (ESR, 2011). Similarly, a study on treated drinking water from 30 private establishments in Sana'a City, Republic of Yemen, reported very high levels of bacteria from faecal and non-faecal sources (Raja'a, Al-Ashwal, & Al-Ghaili, 2001). Although bottled water is believed to be of better quality than ordinary deep well tapped water (Dawson & Sartory, 2000; El-Taweel & Shaban, 2001; Senior & Dege,

2004), studies have shown that some bottled brands failed to comply with the required microbiological standard limits (Hasell & Capill, 2000; Nunes & Fuzihara, 2011; Raj, 2005; Svagzdiene et al., 2010; Tacio, 2005; Zamberlan da Silva et al., 2008).

A similar study in Cebu, Philippines, showed high incidence of infant diarrheal illnesses after consumption of drinking water contaminated with *E. coli* at a concentration of >1.0 x 10^3 MPN/100ml (Moe et al., 1991). In another outbreak of waterborne typhoid illness in Tuburan, Cebu, thousands of patients required medical care, with ~180 hospitalisations, and five fatalities (Rubio, 2012). This outbreak prompted the local government to issue warnings, instruct chlorination of distribution pipelines, release relief goods and order an immediate investigation. Initial results indicated an old water pipeline laid in the 1920s as the source of contamination. These events demonstrate the importance of good processing and maintenance programs in water treatment plants. Moreover, it shows the need for stringent monitoring systems, control measures and regulations, to ensure good drinking water quality is sustainably maintained (Herath et al., 2012; Varga, 2011).

2.6 Microbiology of drinking waters

2.6.1 Microbiology of bottled water

Drinking water in its natural state is not a sterile product (Edberg & Allen, 2004). This is especially true for natural mineral waters and spring waters harvested from subterranean sources. A highly diverse naturally occurring microflora is always present in these underground water sources and subsurface environments. These microorganisms are often called autochthonous organisms (Casanovas-

Massana & Blanch, 2012; Leclerc & Moreau, 2002), and this microflora is dominated by various species of bacteria, such as the pseudomonads of the proteobacteria group, the Bacteroides-Cytophaga-Flavobacterium phylum members, the proteobacteria's prosthecate group, and the actinomycetes subclass Gram-positive bacteria (Leclerc & Moreau, 2002). These bacterial groups are generally considered harmless (Allen, Edberg, & Reasoner, 2004; Edberg & Allen, 2004; Edberg, Kops, Kontnick, & Escarzaga, 1997; Sartory, 2004). Some have even been shown to possess important health benefits for humans (Lucas & Ducluzeau, 1990). In contrast, microorganisms that are not normally found in natural waters and are only acquired from external sources either through groundwater intrusion or processing contamination are termed allochthonous organisms (Leclerc & Moreau, 2002). These include all other microorganisms and pathogens, such as *E. coli*, *Salmonella*, *Shigella*, and *Enterobacter* species.

The autochthonous microorganisms in groundwaters are a diverse group of bacteria not known to cause diseases in humans (Allen et al., 2004). Delabroise and Ducluzeau (1974) highlighted the inability of heterotrophic bacteria found in mineral waters to colonize a human gastrointestinal tract, and this finding was corroborated by many other studies (Allen et al., 2004; Edberg & Allen, 2004; Edberg, Gallo, & Kontnick, 1996; Edberg et al., 1997). This has been supported by results of cytotoxicity and virulence studies of bacterial strains isolated from drinking waters (Edberg & Allen, 2004; Edberg et al., 1996; Edberg et al., 1997). Furthermore, beneficial effects, including antagonistic activities against pathogenic microorganisms, have been documented. For instance, Moreira, Agostinho, Morals, and da Costa (1994) demonstrated a reduction in the levels

of pathogen-associated faecal coliforms, such as *Klebsiella pneumoniae*, *E. coli*, and *Enterobacter cloacae,* and environmentally occurring *Pseudomonas aeruginosa* after inoculation of these organisms into bottled waters containing indigenous heterotrophic bacteria. This is the reason why water in its natural state is normally not sterile. For this reason, most national regulatory standards do not require zero heterotrophic plate count (HPC) limits for drinking water. Furthermore, when the source water can be guaranteed to be safe through intensive source protection and monitoring schemes, these drinking waters can be bottled without treatment as in the case of bottled natural mineral water and spring water (European Council, 1998; Hunter, 1993. However, this is not always the case in many countries where the entry of extraneous microbial contaminants, also referred to as allochthonous organisms (including pathogens), into water sources can occur. This contamination in various pathways from both environmental and faecal sources are posing impending threats to the underground water quality and making the safety of drinking water questionable. Hence, in order to mitigate the uncertainty of the source water's bacteriological quality and improve the assurance of safety, the drinking water supply, in general, has undergone processing through various treatment mechanisms (Edberg, 2005; Senior & Dege, 2005).

Autochthonous organisms are also known as heterotrophic organisms because photosynthesis cannot occur in groundwater environments, resulting in a microbial food chain that is dominated by heterotrophic nutrition (Leclerc & Moreau, 2002). Organisms of this nature include almost all bacterial groups and some fungi. Heterotrophs cannot fix inorganic carbon (namely, CO_2), but can use other reduced organic compounds available in underground substrates. This is

the reason that studies on the quality of autochthonous microflora in water uses the HPC test. Accordingly, HPC is one of the assessment parameters in evaluating the bacteriological quality and safety of drinking water for human consumption.

There were evidence that the presence of naturally occurring bacteria in original source waters creates inhibitory impacts on allochthonous organisms contaminating these waters (Ducluzeau, Hudault, & Galpin, 1976; Kerr, Fitzgerald, Sheridan, McDowell, & Blair, 1999; Leclerc & Moreau, 2002; Lucas & Ducluzeau, 1990; Ramalho, Afonso, Cunha, Teixeira, & Anthony Gibbs, 2001; Rosenberg, 2003). Some reasons for this include microbial antagonism, predation, and inhibition as a consequence of the purifying effect of freshwaters. The decrease in allochthonous bacterial numbers varies between genera and could be influenced by environmental parameters such as light, temperature, and dilution (Leclerc & Moreau, 2002). Nonetheless, Leclerc and Moreau's findings suggest that the accumulation of inhibitory substances in the water as metabolic by-products of continued growth cycles and death of autochthonous bacteria is more likely the cause of the microbial antagonism against the pathogens and not because of the effect of the chemical and physical characteristics of the water. In addition, other known mechanisms of bacterial competition have been observed in other studies (Delabroise & Ducluzeau, 1974; Leclerc & Moreau, 2002). This includes the production of antibiotic substances by species of *Pseudomonas* and related genera. These secondary metabolites possess toxic activity against competitors (Lucas & Ducluzeau, 1990; Moreira et al., 1994). In the field of bioremediation, the *Pseudomonas* group emerged as one of the most

promising group of microorganisms because of their high biological degradation potential to prevent the invasion of harmful and pathogenic microorganisms.

Researchers have argued that autochthonous bacteria in water should be further investigated and assessed for possible human health risks (Pavlov, de Wet, Grabow, & Ehlers, 2004). This can be studied using the heterotrophic plate count (HPC) test. Literature has shown that these organisms are psychrotrophic (adapted to lower temperatures between 25–30°C), prototrophic (no specific nutritional requirements but mostly inorganic compounds), and oligotrophic (thrive in low nutrient environments) (Leclerc & Moreau, 2002; Morita, 1997). These features are in contrast to human body conditions (high nutrient levels in complex forms and moderately high temperature) and, at the same time, these bacteria are vulnerable to the natural barriers of the gut, such as the gastric trap, mucous membranes, intestinal cytoprotection, and immune response, making colonization by these bacteria impossible. Thus, these naturally occurring microorganisms found in natural mineral waters, spring waters, and other untreated potable waters cannot grow in the human gastrointestinal tract (Leclerc & Moreau, 2002). Accordingly, these generally do not cause diseases to humans (Edberg et al., 1996). Nevertheless, emphasis should be put on allochthonous microorganisms and pathogens that are contaminating drinking water because of pollution at the source, inefficient water treatment, and inadequate processing methods. Importantly, while gastrointestinal protection against pathogens is evident, water consumed in between meals can rapidly pass along the stomach because of the relaxation of the pylorus area (stomach's lower part leading to the small intestine), thereby eluding the stomach's intragastric retention and bactericidal effects (Leclerc & Moreau, 2002; Levine & Nalin, 1976; D. Mossel &

Oei, 1975). Hence, ingested water contaminated with pathogens even in low levels can cause disease.

A wide variety of pathogens can grow in drinking water environments and evade water treatment defences (Odonkor & Ampofo, 2013; Soriano-Pasumbal & Ong-Lim, 2005). Most of these organisms are biofilms (aggregates of bacterial cells that are attached to surfaces and enclosed in an exopolysaccharide matrix) that are formed over time and are protected by their self-produced polysaccharide matrix (Mah & O'Toole, 2001; van der Merwe, Duvenage, & Korsten, 2013). This makes biofilms resistant to ordinary cleaning and treatment agents. Hence, systematic monitoring and identification of microbiological hazards in drinking water is paramount. The important bacteria of concern are the enteric pathogens, such as the enterohemorrhagic *E. coli, Salmonella spp., Vibrio cholera*, *Shigella spp*., and *Campylobacter jejuni* (Hassan, Farhad, Ebrahim, & Amin, 2013; Leclerc & Moreau, 2002). There are also environmental pathogens that are introduced from surface waters to the drinking water distribution system, such as the bacteria *Legionella spp.*, *Aeromonas spp.*, *Mycobacterium avium* complex, and *Pseudomonas aeruginosa* (Bartram, Cotruvo, Exner, Fricker, & Glasmacher, 2004; Pavlov et al., 2004). Again, colonisation of the water contact surfaces with these bacteria is aggravated by biofilm formation in the distribution system. In addition, enteric protozoans, most notably *Cryptosporidium parvum*, *Giardia lamblia,* and its emerging opportunistic relatives like *Isospora* and *Cyclospora,* along with pathogenic viruses, such as norovirus, rotavirus, Hepatitis A, and E viruses from sewage, can be transmitted through these waterborne pathways (Fong et al., 2007; Leclerc & Moreau, 2002). While bacterial pathogens possess abilities to grow in large numbers, protozoans and viruses do not normally

multiply in receiving waters. However, viruses and protozoans only require low infectious doses to cause diseases. The infectious doses of these microorganisms vary widely. For instance, Leclerc and Moreau (2002) reported that the pathogenicity of *Salmonella* spp. is 10^7 – 10^8 cells while *Campylobacter* and enterohemorrhagic *E. coli* requires only ~10^2 cells. Similarly, *Cryptosporidium* protozoans need only around 10 – 100 oocysts and 1 – 10 infective units for viruses to cause clinical illnesses.

The significance of any positive bacteriological test result on water is always based on the potential health risk this may cause to consumers. The health risk posed by the presence of indicator organisms and pathogens in drinking water samples is calculated based on the number of bacteria present, the virulence of the bacterium, and the immune system condition of the host (Edberg & Allen, 2004). The ability of pathogens to cause a disease is a function of these factors. Some organisms are more pathogenic than others based on their virulence characteristics, such as the presence of exotoxins in most Gram-positive bacteria as by-products of their metabolism (Abdulraheem, Mustafa, Al-Saffar, & Shahjahan, 2012; Edberg et al., 1997). These virulence factors may be released by the bacteria in the water or inside the body of the host when the water is ingested (Levine & Nalin, 1976). Consequently, these can cause damage to host cells in the intestines that trigger chain reactions and lead to disease symptoms ranging from mild, such as nausea, vomiting, and diarrhoea, to more fatal conditions, such as bloody diarrhoea, kidney failure, paralysis, and toxic shock syndromes (Dawson & Sartory, 2000; Edberg, Rice, Karlin, & Allen, 2000; Odonkor & Ampofo, 2013; Payment et al., 1997).

In bottled waters, the level of microflora can be altered drastically because of the difference in the water's microenvironment compared with the original underground source. Studies show that placing water in bottles increases the surface area of the water environment compared to the water's interstitial underground source and disrupts the natural dynamics of metabolite and nutrient exchange between bacterial cells and the *in situ* environment (Leclerc & Moreau, 2002; Zobell & Anderson, 1936). This phenomenon is called the 'bottle effect'. The bottle effect is generally related to the availability of organic carbon and the bottle surface to water volume ratio. Leclerc and Moreau (2002) stated that organic carbon is essentially the most limiting nutrient affecting the activity and growth of bacteria. In subsurface environments, the supply of readily available carbon is typically low. However, this complex organic matter can be modified and increased through oxygenation and increasing temperature during bottling (Leclerc & Moreau, 2002; Morita, 1997). It was observed that bacteria grow rapidly when the surface area is larger than the volume of water because nutrients present accumulate and adsorb on the surface which makes it more readily available for the bacteria. However, this theory of 'bottle effect' also known as 'volume effect' was contradicted by the work of Hammes, Vital, and Egli (2010), who argued that evidence on the 'surface area to water volume' relationship was inconclusive. They disputed that the real factor may only be the amount of organic nutrients adsorbed onto surfaces, regardless of container size. Hence, these 'surface-adsorbed organic nutrients' influence the levels of bacteria present as a result of the organic carbon-contaminated glasswares used by the 'bottle effect' authors in their experiments. Nevertheless, it is difficult to compare these studies as the results may have been confounded by the testing methods

used by the different researchers. Importantly, the type of culture media, time of incubation, and temperature when culture-based methods are used can also affect the bacteriological results of a study, making comparisons impractical or unreliable. Whatever the most plausible explanation of the so-called 'bottle effect' is, the commonality is that bacteria undergo considerable increase in numbers after bottling, and this is influenced by the availability of utilisable nutrients. This was corroborated by a number of bottled water storage and stability studies (Delabroise & Ducluzeau, 1974; Ducluzeau et al., 1976; Duranceau, Emerson, & Wilder, 2012; Raj, 2005) and it was also supported by the study of Jayasekara et al. (1999), who demonstrated the occurrence of variations in bacterial counts adhering to the bottle surface among different bottles from the same manufacturer. The bacteria attached to the surfaces reached 83% of the total bacterial organisms in the bottle and was around 10^6 – 10^7 colony forming units (cfu). In contrast, one study showed low bacterial levels, around 632 cfu per cm^2, adhering onto the surface of polyethylene terephthalate (PET) bottles (Jones, Adams, Zhdan, & Chamberlain, 1999). Hence, the type of bottle material can also influence the levels of bacteria in the water. For instance, water packed in containers made of glass usually contains lower bacterial counts than that in polyvinyl chloride (PVC) plastic bottles. Possible reasons for this effect are the higher oxygen diffusion rates, organic matter migration, and rougher material surface characteristic of PVC bottles; all promote the growth and further adhesion of bacterial cells on the surface (Leclerc & Moreau, 2002). Likewise, the presence of residual cleaning substances in glass bottles after washing can cause bacteriostatic impacts, resulting in lower bacterial levels (Bischofberger, Cha, Schmitt, Konig, & Schmidtlorenz, 1990). The most notable bacteria isolated

from bottled waters are the Gram-negative aerobic bacilli that belong to the Flavobacterium-Cytophaga phylum (Jones et al., 1999).

The integrity of the bottles in the stores could also be compromised by poor storage conditions, for instance those in high relative humidity in non-air-conditioned and/or inadequately ventilated storage rooms (Duranceau et al., 2012; Geldreich, Nash, Reasoner, & Taylor, 1974). These conditions may result in water condensation, which can lead to the development of biofilms penetrating the internal surface of the bottle, especially in bottles with inconsistent sealing systems after bottling (Kohnen et al., 2005; Mah & O'Toole, 2001). Also, the possibility of re-growth of sub-lethally injured bacterial cells or low-level bacterial species in the bottles could occur even after being sealed (Jayasekara, Heard, Cox, & Fleet, 1999; Marshall, 1988; Payment, 1995).

In regard to the effect of storage duration on bottled water bacterial counts, Duranceau et al. (2012) show that viable colony counts rapidly increase after three to seven days of bottling. They also observed in their bacterial growth modelling that longer storage durations from three to seven years proportionately resulted in enormously high HPCs for bottled waters stored at an average ambient temperatures of 28 – 37°C. Their study used logistic growth models to describe the long term (in years) persistence of bacteria in PET bottled waters stored in porch storage conditions. The model associating the observed and predicted bacterial growth using a HPC test resulted in a high correlation coefficient ($R^2 > 0.99$). While their experiments and objectives differ from the present study, some common results were observed. First, Duranceau et al.'s study showed that bacteria can increase in a bottled water environment through

time. Second, they used a HPC test and revealed its usefulness to monitor bacterial growth and, finally, the viability of bacteria to consistently increase in PET packaging format was verified because the bottled water manufacturers in the current study also used PET packaging material. However, some contrasting results in other studies revealed that long storage times can result in a bottled water product being free of any microorganism. Increases in bacterial cell numbers obey certain microbial growth curves and cycles composed of adaptive lag, exponential increase, stationary, and decrease phases (Duranceau et al., 2012). It is difficult to establish absolute intervals between phases because of the presence of slow growing and fast growing bacterial cells in water samples. In addition, microorganisms respond differently to various types of culture media, which makes comparability between studies challenging. Also, many bacteria, including pathogens, can enter into a particular metabolic stage known as a 'viable but non-culturable'(VBNC) state in response to adverse environmental conditions, nutrient deprivation, starvation, and other metabolic stress. Hence, results showing zero or low colony counts may actually contain high numbers of live organisms that do not respond to the culture media, equipment, and incubation conditions of the study's test methods. However, this has been currently addressed by the development of more sensitive culture media and molecular test methods (Abo-Amer, Soltan, & Abu-Gharbia, 2008; APHA, AWWA, & WEF, 2012b; Rompre, Servais, Baudart, de-Roubin, & Laurent, 2002).

In relation to water temperature, bacterial levels in bottled water tend to increase in numbers when stored at 25 – 37°C. No significant bacterial levels were observed at refrigeration temperatures of 4°C, while higher temperatures of up to 42°C proved fatal for most heterotrophic bacteria (Duranceau et al., 2012; Leclerc

& Moreau, 2002; Raj, 2005). Leclerc and Moreau (2002) described experimental evidence suggesting that growth was highest when bottled waters were stored at an ambient temperature of ~20°C. However, at refrigerated conditions (~4 - 6 °C), the growth of bacteria was sustained, albeit at slower rates.

Furthermore, it has been shown that bottled waters after production from the factory are normally shelf-stable because of the protective effect of treatment, bottling, and sealing so that the integrity of the product is generally maintained (Edberg, 2005; Leclerc & Moreau, 2002). Therefore, any post-treatment bacteriological change in treated bottled waters is more likely to be due to the effect of factory production conditions rather than a result of post-processing and handling circumstances in proper storage, well ventilated stores, and normally handled by consumers. However, this was not the case in the results of Duranceau et al.'s (2012) study where they concluded that bottled water's bacteriological quality deterioration likely occurred after consumer purchase and upon subsequent storage. Their results were, however, disputable because the bottled water storage under study was subjected to extreme time and temperature abuse conditions (namely, storage in a car trunk or on a house porch in summer conditions), resulting in loss of bottle integrity, contamination, and microbial growth that could have led to higher bacterial counts.

2.6.2 Microbiology of tap water

Like the various water sources for the bottled water brands, municipal water system sources also contain naturally occurring microorganisms overwhelmingly dominated by the bacterial group (Casanovas-Massana & Blanch, 2012; Edberg & Allen, 2004). Depending on the source, either surface freshwater or

underground water, the bacteriological quality of drinking water is influenced by different sets of factors (Leclerc & Moreau, 2002; Senior & Dege, 2005). Municipal water sourced from groundwaters and distributed through pipe networks are typically inhabited by heterotrophic microorganisms. Compared with surface waters that are governed by suspended particles and their attached microflora, groundwaters are generally confined in high pressure interstitial spaces underground that have greater saturation resulting to decreased likelihood of subsurface contamination, low porosity (lesser interstitial space for microbes to thrive), and oligotrophic chemistry (low nutrient) (Leclerc & Moreau, 2002; Perk, 2006). Because of these factors, microbial growth are greatly limited in groundwaters in contrast to surface waters which are virtually open to the atmosphere and likely contamination. Hence, there is some degree of microbial exclusivity (only highly- tolerant organisms can survive that are usually non-pathogenic) in these systems that contribute to the natural protection of these groundwater habitats against pathogens compared with surface waters. Moreover, unlike packaged waters that acquire relative stationary residence in bottles, tap water flows in a continuous phase along a distribution network of pipelines. The pipeworks are usually made of metal materials, mostly iron and copper, as well as lead coming from solders that join copper pipes (Carter et al., 2000). In most typical scenarios, the delivery of water requires adequate amounts of pressure to reach treatment plants (if treated), reservoirs, substations or distribution loops, and ultimately to each single dead-end distribution loop. The water typically remains in the dead-end loop until extracted by a consumer through taps in private households, and commercial and industrial establishments (Percival et al., 2000). In these conditions the waters are

removed from their original source and transported to a different environment and accompanying microflora. More specifically, the type and levels of bacterial flora in municipal water systems are not the same as in bottled waters. The dominant bacterial types in municipal water supplies include many of the acid-fast bacilli, Gram-negatives and Gram-positives and spore formers, as described by Geldreich (1996).

Kumpel and Nelson (2014) studied the multiple mechanisms affecting water quality and contamination with coliforms and *E. coli* in the pipes in cases of water interruption and intermittent supply. They demonstrated that low water pressure in distribution pipes increases the levels of coliforms even in the presence of chlorine residuals. In contrast, high pressures, together with the presence of disinfection residuals, reduced the levels of coliforms and no *E. coli* was detected. Possible explanations for these contaminations during low water flow are the occurrence of external intrusion of contaminants into the pipes, internal backflow, internal pipe wall particulate release, and sloughing of bacteria from attached biofilms as a consequence of low flow (Kumpel & Nelson, 2014). This was hypothesised in the study of Carter et al. (2000) that when water pressure and residual chlorine are high in a municipal distribution system, bacterial counts seemed to decrease. However, the levels of bacteria along the pipe network varied depending on the location. Carter et al. (2000) showed that bacterial levels increased with distance from the treatment plant as a consequence of reduced effective chlorine residuals. In contrast, bacterial numbers decreased from booster pump stations to dead end supply loops which supply the household concessionaires (Carter et al., 2000).

It is also important to consider that bacterial contamination in distribution pipes is aggravated by the presence of biofilms, which are characterised by the presence of typical microorganisms, such as *E. coli*, *Legionella*, and *Pseudomonas* (Berry, Xi, & Raskin, 2006; Brettar & Höfle, 2008; Leclerc & Moreau, 2002). Problems with plumbing systems, ineffective maintenance of pipes, inefficient disinfection, low water pressure, and flow interruption are factors that could significantly contribute to the proliferation of biofilms at the periphery of long pipes, making treatment systems ineffective and unsustainable. This is in agreement with the studies of Carter et al. (2000), Edberg et al. (1997), and Kumpel and Nelson (2014), which support the idea that bacterial contamination in municipal tap waters is affected, not only by the microbiological properties of the sourced water, but also by defects in distribution, maintenance, and management. It has also been established that most disinfectants and sanitizers are ineffective against bacteria that form biofilms because of the relative protection and resilience of microorganisms capable of entering this matrix (Mah & O'Toole, 2001; Marshall, 1988; van der Merwe et al., 2013). It was also shown that the effective disinfection residuals of chlorine are greatly reduced by the chlorine-demanding organic matter found in soils and sewage that infiltrate the system (Edberg et. al., 2000). Furthermore, *E. coli* can survive in distribution pipes when chlorine residuals dissipate. Specifically, *E. coli* bacteria can survive for about 4 – 12 weeks in drinking water distribution systems (Edberg et. al., 2000). Similar to viruses, bacteria are sensitive to chemical oxidation treatments (for example, chlorine), but survive longer than viruses in the water. Hence, the presence of *E. coli* alone can provide information about the effectiveness of treatments applied. In addition, Edberg (2005) and Edberg et al. (2000) indicated that the

susceptibility of these organisms to the bactericidal effect of chlorine is markedly reduced because of the protective effect of faecal material and biofilms contaminating the water and pipes. Therefore, in addition to chlorination, proper application of sanitation and maintenance programs and engineering are major factors in the assurance of bacteriologically safe municipal water supply.

Many studies have shown the high disinfection power of chlorine, which yields effective microbicide residual in municipal water systems (Edberg et al., 1996; Edberg et al., 2000; Kumpel & Nelson, 2014; Senior & Dege, 2005). In contrast, treatments such as ozone, reverse osmosis, and distillation applied on bottled waters leave no effective disinfection residuals (Edberg et al., 1996; Kumpel & Nelson, 2014; Percival et al., 2000; Senior & Dege, 2005). Whilst most urban municipal waters require disinfection treatments, the application of source protection and monitoring is paramount to facilitate the efficiency of the water treatment regime applied (Edberg, 2005). It should be noted that once water enters the distribution system from the underground source, a new microecosystem is formed because of the effect of pipes and production materials on the original microflora of the water. One of the most influencing materials is the organic matter that serve as nutrients for heterotrophic bacteria, facilitating their colonization on the distribution pipe networks (Leclerc & Moreau, 2002; Senior & Dege, 2005).

As earlier mentioned, bacterial growth and survival is affected by the organic matter present originating not only from the raw water, but also from the distribution materials in pipes, such as sealants, lubricants, and joints (Geldreich, 1996). The colonisation of pipes with heterotrophic bacteria is facilitated by these

organic nutrients. More importantly, this material promotes the formation of biofilms that can contaminate pipe networks and edges where water stagnation occurs (Brettar & Höfle, 2008). Studies showed that the most widely used bacterial indicator, *E. coli,* is able to survive in water for a period of 4 – 12 weeks at a temperature range of 15 - 18°C (Edberg et al., 2000; Odonkor & Ampofo, 2013; Schets et al., 2005). Further, *E. coli* survives for up to 100 days in groundwater at a temperature of 10°C (Filip, Kaddumulindwa, & Milde, 1987). Thus, the transport of pathogens from these sources through municipal pipes is inevitable in the absence of source protection and effective treatment methods. Hence, these groundwater sources, and distribution networks with leaks in pipes and joints, can serve as intrusion points and ideal spots for the growth of enteric pathogens. Consequently, pathogen contaminated drinking water delivered from this distribution source poses potential health risks to consumers.

Therefore, various water disinfection methods are usually applied on drinking water originating from sources not guaranteed as being microbiologically pure and pathogen free (Edberg, 2005). For municipal water supplies, chlorination is the most appropriate treatment method because of chlorine's effective disinfection residual consistently being present throughout the distribution network to ensure the destruction of pathogens (Kumpel & Nelson, 2014; Leclerc & Moreau, 2002). Nonetheless, as said above, the effectiveness of this method is affected by the pipe network reticulation and the type and levels of microorganisms present.

In the above discussion it was shown that there are fundamental differences between the bacteriological quality of municipal tap water supply and bottled

waters. However, both water types must not contain harmful bacteria or pathogens and must be safe (potable) for human consumption, regardless of the presence of a natural microflora (Senior & Dege, 2005). Therefore, microbiological testing methods are similar for both water types. Municipal water supplies usually achieve water safety by chlorine disinfection and the presence of chlorine residuals in the distribution system. This chemical treatment, however, cannot be applied to bottled water because of the organoleptic changes it causes to the bottle surface and the water itself.

2.7 Bacteriological assessment of water quality

The bacteriological quality of water from a source and the efficiency of disinfection treatments can be assessed by monitoring the presence of pathogens or indicator organisms. The commonly used bacteriological analysis and testing parameters include isolation of *Escherichia coli or E. coli* count, total coliforms count, faecal coliforms count, *Pseudomonas sp*. count, and *Enterococci sp. count* (Schets et al., 2005; Svagzdiene et al., 2010). Rompre et al. (2002) studied various methods for the enumeration of coliforms [*E. coli*, faecal, and total coliforms] in water, including the conventional multiple tube fermentation [MTFT] and membrane filtration [MF] techniques. They also studied these organisms using molecular methods which are more rapid such as the immunological assays [IA] and polymerase chain reaction [PCR] techniques. Despite the shorter testing duration and practicality of the rapid techniques, the traditional bacteriological methods are more cost-effective, are considered more precise and yield a better recovery rate (per cent viability of microorganisms to be detected and enumerated). The authors concluded that, although many detection methods for the coliform-group have been developed, only a few have the potential to become

the gold standard method for detection of coliforms in drinking water. In fact, studies showed that when lactose-based coliform media are used, a relatively high rate of false-negative coliform results occurred as a result of high HPC counts in the sample (Allen et al., 2004; Bartram et al., 2004; Duranceau et al., 2012). This effect is due to the inhibition of the growth of the coliforms in lactose-based coliform media when HPC bacteria reaches 500 – 1000 colony forming units per millilitre (cfu/ml) (Allen et al., 2004; Duranceau et al., 2012). Factors that influence choice of the methods largely depend on the purpose of the test, type of sample, validity of the developed test method to identify the bacteria, and cost considerations (Rompre et al., 2002).

Another bacteriological parameter used in drinking water quality assessment is the heterotrophic plate count [HPC] testing (Varga, 2011). Sartory (2004) demonstrated the usefulness of this test in the assessment of microbial growth in treated drinking water in the United Kingdom (UK). The authors showed the appropriateness of HPC as a management tool in water treatment and distribution but not as a direct health-risk parameter. Upon completion of the testing, the results are interpreted according to their potential implications on public health risk. In this study, the following four commonly used tests were used: (1) *E. coli* count; (2) thermotolerant or faecal coliform count; (3) total coliform count; and (4) heterotrophic plate count.

2.8 Characteristics of indicator organisms

There are a multiple species of pathogens capable of contaminating water samples, hence, the analysis of each species at regular intervals is regarded as impractical and expensive. Therefore, the use of indicator organisms was

introduced to allow a cost effective, more frequent, less sophisticated routine analysis of water samples. An indicator organism is an organism that suggest the occurrence of other organisms especially pathogens when the indicator is present in a particular sample (Odonkor & Ampofo, 2013). Hence, instead of identifying all pathogens of concern, a simple but scientific approach is to only analyse for the indicator bacteria. Whereas indicator organisms may not be pathogenic, the presence of a single indicator organism in drinking water indicates that contamination with a pathogen may be present. This does not, however, immediately indicate a potential health risk problem (Allen et al., 2004). To effectively evaluate the safety and health risk of a contaminated water sample, monitoring must be based on a combination of multiple marker organisms accompanied by thorough analysis of each combination of results. In addition, the specific objectives of the monitoring should also be carefully considered. The concept of 'index' and 'indicator' systems in the monitoring of the bacteriological quality of drinking water is generally used to improve the interpretation of the implications of various bacteriological test results (Leclerc & Moreau, 2002; Odonkor & Ampofo, 2013). The presence of an 'Index' organisms indicates the probable presence of a related pathogen, with the associated health risks, whilst the presence of an 'indicator' more commonly reveals a treatment failure or processing deficiency (Ingram, 1977, as cited in Leclerc & Moreau, 2002). The attributes of an index or indicator are: (1) their concomitant presence with the pathogens; (2) they should be easy to detect and quantify; and (3) their test methods should be widely available and cost-effective (Edberg et al., 2000, Odonkor & Ampofo, 2013, Sartory, 2004). These organisms, which are also the most routinely used in the bacteriological assessment of water quality, include *E.*

coli as the generally used index organism*;* and coliforms, thermotolerant or faecal coliforms, heterotrophic bacteria, *Pseudomonas aeruginosa*, faecal streptococci, and sulphite-reducing anaerobes as the indicators (Edberg et al., 2000; Leclerc & Moreau, 2002; Odonkor & Ampofo, 2013) . For non-routine investigations, tests for viruses and protozoans are also recommended. Unfortunately, bacterial indicators cannot yet be used to predict the presence of enteric viruses as there is still no direct correlation between viruses and bacteria due to the variability and unpredictability of viral behaviour (Leclerc & Moreau, 2002).

The *E. coli* test is considered an extremely important test to reveal a recent faecal contamination of water and food products or unsanitary processing, and the presence of other enteric bacterial pathogens (Odonkor & Ampofo, 2013; USFDA, 1998). This test was, however, challenged because other organisms can mimic its biochemical characteristics. For instance, *E. coli's* ability to ferment lactose, which is the basis for the identification of *E. coli* in many standard laboratory tests, can be a feature of other enteric bacteria, such as *Enterobacter*, *Citrobacter*, and *Klebsiella spp.* (Edberg, 2000; USFDA, 1998). In addition, these organisms may show phenotypic characteristics similar to those of *E. coli,* making the distinction between *E. coli* and other organisms difficult. This resulted in the development of a test for the presence of the so- called 'coliforms' and 'faecal coliforms' (later defined 'thermotolerant coliforms'), which was widely adopted as a suitable index of sanitary significance by the USA Public Health Service, the International Commission on Microbiological Specifications for Foods, the Philippines, and in many other countries (DOH, 1993, 2007; USFDA, 1998). Nevertheless, *E. coli* was still considered the most specific indicator of recent faecal contamination because studies have shown that that some of the coliforms

can also be found in natural environments, even in the absence of faecal materials (Odonkor & Ampofo, 2013). The presence of more recent analytical methods has also facilitated the reinstatement of *E. coli* as an indicator of public health and sanitary significance. In addition, *E. coli* is now regarded as the most recognised indicator of faecal contamination in drinking water (Edberg, 2000; Odonkor & Ampofo, 2013).

The total coliform count indicates the general sanitary quality of water, disinfection and treatment effectiveness, and the sanitary condition in food processing environments. The term 'coliforms' refers to facultative anaerobic, Gram-negative, rod-shaped bacteria that ferment the sugar lactose to produce gas and acid at 35°C within 48 hours (USFDA, 1998). However, the validity of the coliform test results should be considered with caution as past waterborne outbreaks from treated waters which have complied with coliform standards have been recorded as reported by Payment et al. (1997). They concluded that negative coliform test results do not always imply a pathogen-free water. On the other hand, the faecal or thermotolerant coliforms are a subset of the coliforms which presence could indicate contamination in aquatic environment where shellfish are grown and harvested (USFDA, 1998). The term 'faecal coliforms' refers to a subgroup of the coliforms that ferment lactose at a temperature of 44.5°C within 24 hours (Edberg, 2000). But this term was reviewed because these organisms were also found in organic-matter-rich environments, even in the absence of faecal contamination, as reported in APHA, AWWA, and WEF (2012). Hence, they are now termed 'thermotolerant coliforms' to refer to its higher growth temperature requirement than non-faecal coliforms.

Another bacteriological test widely used in drinking water testing is the heterotrophic plate count (HPC) test. This test allows the growth of the whole range of bacteria present in water samples (USFDA, 1998). The HPC test was introduced to provide information on the effects of water disinfection and treatment on the bacteriological quality of drinking water. For instance, bottled waters and municipal water supplies that have undergone treatments are commonly assessed for treatment effectiveness using the HPC test (Carter et al., 2000). Likewise, untreated waters that are inherently microbiologically wholesome are also often checked using the HPC test (Leclerc & Moreau, 2002; Sartory, 2004). Bottled natural mineral waters and spring waters may contain some levels of naturally occurring bacteria representative of the source, as indicated by a positive HPC result. Nonetheless, very high levels of HPC and coliform group counts in the water may indicate vulnerability to contamination and deficiency in the natural hydrological protection mechanisms of the source (Duranceau et al., 2012; Leclerc & Moreau, 2002). There is considerable debate on the potential health risks associated with high levels of HPCs in drinking water. Some evidence indicates that without faecal contamination, positive HPC counts alone may not be associated with adverse human health effects (Leclerc & Moreau, 2002; WHO, 2002). Therefore, bacteriological testing of drinking water should apply a combination of tests which include the *E. coli*, faecal and non-faecal coliform tests, and HPC, to better understand the degree of bacterial contamination of the drinking water supply. Several researchers used this approach in the interpretation of the public health significance of the microbiological testing results (Hasell & Capill, 2000; Levesque et al., 1994; Raj, 2005; Svagzdiene et al., 2010).

Other bacteria have also been used as indicators, for instance *Pseudomonas aeruginosa.* This species is an environmental bacterium mostly found in soils and vegetables. Although it can be found in the gastrointestinal tract of humans and animals, studies showed that gut is not its normal portal of entry and requires previously damaged organs such as in immunosuppressed individuals for infection to occur (Allen et al., 2004; Leclerc & Moreau, 2002). Hence, the organism is an opportunistic pathogen in immunocompromised individuals. This bacterium is regularly found in low numbers in drinking water. Because it was reported to be an opportunistic pathogen, its presence in drinking water must be controlled to a minimum. Another indicator bacteria are the faecal streptococci which are a group of microorganisms typically present in human and animal faeces, albeit in lower numbers than *E. coli*; hence, testing for faecal streptococci is often performed as an additional indication of disinfection and treatment efficiency (Edberg et al., 2000). Both *P. aeruginosa* and faecal streptococci have been isolated in some studies in bottled waters and municipal water systems, indicating considerable levels of external contamination is possible in these water sources. Moreover, some authors argue that *Pseudomonas aeruginosa* together with another opportunistic environmental pathogen, *Legionella spp.,* should be monitored separately because some indicator methods, such as the test for coliforms, cannot identify their presence in drinking water (Leclerc & Moreau, 2002). Nonetheless, because these bacteria are heterotrophic, they usually grow in the HPC test. It should be noted that a combination of the index and indicator organisms is the more suitable and cost-effective monitoring system in the assessment of the bacteriological quality of drinking water. Moreover in monitoring drinking water supply for public health purposes, it could be more

important to analyse for indicator organism using simple, inexpensive but reliable methods frequently, rather than a highly sensitive, sophisticated, but expensive test, used occasionally (Edberg et al., 2000; Leclerc & Moreau, 2002; Odonkor & Ampofo, 2013). Notwithstanding their differences, *E. coli*, coliforms, thermotolerant coliforms, HPC, and *P. aeruginosa* are still being used albeit in different applications and purpose.

2.9 Environmental impacts associated with water supply to residential urban areas

The world's current water footprint (use of water resources) is rapidly rising (Hubacek, Guan, Barrett, & Wiedmann, 2009; Yu, Hubacek, Feng, & Guan, 2010). Uitto and Schneider reported that demand for freshwater worldwide (as cited in Yu, Hubacek, Feng, & Guan, 2010) has increased fourfold in the past few decades. In China, agriculture accounts for the biggest water consumption at 79% of the national water footprint (Hubacek et al., 2009); the household and manufacturing sectors ranked second (10%) and third (8%), respectively. In the UK, the consumption of water has greatly increased over the past decades just like in many other developed countries (Yu et al., 2010). The Organization for Economic Co-operation Development (OECD) (1998) estimated that approximately 13-20% of the total world population will be living in water-scarce regions (less than 1000m^3/capita/year) by the year 2050. While general water exploitation and scarcity is an issue of concern, the effects of water use and extraction have also contributed to significant changes in ecosystems' health and water quality in many countries (Perk, 2006).

One major issue affecting water quality is the pollution of various drinking water sources, such as groundwater and surface waters (An & Breindenbach, 2005;

Barrell et al., 2000; Perk, 2006). Intensive agriculture for instance the expansion of farming areas and the increasing use of pesticides and fertilizers have significantly affected the water quality of surface water bodies, such as in rivers and streams (Grabowski, Wharton, Davies, & Droppo, 2012; Sharpley, Kleinman, Flaten, & Buda, 2011; Sharpley, Kleinman, Jordan, Bergstrom, & Allen, 2009; Wang, Magesan, & Bolan, 2004). The pollution of water bodies is a major problem in many highly industrialized and agriculture-intensive countries. In New Zealand, for instance, contamination of rivers by nutrients, such as phosphate and nitrates, occurs due to intensive dairy farming and inefficient farm management practices (McDowell, Sharpley, Crush, & Simmons, 2011; Sharpley et al., 2011; Sharpley et al., 2009). In addition, pollution coming from industrial manufacturing sectors is also affecting various groundwater and surface water bodies (Perk, 2006). In China, approximately 50% of freshwater resources available annually were reported in year 2000 to be polluted with wastewater emissions from the pulp and paper industry (Hubacek et al., 2009).

Poor drinking water quality as a result of pollution has resulted in various infectious waterborne illnesses (DOH, 2011; Fong et al., 2007; Moe et al., 1991; WHO, 2002). Hence, efforts to improve and maintain drinking water safety and quality are being made through use of various water treatment techniques (Bates, 2000; Besic et al., 2011). Alternative drinking water treatment techniques (such as chemical oxidation, microfiltration, membrane processing, ozonation, and reverse-osmosis) and delivery modes (example: bottled water versus piped distribution; sale in supermarkets versus vending machines) are associated with different environmental impacts. Studies have shown that bottled drinking water can have greater environmental impacts than the conventional municipal water

treatment and delivery systems (namely, via piped distribution to household faucets) (Bonton, Bouchard, Barbeau, & Jedrzejak, 2012; Jungbluth, 2005; Raluy, Serra, & Uche, 2005; Senior & Dege, 2005). Because of the relatively intensive use of resources, such as water and energy, the extra use of chemicals, longer transport, and more waste disposal, such treatment and delivery modes may contribute to resource depletion, global warming, eutrophication, acidification, and ozone layer depletion, as well as environmental toxicity, compared with conventional municipal treatment and delivery systems (Crettaz, Jolliet, Cuanillon, & Orlando, 1999; Friedrich, 2002; Raluy et al., 2005).

Considering water treatment, bottled water employed more rigorous processes and techniques, including the addition of chemicals aimed at ensuring safety and quality of the product water. The use of energy and material resources, and the associated emissions have resulted in varying degrees of water pollution and other negative environmental impacts (Jungbluth, 2005; Senior & Dege, 2005). With regards to the delivery mechanism, the alternative methods usually use various packaging forms, most notably bottles made of PET, to ensure product protection, integrity, safety, as well as convenience for consumers (Senior & Dege, 2005). This involves the use of transportation for both the raw materials and finished products, resulting in high energy use and gas emissions. Furthermore, when the water has been consumed, the packaging material becomes unwanted waste and ends up in landfills, representing an additional environmental problem (Ferrier, 2001; Foolmaun & Ramjeeawon, 2012; Friedrich, 2002).

Unjustified overuse of bottled water consumption leads to an immense increase in packaging waste that, notwithstanding its recyclability, still poses significant, global solid waste management challenges and environmental pollution concerns (Tacio, 2005). Recent technologies and mechanisms of drinking water delivery, production, and processing have generated negative environmental impacts as a result of resource use, emissions, and waste disposal. In fact, international campaigns urging consumers to drink tap water instead of bottled water were conducted (AllAboutWater.org, 2004; Doole; Doria, 2006; International Water Association & World Health Organization, 2003; Senior & Dege, 2005). These were based on the premise that the quality of some bottled water brands was similar to that of tap water (Raj, 2005; Svagzdiene et al., 2010). However, continued negative public perceptions on the quality of the tap seem to hinder most people (Johnstone & Serret, 2012), especially in developing countries such as in the Philippines (Francisco, 2014), from using this water type. Hence, this current study while primarily intends to understand the bacteriological quality of bottled and tap water in the context of consumer safety, may have some positive contribution from an environmental management perspective when results show that both drinking water types have similar bacteriological quality and safety. Hence, consumers may consider the use of the tap as the preferred drinking water source thereby reducing the environmental impacts of bottled water overconsumption.

CHAPTER 3 - MATERIALS AND METHODS

3.1 The study area

3.1.1 Brief introduction

The study was performed in the mega-urban centre of Cebu City, Philippines (Figure 3-1 and Figure 3-2). The city is situated in the central eastern section of the province of Cebu, an island at the centre of the Visayas in the southern part of the country, with geographic coordinates of 10° 17' north and 123° 54' east. The total land area of the city is 29,124.78 hectares, with an urban zone of 5,598.53 hectares (CPDO, 2008).

Administratively, Cebu City is divided into north and south districts with a combined total of 80 'barangays', or villages, consisting of 46 in the north and 34 in the south districts. In addition, 50 of these villages are classified as urban barangays of the city. Within the urban zone lies the mega-urban centre, defined as the central business district which hosts the biggest commercial and trading facilities of the city, and among the biggest in the country (CPDO, 2008). This made Cebu City the second largest growth centre in the country after Metro Manila (the country's capital). Along with this vibrant growth and development, however, comes the increasing demand for basic utilities and infrastructure, in particular the drinking water supply.

Water resources and quality depend on climatological, hydrological, topographical characteristics of a region. However, this is also influenced by the rate of water extraction, especially in areas of high water demand typical in many highly urbanized and industrialized areas such as in Cebu City. There are

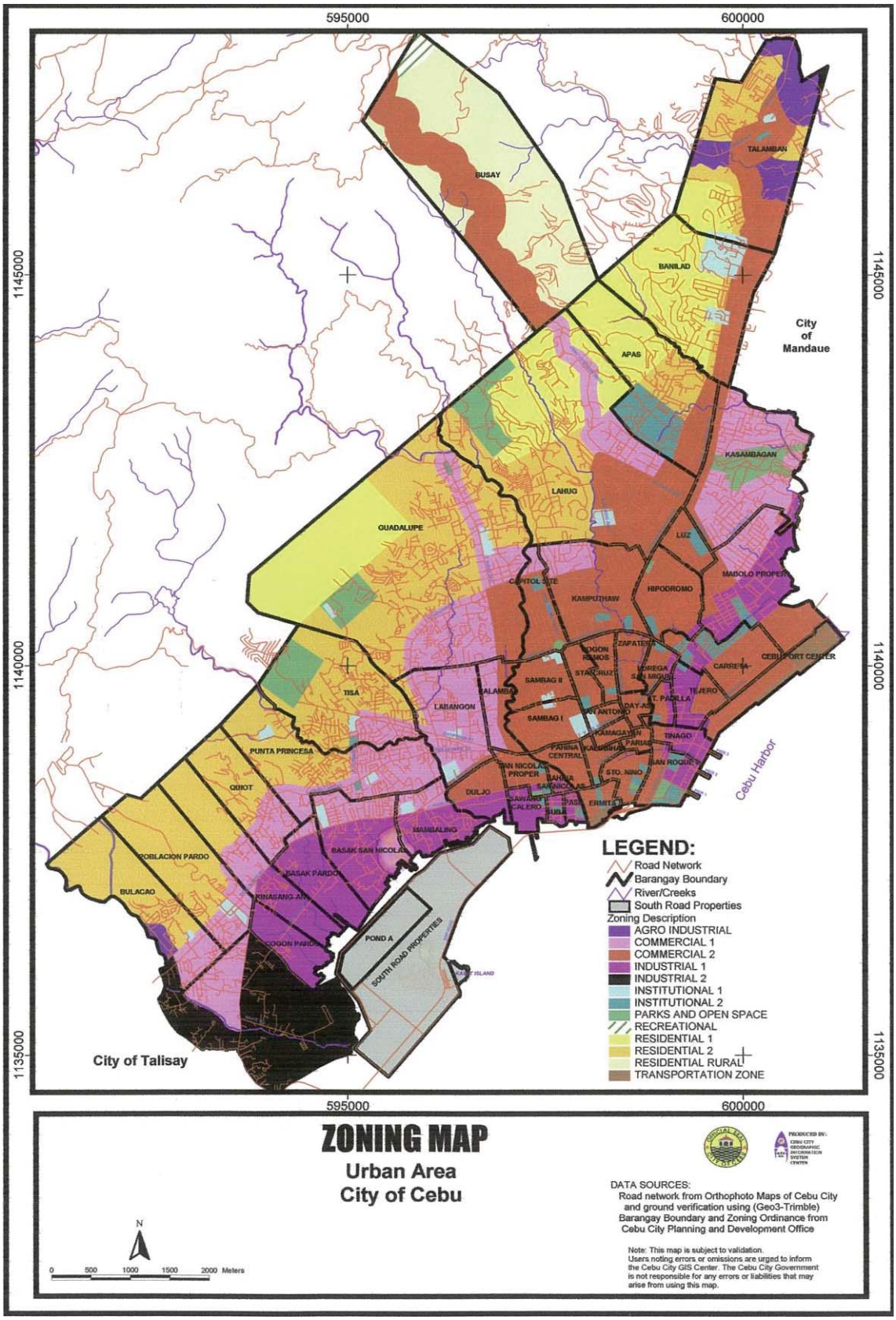

Figure 3-1 Map of the mega-urban centre of Cebu City, Philippines.

Source: adopted with permission from Cebu City GIS Center, 2013

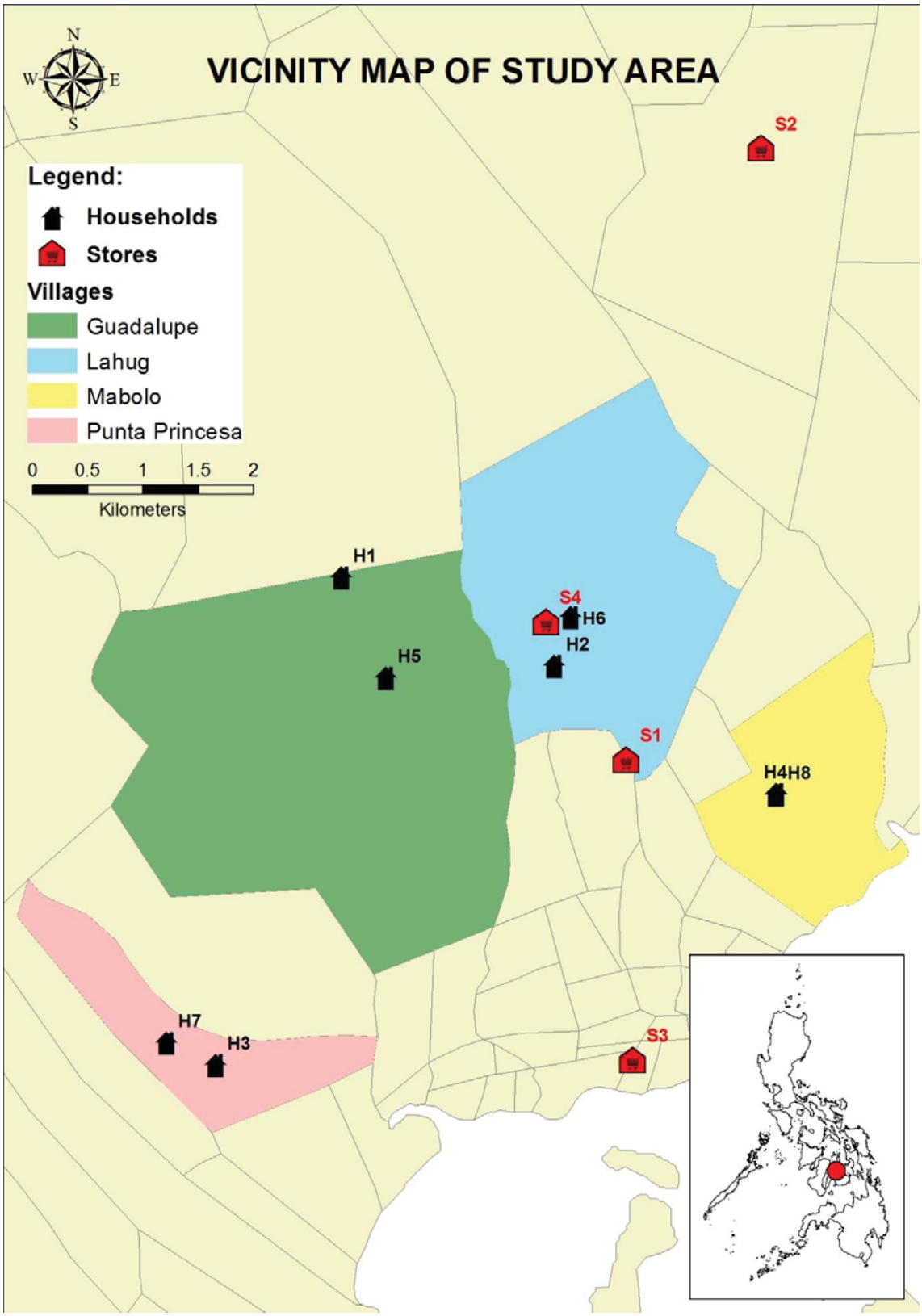

Figure 3-2 Map of the study area in Cebu City, Philippines (created using GIS software).

The map shows the location of households and stores. The relative position of the study site on the Philippine map is also shown in the insert.

hydrological and physical limitations to Cebu's watersheds which have resulted in relative water scarcity and over dependence on groundwater (CPDO, 2008). It has been estimated that approximately 84% of the local water supply is sourced from groundwater, mostly from coastal aquifers (CPDO, 2008; McGlynn, 2011). Current data reveals that these sources are already experiencing seawater and domestic sewage contamination (CPDO, 2008). Cebu City is characterized by a mountainous and rugged topography with highest elevations up to 900 meters above mean sea level. In terms of weather and climate, the city and country as a whole has a tropical climate with an average annual temperature of 26.5°C and mean relative humidity of 75%. Annual average precipitation is registered at 1,636.70mm. This decreases during February to April (dry season) and increases in May to July (start of wet season).

Population growth and urbanization have resulted in major urban development of the city's flat lands. Based on the 2010 census, the total population of Cebu City was 866,171, an increase of 20% since the previous census done 10 years earlier. This number corresponds to 42.4% of the metropolitan population and 30.2% of the entire Cebu province. Since municipal water use is directly related to the size of an urban population and the amount of water withdrawn, Cebu City is faced with problems relating to availability of good quality water.

3.1.2 Water infrastructure in the study area

The development and maintenance of infrastructure and utilities will play a significant role in the city's future sustainable development. One of the major utilities is the water supply. The water supply in Metropolitan Cebu undergoes various processes of treatment, transmission, and delivery to consumers.

Distribution is mainly piped though in few cases it is via transport vehicles. In Cebu City, water supply, maintenance and management is under the jurisdiction of the Metro Cebu Water District (MCWD) (CPDO, 2008; Husayan, 2013). The MCWD was created through presidential decree 198 in order to develop water supply and sewerage systems in the vicinity of Metro Cebu, hence it is regarded as the main local water utility in the area (CPDO, 2008). For this reason, the tap water provided by MCWD was selected. The MCWD covers four cities, namely: Cebu, Talisay, Mandaue, and Lapulapu, as well as the four towns of Consolacion, Liloan, Compostela, and Cordova, but all tap water was collected from Cebu City.

3.1.3 Selection of water types

This study used bottled and tap water samples collected in the mega-urban centre of the City of Cebu and the urban barangays or villages of Guadalupe, Lahug, Punta Princesa, and Mabolo (Table 3-1). Bottled water samples were acquired from four private stores, whilst tap water samples were collected from eight households in four barangays. Specifically, for tap water the study used water samples collected from two households on each of four the barangays with the greatest number of local water district concessionaires (B. Gabril, personal communication, July 5, 2013), for a total of eight households (Table 3-1). Because of the presence of tens of bottled water brands in the Philippines, this study focussed on the six brands with the greatest market shares, based on a study by Euromonitor International (as cited in Manila Bulletin, 2013) and Campaign-Nielsen's Top Brands Report (Campaign-Nielsen, 2011). Euromonitor's results showed the percentage market share of each of the top four brands whilst the 5th brand was based on Campaign-Nielsen's study. In addition, these brands were the most available in the shelves of large

supermarkets and small retail stores in the study area. The last brand identified was the only non-nationwide available brand as it was consistently present in small stores whilst at the same time represents the small local brands and companies. This information was verified during visits conducted prior to the study. Together the six brands accounted for ~80% of the market share in the Philippines (Campaign-Nielsen, 2011; Euromonitor-International, 2012; Manila-Bulletin, 2013). In order to capture the variability due to the different batches produced, the bottled water brands were collected from two large and two small city stores in the study area.

These stores were selected based on the investigator's personal knowledge on the location and size of stores in the study area, and according to the following selection criteria: (1) presence of a valid business permit (as confirmed by Cebu City local government office); (2) identification of all six pre-identified bottled water brands in these stores during a preliminary visit to the stores (not all brands were present in all stores); and (3) location within the study area. Table 3-2 displays the business profiles of the selected stores (CPDO, 2008).

Table 3-1 Demographic information of the selected barangays in the study area.

Name of barangay	Sampled households	Population (2010 Census)	Ranking according to the number of MCWD concessionaires*
1. Guadalupe	H1 and H5	60,400	1st
2. Lahug	H2 and H6	35,157	2nd
3. Punta Princesa	H3 and H7	22,270	3rd
4. Mabolo	H4 and H8	21,842	4th

*Data obtained from COREPLAN Department of Metro Cebu Water District

Table 3-2 Business profile of the selected stores in the study area.

Store Name**	Description	Availability of top bottled water brands
S 1	Large Store 1 (LS1)	Top 5 of 6
S 2	Large Store 2 (LS2)	Bottom 1 of 6
S 3	Small Store 1 (SS1)	Top 5 of 6
S 4	Small Store 2 (SS2)	Bottom 1 of 6

** Data confidentially requested from Cebu City Government, Mayor's Business Permits Office

3.1.3.1 Sampling strategy and justification

The objective of this study was to assess the bacteriological quality of bottled and tap water in the study area at the point of sale. Tap and bottled water have discrete life cycles, thus a formal statistical comparison between the bacteriological qualities of these two drinking water sources is not feasible. An intuitive unit of reference could be a single drinking water 'portion' likely to be acquired for consumption by an individual person on a single drinking occasion. In this case, the units of reference can be the single bottle of commercially available bottled water, and a similar volume of tap water collected into a container.

Therefore, a strategy based on sampling and bacteriological testing of similar volumes of water was designed, based on a budget allowing the testing of up to 250 - 270 sample units. In this study, a sample unit is defined as the individual portion or container of water sample collected from each source. For bottled water, this refers to the individual bottle per brand purchased from a store. For tap water, a sample unit refers to the collected water in one sterilized glass bottle from a tap.

3.1.4 Collection of bottled water and tap water samples

This study employed a survey methodology in the gathering of data through the conduct of field sampling and laboratory testing (Hasell & Capill, 2000). For the collection of water samples, a stratified random sampling technique was employed (Abo-Amer et al., 2008; Levesque et al., 1994). Thompson (2002) says such a technique results in a representative sample as required for this collection of water samples from household taps and bottled water brands. The collection of samples from each water type was repeated in multiple sampling occasions. A sampling occasion is defined as the collection of samples from all water types on a particular period of time. For instance, a sampling occasion for bottled water refers to the purchase of all bottled water brands in all stores covered in the study throughout a one-month period. Likewise, a sampling occasion for tap water is the collection of the water from taps in all households identified in the study in the same period of time. Thus, in this study a one sampling occasion covers the total combined collection of all bottled and tap water sample units from all these sources over a one-month period. Another sampling occasion is essentially a repetition of this activity.

Three sampling occasions for tap water is a visit to each household three times to collect the water samples. On the other hand, a two and three sampling occasion for bottled water is a visit to each store two and three times, respectively, to purchase the bottled water brands.

3.1.4.1 Sampling reference criteria

For the purpose of this study, the unit of sampling was the 500 mL water bottle. Based on the laboratory costs for the processing of each bottle a maximum

sample size of 270 bottles was estimated, including 150 bottled and 120 tap water bottles. In this study, the functional lot of tap water was defined as a tap-sampling occasion combination and that of bottled water was a store-sampling occasion combination. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986) and the Philippine Standards of Quality and Requirements for the Processing, Packaging, and Labelling of Bottled Drinking Water (DOH, 1993), five bottles is the minimum number of units required for the bacteriological testing of functional lots of drinking water. Therefore, 500 mL water bottles were sampled in multiples of five, for a total of 30 bottled and 24 tap water functional lots. Over all, eight taps (two for each barangay) on three sampling occasions were tested (8x3x5=120 bottles). Conversely, six bottled water brands were tested. For the first three brands, there were three sampling occasions from each of two stores (3x2x5x3=90). For the other three brands there were two sampling occasions (2x2x5x3=60 bottles). No blocking for batch was considered for the sampling, as it was hypothesised that only one or two production batches of bottled water will be found for each brand on store-sampling occasion. However, the production batch of each bottle was registered and considered in the analysis.

For tap water, each tap was considered an independent production unit, as no information about the water supply reticulation was available.

3.1.4.2 Sample collection and schedule

The sampling plan was designed to allow collection of both bottled and tap water samples in the same period of time. Sampling of each water source was performed once a week, every Sunday (bottled water) or Thursday (tap water),

for a total of 10 consecutive weeks for bottled and 12 weeks for tap water. The sampling schedule is presented in Table 3-3.

Table 3-3 Sampling schedule for the bottled water brands (B) purchased from stores (S) and household tap water samples (H) collected from villages (V) in the study area.

		Bottled water samples [a]		Tap water samples [b]	
Sampling occasion	Sampling week	Brand and store	No. of bottles (unique sample code)	Household and village	No. of samples (unique sample code)
#1 Jul. 28 - Aug. 24, 2013	**1**	B1 (S1); B2 (S1); B3 (S1)	15 (1 – 15)	H1 (V1); H2 (V2)	10 (16 – 25)
	2	B4 (S1); B5 (S1); B6 (S2)	15 (26 – 40)	H3 (V3); H4 (V4)	10 (41 – 50)
	3	B1 (S3); B2 (S3); B3 (S3)	15 (51 – 65)	H5 (V1); H6 (V2)	10 (66 – 75)
	4	B4 (S3); B5 (S3); B6 (S4)	15 (76 – 90)	H7 (V3); H8 (V4)	10 (91 – 100)
#2 Aug. 25 - Sept. 21, 2013	**5**	B1 (S1); B2 (S1); B3 (S1)	15 (101 – 115)	H1 (V1); H2 (V2)	10 (116 – 125)
	6	B4 (S1); B5 (S1); B6 (S2)	15 (126 – 140)	H3 (V3); H4 (V4)	10 (141 – 150)
	7	B1 (S3); B2 (S3); B3 (S3)	15 (151 – 165)	H5 (V1); H6 (V2)	10 (165 – 175)
	8	B4 (S3); B5 (S3); B6 (S4)	15 (176 – 190)	H7 (V3); H8 (V4)	10 (191 – 200)
#3 Sept 22-Oct 14, 2013	**9**	B1 (S1); B2 (S1); B3 (S1)	15 (201 – 215)	H1 (V1); H2 (V2)	10 (216 – 225)
	10	B1 (S3); B2 (S3); B3 (S3)	15 (226 – 240)	H3 (V3); H4 (V4)	10 (241 – 250)
	11			H5 (V1); H6 (V2)	10 (251 – 260)
	12			H7 (V3); H8 (V4)	10 (261 – 270)
		Sub-total	150	Sub-total	120
		Grand total samples	270		

[a] Brands 1, 2, & 3 x 5 sample units (bottle) per brand x 2 stores x 3 sampling occasions; Brands 4, 5, & 6 x 5 sample units (bottle) per brand x 2 stores x 2 sampling occasions

[b] 8 households (4 villages x 2 households per village) x 5 sample units per household x 3 sampling occasions

As mentioned above, a total of 30 bottles were sampled from each one of the top three brands (Brands 1, 2, 3), and 20 bottles from each of the remaining brands (Brand 4, 5, 6) (market share data for the identification of the top three brands were obtained from Euromonitor International as cited in Manila Bulletin, 2013). For the bottled water sampling, 25 bottles were initially collected at a random fashion from the store shelf on every sampling occasion. The brand name and batch/lot code were registered and the bottle's surface temperature measured using an infrared thermometer (Raytek, Beijing, China). Bottles with damaged packaging and expired shelf life were excluded from the sampling. Twenty bottles were returned to the shelf and 5 were purchased and transported to the laboratory

by the author, where they were tested on the same day of sampling (see Appendix 1 for the detailed bottled water sampling procedure).

For tap water, 30 bottles from each one of four villages with the greatest number of water concessionaires were sampled. All tap water samples were collected following standard collection protocols (APHA et al., 2012d). Two concessionaires per village were sampled.

Concessionaires were recruited by letters sent out to village concessionaires and two concessionaires expressing an interest in participation from each village were eventually selected by the investigator, based on their location, so as to avoid the inclusion of adjacent concessionaires. The four villages were: Guadalupe; Lahug; Punta Princesa; and Mabolo. Based on the same sampling plan, 5 samples per concessionaire per sampling occasion were collected from the household taps in these four villages (Table 3-3).

Tap water sampling involved the collection of 500 mL water samples using an aseptic procedure. From each household, water samples were collected in a sterilised, re-sealable, non-reactive borosilicate glass bottle. Before each bottle was filled, the tap was opened fully to let the water run to waste for 2 – 3 minutes to allow clearing of the service line. Subsequently, the faucet was disinfected with 70% ethanol and flamed. The tap was fully opened again to let water run to waste for another 2 – 3 minutes. The flow was reduced to allow collection of water without splashing. The water sample was collected in the bottle and the sterile water-proof cap applied. At least a 15-minute interval was observed between the samples (see Appendix 2 for the detailed tap water sampling procedure).

The temperature of the bottle's surface was measured as above and filled bottles were labelled with collection date and time, temperature, village, and concessionaire's name. Additional information on each water sample's visual turbidity was recorded and the concessionaire was asked to provide information on any unusual water supply event occurring in the preceding days. In each sampling week, a tap from one household in each one of two villages was sampled.

All the water samples were transported using cleaned, sealed, and cooled sample collection buckets at a temperature of 4 - 10°C to the Microbiology Section, Regional Standards and Testing Laboratories, Department of Science and Technology, Regional Office 7, (DOST 7), Cebu City within 4 - 6 hours after collection. The DOST 7 - RSTL is a non-regulatory government agency and an internationally accredited testing centre currently certified to the requirements of ISO 17025 quality management standards. The laboratory has been operational since 1985, and provides analytical services to various industrial bodies, including bottled water processors, food service and processing companies, marine and aquaculture, and pharmaceutical industries. It complies with the elements of a quality management system as required by the Philippine National Standards, International Standardization Organization – International Electrotechnical Commission (PNS ISO/IEC 17025:2005).

This laboratory has an established Quality Management System (QMS) currently accredited by the International Organization for Standardization or ISO/IEC 17025 and the Department of Health, Philippines in the field of laboratory testing

(APHA et al., 2012d; DOST, 2009; USFDA, 1998). Once in the laboratory, water samples were stored at 4 ±2°C and tested within 6 hours of sampling.

3.2 Bacteriological analysis of water samples

The bacteriological parameters assessed in this study were: the number of viable *Escherichia coli* (ECC), thermotolerant coliforms (formerly faecal coliforms) (TTCC), and total coliforms (CC). In addition, the heterotrophic plate count (HPC) was included to assess the total number of bacteria (coliforms and non-coliforms) present in the samples. These parameters are frequently used as indicators of the bacteriological quality of drinking water, and numerous bacteriological studies of drinking water employ a combination of two or more of these tests (Barrell et al., 2000; Besic et al., 2011; ESR, 2011; Marzano et al., 2011; Moe et al., 1991; Soriano-Pasumbal & Ong-Lim, 2005; Svagzdiene et al., 2010; Wright, Gundry, & Conroy, 2004). These parameters are also included in the microbiological standards for the quality of drinking water set by Philippine National Standards for Drinking Water (DOH, 2007).

Specific standard operating procedures [SOPs] were prepared in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, & WEF, 2012a, 2012c; APHA et al., 2012d) guide and the Bacteriological Analytical Manual published by the US Food and Drug Administration (USFDA, 1998). The former has been the standard reference method adopted by many ISO accredited laboratories (DOST, 2009), as well as in several previous drinking water studies (Gomes, Bastos, & Leite, 2008; Guerrero, Gusils Leon, Ruiz, & Cardenas, 2010; Robles et al., 2011).

The bacteriological testing was performed following these standard laboratory procedures. Testing results were recorded in a coded, traceable and access-restricted logbook. The bacteriological procedures are briefly described below. Thorough descriptions of the methods are reported in Appendix 3 for ECC, TTCC, and CC; and Appendix 4 for HPC.

3.2.1 Viable *E. coli* count test (ECC)

3.2.1.1 Analytical principle and procedure

For the *E. coli* count, a multiple-tube fermentation technique with results expressed as most probable number [MPN] units was used (APHA et al., 2012a; USFDA, 1998). The MPN technique is a statistical multi-stage laboratory analysis based on the assumption of a poisson distribution of the number of bacterial cells present in water. The test is carried out in three phases: 1, presumptive; 2, confirmed; and 3, completed phase. This test is based on the ability of *E. coli* bacteria (and other coliforms) to ferment the sugar lactose present in the Lauryl Tryptose Broth (LTB) media, producing acid and gas within 48 hours of incubation at 35°C during the presumptive phase. In the second phase, confirmation is achieved using *E. coli* medium (EC), which selectively enhances the growth of *E. coli* and other faecal coliforms, while inhibiting Gram-positive and spore-forming bacteria. This results in growth which is indicated by turbidity and the formation of gas resulting from lactose fermentation. In the 3rd phase, a loopful of broth from each positive EC tube are inoculated onto Eosin Methylene Blue (EMB) agar plates. Bacterial isolates are subjected to biochemical identification as *E. coli* by a series of tests known as the IMViC tests, which stands for an indole production test, methyl red for acid indication, a Voges-Proskauer (VP), and a citrate

fermentation test. *E. coli* biotype I strains typically show positive indole and methyl red reaction and negative VP and citrate tests. On the other hand, *E. coli* biotype II strains display negative reactions in all these tests, except for the methyl red. Additionally, Gram staining and microscopic examinations are done to verify the presence of *Gram*-negative rod cells. Final *E. coli* counts are interpreted based on the biochemical results and calculated according to the proportion of positive LTB tubes confirmed by the final screening, using published tables (APHA et al., 2012a).

3.2.2 Thermotolerant coliform count test (TTCC)

3.2.2.1 Analytical principle and procedure

The thermotolerant coliforms are a group of coliforms capable of fermenting lactose to produce gas within 24 hours of incubation at 44.5°C (Hachich et al., 2012; USFDA, 1998). This group of organisms were previously known as faecal coliforms. However, studies have indicated that they also occur in waters rich in organic matter, even in the absence of any recent faecal material contamination. The test for TTCC is performed using LTB media in multiple tubes similar to the presumptive phase in the *E. coli* count test (APHA, AWWA, WEF, 2012). A positive reaction is observed with the production of gas (CO_2) and turbidity (bacterial growth) in the tubes within 48 hours of incubation at 35°C. The confirmation of the presence of TTCC is performed by transferring inoculum from the presumptive-positive LTB tubes to EC medium tubes and incubated at 44.5°C. The appearance of gas and growth in the tubes within 24 hours constitutes a positive reaction in the confirmed phase. Final TTCC counts are

also determined using published MPN tables according to the proportion of LTB tubes confirmed by the EC broth reaction (APHA et al., 2012d).

3.2.3 Total coliform count test (CC)

3.2.3.1 Analytical principle and procedure

The coliform group of microorganisms consists of many genera belonging to the Enterobacteriacea family of bacteria and has been regarded as an index of the sanitary quality of water (Rompre et al., 2002; USFDA, 1998). The CC test is based on the fermentation of lactose by these bacteria resulting in the production of acids, CO_2, and bacterial growth in the tubes, and these positive reactions are indicated by shades of yellow coloration, effervescence, and turbidity, respectively. The test is performed using the multiple tube fermentation technique, most probable number (MPN) method (APHA et al., 2012a). The test for CC uses LTB culture media in the presumptive phase and brilliant green lactose broth (BGLB) in the confirmed phase. Both phases are incubated at a temperature of 35°C for 24 – 48 hours. A parallel confirmatory test is applied using EC medium to determine whether the coliform is of faecal or non-faecal origin. A parallel positive BGLB broth and a negative EC broth indicate the presence of coliforms from non-faecal sources. Conversely, results that show positive EC broth and negative BGLB broth cultures are considered as positive for coliforms from faecal sources. Nevertheless, confirmation and completion of final total coliform counts are calculated according to the proportion of LTB tubes showing BGLB-positive confirmatory results and estimated using a published MPN table (APHA et al., 2012a).

3.2.4 Heterotrophic plate count test (HPC)

3.2.4.1 Analytical principle and procedure

The heterotrophic plate count (also known as standard plate count, aerobic plate count or total viable bacterial count), is a method of enumerating live and culturable aerobic and facultative anaerobic bacteria in water (Allen et al., 2004; APHA et al., 2012d). The method is based on the principle that environments in groundwater are largely heterotrophic, i.e. made of organisms that cannot fix inorganic carbon, hence depend on dissolved organic carbon or other organic compounds available for nutrition. The method is a viable cell count method which assesses the number of colony forming units (CFU) observed visible on solid media after standard bacteriological culture. The method for HPC used in this study was based on the pour plate technique, and used tryptone glucose yeast agar medium, also known as plate count agar (PCA) medium. For this method, the water samples undergo dilution, plating, incubation, and counting as described in Appendix 4. The water samples are diluted with the goal of producing counts within the range of 30 – 300 CFU. Briefly, duplicate water samples of 1ml and 0.1ml from the original water sample, and 1ml and 0.1ml from the same original sample diluted to a factor of 10^{-2} were inoculated onto pre-labelled sterile petri dishes. Immediately after, an amount of 15 – 20 ml PCA was aseptically poured onto each petri dish and thoroughly mixed with the water samples. The petri dishes were then incubated in an inverted position at 35°C for 48 hours in aerobic conditions and the colonies growing on plates showing between 30 and 300 colonies were manually counted using a colony counter. Final results were computed as CFU/mL, using the average CFU count in the

dilution showing 30 – 300 colonies and taking into account the volume of inoculum. (APHA et al., 2012d).

3.3 Analysis of data

3.3.1 Data handling and preliminary exploratory data analysis

The results of the ECC, TTCC, CC, and HPC, and accompanying information about bottled water brands and store names, production lot/batch code, and the village and household names of both bottled water and tap water samples were collated on an electronic spreadsheet, tabulated and plotted. Data were explored and presented in graphical and tabular forms.

3.3.2 Analysis for compliance with the Philippine regulatory guidelines for the bacteriological quality of drinking water

The results of the ECC, TTCC, CC, and HPC were assessed for compliance with the Philippine regulatory requirements. Specifically, for the bottled water samples, the results were compared with the microbiological specifications set by the Philippine Standards of Quality and Requirements for the Processing, Packaging, and Labelling of Bottled Drinking Water (DOH, 1993) (Table 3-4). For the tap water samples, the Philippine National Standards for Drinking Water (DOH, 2007) was used (Table 3-5). The results were evaluated for compliance and scored as complied (passed) or did not comply (failed) with these regulatory criteria.

Based on these guidelines, evidence of bacterial growth in any one of the bacteriological tests may result in the classification of the samples as non-compliant (or 'failed' in this study) according to the criteria presented in Table 3-4 and Table 3-5. For a compliant (passed) decision to be made there should only

be a maximum of 1/5 of the sample units exceeding the maximum acceptable levels for bottled water. Conversely, for tap water, no sample unit is allowed to exceed the maximum acceptable level in order for a sample to pass. In addition, the individual sample units of bottled water and tap water were also evaluated per bacteriological test or parameter, and the differences between the proportions of failing sample units were compared between brands and between taps.

Table 3-4 Microbiological specifications for purified bottled water.

Microorganism	n	c	m	M	measurement units
Total coliforms (CC)	5	1	<1.1**	1*	MPN/100ml
Heterotrophic plate count (HPC)	5	1	103	105	colony forming units (cfu)

n = number of sample units tested

c = maximum number of samples m but not more than M

m = guide level

M = maximum acceptable level

*shall not be *E. coli*

**using Multiple Tube Fermentation Technique – MPN method as used in this study

Source: Phil. DOH-FDA, A.O. 18 s. 1993

Table 3-5 Microbiological values for consumer's tap water.

Microorganism	n	c	M	measurement units
Thermotolerant coliforms (TTCC)	1	0	<1.1	MPN/100ml
Total coliforms (CC)	1	0	<1.1	MPN/100ml
Heterotrophic plate count (HPC)	1	0	<500	colony forming units (cfu)
E. coli (ECC)	1	0	<1.1	MPN/100ml

Source: Phil. DOH, PNS A.O. 0012 s. 2007

3.4 Quality control, verification, and validity of the data

In this study, the bacteriological test methods used by the author were prepared by the DOST 7 – RSTL Microbiology Laboratory Section and have been validated, verified, and performed following quality control activities. The

methods were adopted from the Standard Methods for the Examination of Water and Wastewater jointly published by the American Public Health Association (APHA), American Water Works Association (AWWA), and the Water Environment Federation (WEF) and as such have undergone validation by the Joint Task Group of Experts and Specialists, reviewed by the Standard Methods Committee, and approved by the Joint Editorial Board (APHA et al., 2012c). The quality control and verification activities were performed by the laboratory.

Analytical methods require validation, verification, and quality control activities to ensure their performance is satisfactory according to the intended objectives. Validation is the gathering of evidence that a specified method can provide accurate and reliable data. On the other hand, verification is defined as the process of determining whether an analytical method actually performs according to its expected capability to provide reliable data. Likewise, quality control refers to operational activities and techniques used to fulfil the requirements of quality. This is to ensure the quality of laboratory test results are maintained and are part of a quality management system (QMS). A QMS refers to the establishment, documentation, and effective implementation of a laboratory management and quality assurance program that specifies requirements and operating procedures to ensure the maintenance of high quality laboratory performance and results. In microbiology for example, validation aims at determining whether a method used to quantify or detect a particular microorganism is able to do so in the sample matrix under analysis.

For verification, positive and negative control water samples were used every week in parallel with the water sample testing. Positive controls were obtained

by spiking of Lauryl Tryptose Broth (LTB) with a loopful of working culture of *E. coli* ATCC # 25922 strain. Another LTB spiked with a *Staphylococcus aureus* BIOTECH # 25923 was used as negative control. No verification results requiring corrective actions were recorded during the study. Routine quality control measures included periodic media sterility and performance testing, monitoring of counting variability for each sample, and duplicate analyses for each test. All the laboratory personnel underwent periodic training and proficiency testing according to internal standard operating procedure. The author conducted all the field sampling and laboratory analyses with assistance of six laboratory staff members. The raw data and the results were documented in a laboratory logbook.

3.5 Statistical analysis of data

Most bacteriological testing results were negative and this precluded detailed statistical analyses. However, for one bottled water manufacturer (Brand 6), all its bottled water samples were bacteriologically positive and were subjected to multivariable Poisson regression analysis aimed at analysing sources of variation in the bacteriological counts.

The analysis was performed using the RStudio software (Free Software Foundation, 2012). The outcome variable was represented by the raw, replicated HPC counts and bottle was considered as a random effect to capture and account for variation between replicate samples from the same bottle. Other explanatory variables considered as fixed effects were the production batch (nominal variable), sampling occasion (nominal variable), and store (nominal variable). An analysis of variance (ANOVA) and comparison of nested models using Akaike

information criterion (AIC) was carried out to determine which statistical model will best fit the data. The measured bottle temperature (continuous variable) was considered separately and analysed descriptively because the combined data set with the bottle temperature does not fit the model as well as the presence of only few non-zero bacteriological count data.

Although formal statistical comparisons between bottled and tap water bacteriological results could not be performed due to their different life cycles (Senior & Dege, 2005), a simple assessment of differences between the proportions of bacteriologically-positive bottles between the two sources was carried out using two-tailed Fisher's exact tests (see Appendix 5).

3.6 Survey of water bottling plants and municipal tap water supplier

A survey questionnaire was developed and distributed to the participating bottled water manufacturers (Appendix 6) and water-service providers (Appendix 7). The questionnaire elicited information related to water sourcing, sanitation procedures, premises and environmental location, water packaging and/or piping and treatment processes, preventive maintenance, personnel health and training, product testing and product warehousing and distribution as previously described for bottled water (Jagals & Jagals, 2004; Svagzdiene et. al., 2010). The information gathered was used to describe any relationship between these and the bacteriological test results. However, the modest sample size precluded statistical analysis of these relationships. The questionnaires and letters of participation request and informed consent were electronically mailed to the seven executives of the bottled water companies and major local water district of the study area.

3.7 Ethical Considerations

This research was conducted in accordance with Massey University's Code of Ethical Conduct for Research and a full application was approved by Massey University Human Ethics Committee MUHEC Approval no. 13/21 (see Appendix 8).

The samples of bottled water brands were only purchased from stores while tap water samples were collected from concessionaire households of the local water district in the study area. The Metro Cebu Water District authorized the sampling of tap water from these households. As some information and results might have been commercially sensitive (e.g. poor water quality and sanitary results, etc.), data gathered by the questionnaire were confidential.

CHAPTER 4 - RESULTS

This chapter presents the bacteriological results of the 270 drinking water sample units tested in this study. The sample's characteristics are described in Sections 4.1.1 and 4.1.2. The individual results of the water samples by test are presented in Sections 4.2.1 (bottled water) and 4.2.2 (tap water). The results of the statistical analyses including exploratory data analysis, compliance of the bacteriological results with the Philippine regulatory guidelines, statistical analysis of the contaminated bottled water brands, and descriptive analysis of the contaminated tap water samples are reported in Section 4.3. The results elicited by the sanitation program survey questionnaire are reported in Section 4.4.

4.1 Samples' characteristics

4.1.1 Bottled water sample

The six brands tested in this study were the top brands based on market share in the country and were also the most widely available on the shelves of many large supermarkets and retail stores in the study area. According to the consulted sources (see Materials and Methods chapter), these brands combined, accounted for ~80% of the bottled water sold in the country. Five out of six brands were available nation-wide. All the brands had bottles made of polyethylene terephthalate (PET). The bottled water categories, their distribution among stores and the number of production batches found for each brand are reported in Table 4-1. On each sampling occasion, there were numerous batches present. The distribution of bottles among sampled and analysed batches is reported in Figure 4-1.

Table 4-1 Description of the tested bottled water brands

Brand No.	Bottled water category**	Number of production batches found on store shelves	Stores where these brands were available
1	Purified Water	31	Stores 1 & 3
2	Distilled Water	33	Stores 1 & 3
3	Pure Distilled Water	41	Stores 1 & 3
4	Natural Water	31	Stores 1 & 3
5	Natural Spring Water	39	Stores 1 & 3
6	Ultra-Pure Water	2 + 2 no label	Stores 2 & 4

**As indicated in bottle's label

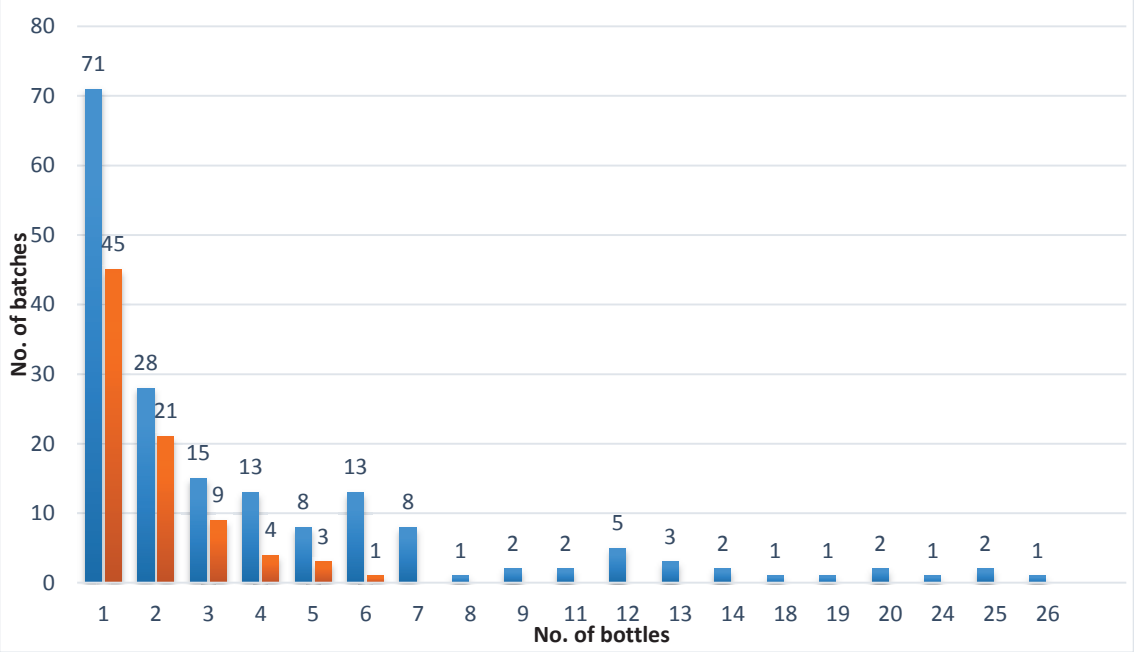

Figure 4-1 Distribution of sampled (blue bars) and analysed (red bars) bottled water bottles among production batches.

4.1.2 Household tap water samples

The eight household taps tested in this study belonged to eight residential houses located in four villages with the greatest number of local water district concessionaires in the study area (Table 4-2). The water supply conditions in all

sampling events were normal except in one occasion wherein a water supply interruption occurred hours prior to the sampling. The type of faucet material and location of the households are reported in Table 4-2.

Table 4-2 Household taps types and location.

Household No.	Type of Faucet	Village
1	Metal (chrome)	Guadalupe
2	Metal (chrome)	Lahug
3	Metal (chrome)	Punta Princesa
4	Polyvinyl Chloride	Mabolo
5	Polyvinyl Chloride	Guadalupe
6	Metal	Lahug
7	Metal (chrome)	Punta Princesa
8	Metal (chrome)	Mabolo

4.2 Bacteriological results

4.2.1 Bottled water

None of the bottled water sample units analysed in this study showed evidence for presence of gas, growth, or acidic reaction after incubation in lauryl tryptose broth (LTB) culture media presumptive analysis for ECC, TTCC, and CC, suggesting the absence of *E. coli*, thermotolerant coliforms, and total coliforms in the samples. Thus, all bottled water brand sample units were reported negative for these indicator organisms.

A total of 21 out of 150 bottled water units showed counts in HPC analysis, suggesting the presence of heterotrophic bacteria in the samples. Interestingly, 20 of these units belonged to a single brand (Brand 6) (Table 4-3). Furthermore, all the analysed sample units from Brand 6 were positive to HPC, with significant differences between batches. In fact, there were two batches with consistent high counts (B6-1 and B6-2) and two (B6-4 and B6-3) with low counts. There were no

batches that occurred in more than one store, making multivariable models with both batch and store variables, unfeasible (Table 4-3). On the other hand, the only positive bottled water unit of Brand 5 showed the highest HPC among all the analysed units. See Appendix 9 for the full bacteriological results.

Table 4-3 Heterotrophic plate counts of bottled water brands.

Bottled brand sample units	Number of positive sample units	Brand number	Store number	Occasion number	Assigned Batch code	Average HPC (cfu/ml)
36 – 40	5	6	2	1	B6-1	1308
86 – 90	5	6	4	1	B6-4	73.8
136 – 140	5	6	2	2	B6-2	1500
186 – 190	5	6	4	2	B6-3	256
135	1	5	1	2	B5-1	4500

The average temperatures of the bottled water units on the store shelves, together with the bacteriological test results, are reported in Table 4-4. Bottled water units purchased in Stores 1 and 2 (which are large stores) had lower temperatures compared with water units purchased in Stores 3 and 4 (small stores). However, regardless of the temperature, Brand 6 always showed counts (100% of the units were positive). Conversely, none of the sample units of Brands 1 to 4 yielded positive results, regardless of temperature and store.

Table 4-4 Temperatures of the bottled water in stores and their bacteriological results.

Store Information		Number (%) of positive bacteriological results					
Store (type)	Average bottled water temperature	Brand 1 (n=30)	Brand 2 (n=30)	Brand 3 (n=30)	Brand 4 (n=20)	Brand 5 (n=20)	Brand 6 (n=20)
S1 (large store)	23.1°C	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	--
S2 (large store)	24.3°C	--	--	--	--	--	10 (100)
S3 (small store)	31.2°C	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	--
S4 (small store)	31.5°C	--	--	--	--	--	10 (100)

-- not applicable
n = number of sample units (bottles)

4.2.2 Tap water

A total of 5/120 tap water sample units tested showed evidence of gas, growth, and acidic reactions after incubation in the LTB media for the presumptive identification of *E. coli* (ECC), thermotolerant coliform (TTCC) and total coliforms (CC), suggesting the possible presence of coliform organisms in the samples. Subsequent inoculations in BGLB and EC broth media followed by IMViC biochemical testing and Gram-staining of isolates confirmed the presence of coliforms, thermotolerant coliforms, and *E. coli* organisms, in the samples. Similarly, these tap water sample units also showed counts after incubation in PCA culture media, suggesting the presence of heterotrophic bacteria in these units. All positive sample units originated from the same household tap and were collected on a single sampling occasion. All five tap water units were positive for total coliforms and heterotrophic bacteria, two were positive for thermotolerant coliform organisms, and one yielded *E. coli* counts (Table 4-5).

Table 4-5 HPC, TCC, TTCC, and ECC of tap water samples.

Sample unit number	Household number	Village name	Occasion number	HPC (cfu/ml)	TCC (MPN/ 100ml)	TTCC (MPN/ 100ml)	ECC (MPN/ 100ml)
116	1	Guadalupe	2	270	1.1	<1.1	<1.1
117	1	Guadalupe	2	280	1.1	<1.1	<1.1
118	1	Guadalupe	2	160	1.1	<1.1	<1.1
119	1	Guadalupe	2	160	1.1	1.1	<1.1
120	1	Guadalupe	2	270	2.6	2.6	2.6

4.3 Bacteriological data analysis results

4.3.1 Exploratory data analysis results

The bacteriological results showed that 21/150 (14%) bottled water and 5/120 (4%) tap water sample units were contaminated with bacteria. In particular, 20 contaminated bottled water sample units represented all the sample units analysed for one brand (Brand 6). Ten of these bottles belonged to two coded batches and the other 10 were uncoded (Table 4-3).

The combined market share of the four uncontaminated brands in the study area was ~78% (Table 4-6). However, the market shares for the two contaminated brands were not reported in the Euromonitor Research Report as cited in Manila Bulletin (2013).

Table 4-6 Comparative summary of the bacteriological quality of bottled water brands.

Brand number	Market Share (%)	Number of sample units (bottles) tested (n=150)	Number (%) of sample units positive
1	21.4	30	0 (0.0)
2	20.9	30	0 (0.0)
3	20.5	30	0 (0.0)
4	15	20	0 (0.0)
5	Ranked 5th (% not available)	20	1 (5.0)
6	Data unavailable*	20	20 (100.0)
Combined brands 1 to 4	77.8	110	0 (0.0)

*the only locally available but popular brand in the study area

The five contaminated tap water sample units belonged to one household (Household 1) and were collected on one sampling occasion. However, these tap sample units were positive for HPC and also for the coliform group of bacteria, and two units were confirmed positive for thermotolerant coliforms. Importantly, one faecal coliform-positive tap water sample unit was also positive for *E. coli* bacteria Table 4-5.

4.3.2 Bacteriological compliance with the Philippine regulatory standards

4.3.2.1 Compliance of the bottled water samples

This section presents the results of the compliance of bottled water samples with the microbiological criteria of the Philippine Standards of Quality and Requirements for the Processing, Packaging, and Labelling of Bottled Drinking Water (Philippine Food and Drug Administration, A.O. 18-A series of 1993). Table 4-7 reports the microbiological criteria applied by the standard.

Table 4-7 Microbiological criteria taken from Philippine FDA, A.O. 18-A series of 1993, used for compliance assessment of bottled water.

Microorganism	n	c	m	M	measurement units
HPC	5	1	1,000	100,000	cfu
TCC	5	1*	0	1*	MPN/100ml

**shall not be E. coli*

n = number of sample units (bottles) tested

c = maximum number of sample units allowed to have bacterial counts > m but not greater than the maximum acceptable level (M)

m = allowable level of microorganisms in bottled drinking water

M = maximum acceptable level

Cfu = colony forming units

MPN = most probable number

All bottled water brands, except Brand 6, complied with the regulatory standards for ECC and TCC in all sampling occasion. All the sample units of Brand 6 had bacterial counts, although not all samples failed to comply with the standard.

Specifically, two samples of Brand 6 exceeded the HPC limits because they had more than one unit (2/5 and 5/5 bottles for Samples 1 and 2, respectively) with bacterial count results between the allowable and maximum acceptable levels of 1,000 and 100,000 MPN/100ml (Table 4-8) (these non-compliant units have been described in 4.3.1). The standard allows a maximum of 1/5 units with counts within these limits (Table 4-7). Although another brand (Brand 5) showed a count in one test (HPC) that exceeded the regulatory limit, this failure only occurred in one sample unit and was within the maximum allowable number of failed sample units required for bottled waters.

Table 4-8 HPC results of non-complying bottled water samples.

Brand number	Store	Occasion	Sample number	Sample unit number	HPC (cfu/ml)	Sample's compliance
6	2	1	1	36 37 38 39 40	620 970 750 2,600 * 1,600 *	Did not comply
6	2	2	2	136 137 138 139 140	1,500 * 1,400 * 1,500 * 1,500 * 1,600 *	Did not comply

*not within m and M

4.3.2.2 Compliance of the tap water samples

This section reports the results of the compliance of tap water samples with the microbiological criteria of the Philippine National Standards for Drinking Water including Consumer's taps (Philippine Department of Health, A.O. 0012, 2007). This standard is specific for consumer's taps in households, refilling stations,

vending machines, haulers, reservoirs, and treatment works. Table 4-9 reports the microbiological criteria applied by the standard.

Table 4-9 Microbiological criteria taken from the Philippine Department of Health, A.O. 0012, 2007, used for compliance assessment of tap water.

Microorganism	n	c	M	measurement units
HPC	1	0	<500	cfu
TTCC	1	0	<1.1	MPN/100ml
TCC	1	0	<1.1	MPN/100ml
E. coli	1	0	<1.1	MPN/100ml

n = number of samples tested (i.e. one 500ml tap water collected from a faucet and placed in a sterilized glass container)

c = maximum number of sample units allowed to have bacterial counts > m but not greater than the maximum acceptable level (M)

m = allowable level of microorganisms in bottled drinking water

M = maximum acceptable level

Cfu = colony forming units

MPN = most probable number

A total of 5/120 tap water samples did not comply with the Philippine regulatory standards for TCC. All the non-compliant samples originated from a single household and were collected on the same sampling occasion (Table 4-10) (these samples have been described in 4.3.1). From these five samples, one did not comply with the ECC and two with the TTCC limits. For HPC, all tap water samples complied with the regulatory standards.

Table 4-10 Bacteriological counts of the non-complying household tap water.

Household number	Village name	Occasion number	Sample number	TCC (MPN/ 100ml)	TTCC (MPN/ 100ml)	ECC (MPN/ 100ml)	Sample's compliance (pass/fail)
1	Guadalupe	2	116	1.1 *	<1.1	<1.1	Did not comply
1	Guadalupe	2	117	1.1 *	<1.1	<1.1	Did not comply
1	Guadalupe	2	118	1.1 *	<1.1	<1.1	Did not comply
1	Guadalupe	2	119	1.1 *	1.1 *	<1.1	Did not comply
1	Guadalupe	2	120	2.6 *	2.6 *	2.6 *	Did not comply

*exceeded the M

4.3.3 Statistical analysis of the contaminated bottled water brands

Based on the multivariate poisson regression statistical model (Table 4-11) for the twenty contaminated bottled water samples belonging to one brand, the possible sources of variation were the following: (1) the stores where the bottled water samples were purchased; (2) their production batches or lots from the factory; and (3) the sampling occasion or month they were collected for laboratory analysis. The effect of temperature was only analysed descriptively, for the reasons described in the Discussion chapter.

Table 4-11 Generalized linear mixed model fit by maximum likelihood (poisson regression) applied on the contaminated bottled water brand (B6) treated as a random effect

Random effects:		
Groups name	Variance	Standard Deviation
Bottle (intercept)	0.18	0.43
No. of observations	136, groups:	Bottle, 20
Fixed effects:		**Significance of the difference in HPCs between batches**
Intercept (Batch B6-1)		$p = 2x10^{-16}$ (significant)
Batch B6-2		$p < 0.54$ (not significant)
Batch B6-3		$p < 0.01$ (significant)
Batch B6-4		$p < 0.01$ (significant)

According to the results from the model for this contaminated brand (Brand 6), the production batch factor was significantly associated with the bacteriological counts and hence cannot be attributed to chance alone ($p = 2x10^{-16}$). Regardless of the occasion or store and after adjusting for the confounding effect that occasion may have, the batch was always significant. In contrast, sampling occasion has no significant effect on the bacteriological counts. Once the batch is included, occasion does not improve the model at all. The time interval between occasions was approximately one month.

In addition, it can be shown from this model that batch 1 is significantly different from batch 3 and batch 4. In contrast, batch 1 is not significantly different from batch 2.

A number of Poisson regression statistical models were developed for these bacteriological results (see Appendix 10). The objective of the models was to analyse the effect of the sources of variation on the bacterial counts per bottle. A combination of the different variables were analysed (batch, occasion, and store) whilst accounting for the limitations of having only few data on this brand. The first model developed accounted only for the random effect of the bottled water sample units on the bacteriological counts with no fixed-effect covariates i.e. no batch, occasion, or store. The second model analysed the fixed-effects of the batch, occasion, or store on the counts in addition to the random effect of bottles. The third model has included two covariates at a time to the bottles, hence analysing the effect of a combination of batch with occasion, batch with store, and occasion with store on the bacteriological counts. Based on this poisson regression model fitting, the effect of the batch and sampling occasion after adjusting for the random effect of the batch were assessed.

In order to identify which model best fit the results of this study, these were tested using analysis of variance (ANOVA) and the Akaike Information Criteria (AIC) value. Consequently, the model with lowest AIC is the best model for the results. Hence, the model which combines counts and the covariate batch was identified as the best fit among the different models developed in this study (Table 4-12).

Table 4-12 Comparison of the 3 applicable models using ANOVA (adopted from R studio statistical software)

ANOVA 1								
Models:								
Model 1:	Counts ~ 1 + (1|Bottle)							
Model 2:	Counts ~ Batch + (1|Bottle)							
Model 3:	Counts ~ Batch + Occasion + (1|Bottle)							
	Df	**AIC**	**logLik**	**deviance**	**Chisq**	**Chi Df**	**Pr(>Chisq)**	
Model 1	2	788.45	-392.22	784.45				
Model 2	5	749.28	-369.64	739.28	45.169	3	<0.00001	***
Model 3	6	750.88	-369.44	738.88	0.401	1	0.5266	
ANOVA 2								
Models:								
Model 1:	Counts ~ 1 + (1|Bottle)							
Model 4:	Counts ~ Occasion + (1|Bottle)							
Model 3:	Counts ~ Batch + Occasion + (1|Bottle)							
	Df	**AIC**	**logLik**	**deviance**	**Chisq**	**Chi Df**	**Pr(>Chisq)**	
Model 1	2	788.45	-392.22	784.45				
Model 2	3	788.91	-391.45	782.91	1.5447	1	0.2139	
Model 3	6	750.88	-369.44	738.88	44.025	3	<0.00001	***

DF = Degree of freedom
AIC = Akaike Information Criteria
LogLik = log likelihood
dev = deviance
Chisq = Chi square
Chi Df = Chi square Degree of Freedom
Pr = probability or p-value
**** = significant*

Model 1 = the null model with bottle included as a random effect
Model 2 = the model that includes the fixed effect of either the batch, occasion, or store with the random effect of the bottles
Model 3 = the model that included two covariates (e.g. batch with occasion, etc. at a time with the random effect of bottles

On the other hand, the effect of the stores as a source of variation could not be assessed because there were no batches of this brand occurring in more than one store. However if there was an effect of the store, it should have been seen in the occasion (as determined by the significance of time between occasions). In addition, these effects would have been amplified with the relatively long time intervals between occasions when the samples were collected. Hence, store

would have confounded the effect of occasion and transforming a non-effect into a significant effect.

If we assumed that store has no effect, this is an indirect evidence suggesting that storage did not affect the bacteriological counts because the longer the bottle was stored, the greater should have been the effect on the counts. However, the results show that there was no significant difference in the bacteriological counts between the two occasions. Therefore, this statistical analysis showed that among the three fixed-effect variables: batch, store, and occasion; only batch has a significant relationship with bacteriological counts.

Furthermore, the contaminated bottled brand was only positive for heterotrophic (HPC) organisms and negative for coliforms, thermotolerant coliforms, and *E. coli* count analysis. It should be noted that HPC's indicate the presence of naturally occurring bacteria that are usually harmless and are characteristic of the water source (Duranceau et al., 2012; Sartory, 2004; Senior & Dege, 2005). Contamination leading to consumer health risks is typically indicated by subsequent positive counts in coliform test for environmental contaminants and *E. coli* analysis for recent faecal contamination.

4.3.4 Analysis of the contaminated household taps

One of the eight household taps during one of the three sampling occasions was contaminated with heterotrophic bacteria. In addition, all these samples were also contaminated with coliform organisms. More importantly, the completed tests also confirmed the presence of thermotolerant coliforms of faecal origin in two of these coliform positive samples as well as the confirmed occurrence of *E. coli* organisms in one of the thermotolerant positive samples. This result only

occurs in one household in one of the three sampling occasions. Nevertheless, this require further consideration because a positive result in all four indicator bacterial tests is a valid evidence that the tap water samples identified can potentially cause serious health risks to consumers (Edberg et al., 2000; Soller, Embrey, Tuhela, Ichida, & Rosen, 2010).

4.4 Sanitation program survey results

4.4.1 Local water district responses

The results of the sanitation program questionnaire delivered to local water district and bottled water manufacturers are presented in Table 4-13 (tap water) Table 4-14 (bottled water).

According to the answers received from the local water district, the same body has been in existence and provided water utility service for 39 years. The MCWD processed and distributed water originating from a number of sources, including an aquifer, one stream, an artesian bore, and one dam. In addition, they had 115 wells and five major reservoirs. All the water was distributed to the concessionaires through reticulation. During water shortage periods and due to increasing consumption, water originating from external sources was transported to the water treatment plants as needed, using transport vehicles (Table 4-13).

Table 4-13 Key sanitation and maintenance program elements implemented by MCWD.

Sanitation program element	Municipal water supplier: the Metro Cebu Water District (MCWD)
Water treatment method	Chlorination using sodium hypochlorite and chlorine gas
Type of sanitation program applied	SSOP on source water protection
Bacteriological parameters monitored	Coliforms, faecal coliforms, *E. coli*, HPC
Water source	Stream, dam, aquifer, artesian bore, wells, and reservoirs

The water from the sources was collected using pumps and distributed after treatment to consumers through galvanized iron pipes. In terms of water treatment and disinfection, the method employed was chlorination using aqueous solution of sodium hypochlorite, as well as application of chlorine gas. The frequency of sampling for bacteriological monitoring of water was based on pre-determined volumes of distribution (which varied among the distribution centres), rather than fixed time intervals.

The bacteriological parameters tested included total coliforms, heterotrophic plate count, *E. coli* count, and faecal coliforms. The adoption of these testing protocols was requisite to gain accreditation by the International Organization for Standardization (ISO). In addition, specific staff development and training programs were implemented.

4.4.2 Bottled water manufacturers' responses

Because of the recruitment of only six bottled water manufacturers, information provided in the survey questionnaire are only analysed descriptively. One bottled water manufacturer representing one brand responded to the survey and answered the majority of the questions. Four manufacturers replied through telephone communication and did not provide some answers which they considered trade secrets, and one manufacturer did not provide any answer (the

latter produced Brand 6, which sample units were consistently positive for the HPC test, (Table 4-3 and Table 4-8). Results presented in this section are based on the five brand manufacturers that responded to the survey and on information available on Brand 6's label. The key sanitation elements are presented in Table 4-14.

Based on the survey questionnaire results, most of the studied water manufacturers had been present on the market for at least twenty years. Brand 1 has been in the bottled water business for 22 years, and Brands 3 and 4 for 20 years.

The manufacturer of Brand 1 purchased groundwater from a private commercial water vendor and bottled it on a plant located in a city adjacent to the study area. Information on the source of water for the other brands was not provided by the manufacturers.

Similar to the local water district, Brand 1 manufacturer used a one-way check valve which was inspected daily, to prevent contamination caused by water backflow. The other manufacturers did not provide information on the methods for prevention of contamination due to water backflow.

Table 4-14 Key sanitation program elements reported by bottled water manufacturers.

Sanitation program element	Bottled water manufacturer					
	1	2	3	4	5	6
Water treatment method	Filtration, Distillation, Ozonation	Distillation	Distillation, Filtration, Ozonation, UV radiation	Filtration, Ionization	Untreated	*Labelled only as ultra-purified*
Type of sanitation program applied	Internal SSOP for bottling, delivery, cleaning of equipment, staff health available	FSMS, HACCP, GMP certified plant	US NSF certified plant	Internal food safety system	ISO 22000, HACCP, GMP certified plant	Not reported
Bacteriological parameters monitored	Faecal streptococcus, HPC, coliform, faecal coliform, *E. coli*, *Pseudomonas aeruginosa*	HPC, TCC, *E. coli*, Faecal coliform	Coliform, faecal coliform, HPC, *E. coli*	HPC, TCC, *E. coli*, Faecal coliform	*E. coli*, coliform, faecal coliform, HPC	Not reported

The bottled water manufacturers reported the use of different water disinfection processes. Brands 2 and 3 manufacturers employed a distillation process for the removal of microorganisms. However, the manufacturer of Brand 3 applied filtration, ozonation, and ultraviolet radiation techniques in addition to distillation. Brand 1 manufacturer employed both filtration and ozonation, whilst brand 4 manufacturer utilized filtration with ozonation. The manufacturer of Brand 5 did not indicate a specific treatment technology; however the bottle label indicated the finished product was bottled directly from a mineral spring source outside the study area, without any treatment.

In regard to transportation of the water, the manufacturer of Brands 1, 3, and 4 used pumps to collect the water from the source and transport it to the plant for processing and treatment. Specifically, Brand 1 manufacturer utilized stainless

steel containers for transportation. However, according to the manufacturer these containers were not sterile. On the other hand, Brands 3 and 4 manufacturers transported water from the source via distribution pipes to a nearby processing facility.

All six bottled water manufacturers in this study used polyethylene terephthalate or PET plastic bottles for water packaging (the material used for Brand 6 was specified in the label). PET is currently the most important packaging material used in bottled waters, worldwide (Senior & Dege, 2005). The main reasons for its popularity are its relatively low price, lightweight and clarity and resistance to cracking, and the ability to be resealed, which allows maintaining water integrity. All the brands were commonly packaged in product volumes of 500 mL and 1 litre. Brands 1, 3, and 4 were also bottled in 1.5L, 4L, 6L, and 10L volumes; and Brands 2 and 5 in 330 ml volumes. In addition, Brands 3 and 4 were also packaged in 5 L volumes. The manufacturer of Brand 1 employed blow moulding of bottles in house, whilst the others did not provide information on the bottle sources. All bottle closures were made of screw caps.

In terms of bottling process, all manufactures of Brands 1 - 5 reported the use of automated machine bottling techniques. In addition, Brands 1 – 5 manufacturers reported the use of product traceability systems allowing monitoring of production lots and batches.

Manufacturers of Brands 1-5 indicated the use of routine water bacteriological testing, and the implementation of the sanitation programs reported in Table 3.4.2.1. Specifically brands 2 and 5 manufacturers implemented Good Manufacturing Practices (GMP), Hazard Analysis and Critical Control Points

(HACCP), and Food Safety Management System programs. The manufacturer of Brand 3 had a sanitation standard operating procedure (SSOP) manual accredited by the National Sanitation Foundation (NSF) of the United States. The manufacturer of Brand 4 also claimed to have an established food safety system, but it did not further elaborate on the type of system. The manufacturer of Brand 1 declared the implementation of a quality assurance system for: (1) water bottling, delivery and dispatch; (2) water protection against contamination during bottling; (3) cleaning of pipeworks and tanks; (4) cleaning of bottling equipment; and (5) personnel health. Details on these implementations were not provided by the other manufacturers.

The manufacturers were asked to provide SOPs concerning protection of water at the source, during transport and during bottling; water sourcing; personnel health and hygiene; and maintenance and cleanliness of storage tanks and pipeworks. However, none of the manufacturers (including the local water district) provided copies of these SOPs.

CHAPTER 5 - DISCUSSION

5.1 Study design considerations

In this study, the bacteriological quality of bottled and tap water samples was assessed in an urban study area in the Philippines at the point of sale. The main objective was to capture the bacteriological variability at the point of sale. For the bottled water, the assumption was that the consumer would check the shelf-life of the bottle. Thus, only bottles within their shelf life were sampled. Although the production batch number was not used as blocking factor for the sampling of bottled water units as this parameter is not likely to be considered at purchase by the average consumer, the recording of the production batch numbers allowed an analysis of the between-batch variability using multivariable models. Conversely, each tap was considered an independent production unit as there were no production batches for tap water and no information about the water supply reticulation was obtained. The main disadvantage of this types of studies is that the bacteriological quality of the two water types cannot be statistically compared as the two types had discrete life cycles and do not share many predictor variables. Therefore the aims of this study were not direct comparison between the two types, but the identification of sources of bacteriological variation within the types.

5.2 Bacteriological results

In view of its intended use as a safe drinking water type for human consumption, it was initially hypothesised that bottled water will have better bacteriological characteristics (i.e. less positive samples in all tests) compared with tap water. This hypothesis derived from the assumption that bottled water undergoes

rigorous processing and treatment, and the storage in bottles preserves water's integrity (El-Taweel & Shaban, 2001; Nogueira, Cardoso, Delgadillo, & Almeida, 2010; Raj, 2005; Raja'a et al., 2001; Senior & Dege, 2004). Moreover, production under strict quality control programs including Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) could improve the bacteriological quality of the finished product (Jagals & Jagals, 2004; Senior & Dege, 2004).

The combined results of the HPC, ECC, CC, and TTCC analyses indicated the presence of bacteria both in bottled and tap water portions, with 21/150 (14%) and 5/120 (4%) samples showing bacterial growth in at least one test, respectively. These proportions could not, however, be statistically compared as the sampling units from both sources were not independent and the level of dependency varied between the two water types. Nevertheless, differences were also observed in the type of contamination, between the two water types. The contaminated bottled water brands were positive for HPC, whilst the contaminated tap water units were positive for all the tests, including *E. coli*, faecal and non-faecal coliforms, and HPC bacteria. One explanation for the absence of the coliform group including *E. coli* in bottled water could be the effect of multiple barrier treatments applied by most bottled water manufacturers, which included at least two of the following treatments: filtration; distillation; ozonation; ultraviolet radiation; and ionization (Table 4-14). Whereas HPC organisms normally undergo alternating cycles of increasing and decreasing growth in the bottle, no introduction of new allochthonous bacteria, including pathogenic organisms, is possible in an intact bottle (Ducluzeau et al., 1976; Lucas & Ducluzeau, 1990). In the case of the municipal tap water samples where

chlorination was the only treatment applied, significant reduction in disinfection residual at any point in the distribution loop could have resulted in regrowth of coliforms, for instance from a biofilm. In fact, Carter et al. (2000) showed that bacterial levels in distribution pipes increased with distance from the treatment plant as a result of reduction in effective disinfection residuals. In the present study, there are indications that growth could have also occurred due to backflow, low and/or intermittent flow, etc., at some point in the complex and long reticulation system of the city (Kumpel & Nelson, 2014; Rosenfeldt et al., 2009), because an episode of water supply interruption occurred hours before the sampling of the contaminated tap water units. Indeed, increasing bacterial levels due to low flow or backflow have been reported in many reticulated drinking water distribution systems in the world because the water is normally supplied to domestic and industrial consumers intermittently and relies on disinfection residuals to maintain the bacteriological quality of water in the pipes (Kumpel & Nelson, 2014). These authors reported that even when effective chlorine residuals was achieved in a municipal water system, elevated levels of coliforms could be detected in the water when water pressure was low, and concluded that persistent contamination from biofilm could exacerbate or external bacterial intrusion occur as a result of backflow. In contrast, at high water pressures there were no *E. coli* and only few coliform levels were detected.

It has been established that waters in underground sources contain naturally occurring microorganisms also known as autochthonous or heterotrophic organisms and are normally considered harmless to humans (Allen et al., 2004). This was supported by the study of Delabroise & Ducluzeau (1974) showing the inability of heterotrophic bacteria found in mineral waters to colonize a human

gastrointestinal tract. Moreover, cytotoxicity and virulence studies of bacterial strains isolated from drinking water corroborated these findings (Edberg & Allen, 2004; Edberg et al., 1996; Edberg et al., 1997). Therefore, when the source water quality can be assured, these drinking waters can be bottled without treatment (EuropeanCouncil, 1998; Hunter, 1993). However, when the source quality is dubious because of impending environmental contamination risks, the water is usually treated using various disinfection mechanisms (Senior & Dege, 2005).

5.2.1 Bottled water

In this study, the water samples from different bottled brand manufacturers (except for brand 5) and from household taps could be categorised as treated drinking water based on their survey questionnaire responses and the information present in the bottle labels. In contrast to untreated drinking water, treated water is usually expected to contain no or only low levels of microorganism because of the antimicrobial effects of the disinfection treatments applied. In fact, the four bottled water brands produced by the manufacturers that declared treatment were found negative for any bacterial growth, and Brand 5 (the only brand without treatment declared) had HPC in 1/20 bottles. As mentioned, this could be attributed to the effectiveness of the treatments employed and the application of good manufacturing and sanitation practices which the four manufacturers reportedly implemented. Brand 5 was expected to contain some naturally occurring bacteria because of the absence of water treatment. In untreated drinking waters, the bacterial numbers after bottling usually rise because of the greater surface area in bottles compared with the original water source' little underground interstitial spaces (Bischofberger et al., 1990; Heukelekian & Heller, 1940). Moreover, although not verified in this study, the type of bottle material

used can also influence the microbial counts of the water as described in the Literature Review chapter (Morita, 1997). It was reported by Morita (1997) that PET bottles as used by all brand manufacturers in this study promote greater bacterial growth than glass bottles. Moreover, when source protection and bottling integrity is sustained, the natural flora of the water is preserved and free of pathogens. In contrast, one bottled brand (Brand 6) consistently showed positive bacterial counts in HPC in all the sample units tested, regardless of the batch, store and sampling occasion. This brand failed the Philippine regulatory requirements across all batches tested. Interestingly, the manufacturer of Brand 6 did not respond to the survey questionnaire and was not willing to co-operate after a telephone follow up done during the sampling period. Furthermore, Brand 6 product's label claim of 'ultra-pure' water is not contemplated in the Philippine regulations, whereas the other five manufacturers declared water categories considered in these regulations.

The contaminated bottled water samples of Brand 6 were positive for HPC, which does not necessarily confirm the presence of pathogens. However, all its samples contained bacterial contaminants and half of them were considered containing high HPC counts. In fact, the bottled water units with high counts did not comply with the Philippine regulatory standards and according to the same regulations they could pose health risks to consumers (DOH, 1993). Low HPC levels (<100 cfu/ml) can indicate a generally safe microbiological quality. However, samples with HPCs >500 cfu/ml may require water system investigation because of possible contamination risks (Duranceau et al., 2012). It is important to note that several authors have argued that when lactose-based culture media are used, samples with high HPCs (500 – 1000 cfu's/mL) may

inhibit coliforms due to desensitization (Allen et al., 2004; Edberg et al., 2000), leading to false negative coliform count results. The water bottles containing high HPC counts may have originated from a poor-quality water source or due to the ineffectiveness of the water treatments applied. In addition, contamination during processing could have also occurred due to inadequate sanitary facilities and procedures, processing breakdown during production, and/or improperly implemented quality control programs. Whilst sanitation programs should provide suitable hygienic conditions in the production of drinking water, only a few studies combined an assessment of the microbiological quality and sanitation programs applied. One study in New Zealand using 38 bottled water brands failed to establish a statistically-significant link between the microbiological and sanitation survey results (Svagzdiene et al., 2010). According to the authors, the reason was the low response rate to the sanitation survey questionnaire (only 4 manufacturers responded). However these authors used binary regulatory pass-fail criteria as the outcome variable in the analysis. In the current study multiple batches of only six brands and four manufacturers were sampled, with the intention of modelling the outcome variable of the HPC count with powerful multivariable analyses. However, statistical assessment of the effect of sanitation programs on this outcome was not possible as only samples originating from one manufacturer had positive counts. Nonetheless, the manufacturer with contaminated water bottles was the only one unwilling to co-operate in this study. Further, the brand produced by this manufacturer did not display a production batch number or a description of the type of water treatment on the bottle labels. These deficiencies raise questions about the ability of this manufacturer to comply with modern regulatory requirements aimed at safeguarding public

health, and indicated the need to improve monitoring and enforcement at the point of sale.

An interesting aspect of this study was the high diversity of the bottled water production batches found on the store shelves. This suggested a lack of proper stock rotation, which could increase the risk of selling expired batches. Good commercial practice (GCP) such as the application of First-to-Expire, First-Out (FEFO) policy requires an effective rotation of food items to prevent the storage of expired products (Codex, 2001, 2009). The simultaneous presence of such a large number of batches on the shelves resulted in the random collection of bottles originating from many different batches. Nevertheless, multiple bottles sampled from 38/83 analysed batches were represented by more than one bottle (Figure 4-1), which would have allowed statistical assessment of between-batch variability across all brands if bacterial growth was found. Unfortunately, bacterial growth was only found in one brand.

One of the working assumptions of this study was that the storage conditions could influence the bacteriological results of bottled water, for instance by the formation of biofilms as described in the Literature Review chapter. Thus, different stores and occasions were sampled. The multivariable Poisson regression used to model HPC counts in the contaminated bottles indicated a significant association between bottled water production batches and HPC counts ($p<0.0001$). Heterotrophic bacteria were observed to increase in numbers after considerable time of storage after production (Bischofberger et al., 1990; Duranceau et al., 2012; Raj, 2005; Sefcova, 1997). The effects of the storage conditions in different stores could not be directly assessed due to complete

separation of batches among stores. However, the effect of the sampling occasion was not significant, suggesting that duration of storage did not significantly influence the counts. It is important to note that the bottled waters sampled in this study were within its quality shelf life periods, hence, were expected to contain low or no microbial counts. The lack of a significant effect of the storage duration did not support a 'store effect', as in such case the effect of storage was expected to be directly correlated with the duration of storage, and this would have been seen as a significant effect of the sampling occasion.

Another point to consider was the temperature of the bottled water samples on the shelves which varied among the stores, from an average of 24°C in large stores, to 31°C in the small stores (Table 4-4). This difference was due to the absence of air-conditioning systems and adequate ventilation in the small stores. In relation to the bacteriological test results, Brand 6 showed consistent positive counts, regardless of the store. In regard to the effect of water temperature on bacterial counts, some studies have showed that exposure of bottled water to ambient temperature between 25 and 37°C for three days can result in marked increase in bacterial counts (Duranceau et al., 2012; Leclerc & Moreau, 2002; Raj, 2005). In the study of Leclerc and Moreau (2002), maximum bacterial growth was observed at ~20°C that coincided with the contaminated brand's higher count storage temperature. It can be recalled that these heterotrophic bacteria are psychrotrophic (~25°C). In the present study, all the non-contaminated bottled water from Brands 1- 4 and the high HPC count bottles from Brand 6 originated from stores where the average temperatures were lower than the temperatures recorded in the other stores. This apparent contradiction, however, was likely confounded by the production batch as previously discussed for the effect of the

stores. The effect of the temperatures was modelled as a co-variable in the Poisson models, resulting in a large Akaike information criterion value (not shown), perhaps due to the big differences observed in storage temperatures between the stores.

5.2.2 Tap water

In this study, the contaminated tap water samples were positive for *E. coli*, thermotolerant coliforms, total coliforms, and the HPC bacteria. This indicated a possible health risk to consumers, due to the potential co-occurrence of pathogens such as *Salmonella*, *Yersinia*, and *Shigella* (Edberg et al., 2000; Odonkor & Ampofo, 2013; Soller et al., 2010). The presence of coliforms indicates an unsanitary condition of this water at the specific sampling occasion (An & Breindenbach, 2005; APHA et al., 2012a). Furthermore, it indicated faecal contamination of the water at the distribution point, either from the source water, the incoming distribution pipes or as a result of poor household hygiene. Such contamination could have also occurred as a result of operational and maintenance problems of the MCWD. Interestingly, information obtained from the questionnaire indicated sudden water interruptions occurred occasionally in the study area, and a water supply interruption was registered on the night preceding the sampling occasion which resulted in the finding of *E. coli* in water (4.1.2). The fact that all the other tap water samples analysed were negative for any of the indicator organisms suggests that the overall disinfection treatment applied on the municipal water supply was effective (Jagals & Jagals, 2004; Kumpel & Nelson, 2014; Rosenfeldt et al., 2009), but no treatment can completely eliminate the risk in the event of an interruption of the water supply. Furthermore, the five contaminated tap water sample units positive for heterotrophic organisms

were supported by some studies which revealed that most of the microorganisms present in municipal pipes are heterotrophic bacteria (Edberg et al., 1997; Rosenfeldt et al., 2009). Had these samples been HPC positive but negative for coliforms or *E. coli*, this would not have necessarily pose a health risk (Edberg et al., 1997). Compared with the contaminated bottled water's HPC results, the tap water's HPC were significantly lower. This could be attributed to the chlorination treatment's high disinfection residual maintained throughout the distribution network as suggested in numerous studies (Carter et al., 2000; Edberg & Allen, 2004; Edberg et al., 1996; Edberg et al., 1997; Edberg et al., 2000; Kumpel & Nelson, 2014). Hence, negative or low HPC is seen as an indicator for the presence of chlorine residuals in water distribution systems.

Based on the Philippine regulatory requirements, water showing bacteriological results beyond standard limits are regarded as potentially unsafe for human consumption (DOH, 2007). In the present study, one household tap in one occasion did not comply with the regulatory requirements for *E. coli*, total coliforms, and thermotolerant coliforms. Similarly, one bottled water brand (brand 6) in two occasions did not comply with these regulations.

As mentioned, the significance of any positive bacteriological test result is always related on the potential health risk this may cause to consumers (Edberg & Allen, 2004). Drinking waters that contain some levels of pathogens may not necessarily cause illness to healthy individuals and the probability of an adverse health effect depends on the interaction of the organisms with the immune system of the host (Allen et al., 2004; Edberg et al., 1997; Leclerc & Moreau, 2002; Payment, 1995). For instance, some bacteria which are not normally pathogenic,

such as *Pseudomonas aeruginosa,* are capable of causing infectious diseases in immunosuppressed individuals. Therefore, a thorough assessment of both the level of microorganisms present in the water and the virulence of the microbe identified and the immune condition of the consumer is needed to evaluate the likelihood of a disease occurring as a result of consuming contaminated drinking water. Nonetheless, many highly virulent enteric pathogens, such as *Shigella spp.*, *Salmonella spp.*, *Vibrio cholera*, enteropathogenic *E. coli,* and *Campylobacter jejuni,* can cause serious waterborne diseases also in immunocompetent individuals (Edberg et al., 2000; Muellner et al., 2010; Odonkor & Ampofo, 2013; Rajkowski & Rice, 1999; Soller et al., 2010; WHO, 2012).

5.3 Sanitation survey results

All except one bottled water manufacturer cooperated in this study by responding to the questionnaire, and declared the implementation of at least one sanitation program and food safety system. The programs declared by the five brand manufacturers, including the food safety management system and GMP programs applied by the manufacturer of Brands 1-5 (Table 4-14), if properly implemented, can effectively enhance the delivery of bacteriologically safe bottled drinking water (Mossel & Struijk, 2004). Indeed, except for a single bottle which showed high HPC, the brands produced by these manufacturers showed no bacteriological counts. Conversely, the only manufacturer that did not cooperate in this study produced a brand (Brand 6) that failed the Philippine national standard for bacteriological quality of drinking water in 50% of the production batches. Furthermore, this manufacturer produced bottles without production batch identification and did not indicate the type of water treatment on

the bottle's label. These failures across several areas of production raise serious questions about the safety of the product sold by this manufacturer.

In regard to water treatments applied, Brands 1 - 4 are considered as treated bottled waters, whilst Brand 5 is untreated because it was harvested naturally from protected underground spring sources and bottled without further treatment. Further treatments such as filtration can eliminate or reduce bacterial hazard, but it is not a good hygienic practice to harvest water from highly contaminated sources, especially when efficient multiple barrier systems are not in place (Senior & Dege, 2005). Based on the disinfection treatment methods applied by the treated water manufacturers in this study, the bottled waters produced may have no longer contained pathogens or naturally occurring bacteria. On the other hand, the untreated brand (Brand 5) may have contained naturally occurring bacteria representative of its original spring source. In fact, one bottle from this Brand was positive to the HPC (but the batch still passed the standard). Edberg and co-workers (1997) analysed the invasiveness and cytotoxicity of bacteria present in natural mineral waters and concluded that these organisms did not possess significant pathogenic characteristics threatening human health.

Regular monitoring and routine testing schemes should be implemented by drinking water manufacturers. In this study, all the five bottled water brand manufacturers that responded to the survey declared performing periodic analysis of their bottled waters for the presence of coliforms, thermotolerant coliforms, heterotrophic plate count, and *E. coli* organisms. In addition, Brand 1 also analysed the water for faecal streptococci and *Pseudomonas aeruginosa*

which are also sometimes used as indicators of faecal pollution (*E. coli* and thermotolerant coliforms) and environmental contamination (*P. aeruginosa)*.

It would have been helpful to be able to also identify the source of the bottled water (e.g. surface waters, groundwater, rainwater, etc.), which was explicitly asked in the questionnaire. However, for unknown reasons, the water source was not provided by any of the manufacturers, even when successively contacted by phone.

In this study, the municipal water supply delivered by MCWD was mostly sourced from groundwaters, which usually possesses superior natural protection against bacterial contamination compared with surface waters. Nonetheless, the municipal water system in the study area employed standard disinfection treatments to ensure safety of the water supply. Hence, just like most of the bottled water brands tested in this study, the municipal water sampled should be considered as treated drinking waters. The treatment applied by the MCWD was chlorination, which kills microorganisms by chemical oxidation. In addition, the MCWD implemented a quality control and water safety preventive maintenance program which covered the whole city's water supply network in the study area. Thus, the drinking water distributed to the concessionaire's taps in domestic households and commercial and industrial establishments are intended to be free from pathogens and safe for consumption, and this was reflected by the bacteriological results, except on one occasion in which a water supply interruption was recorded.

This study also found that GMPs, SSOPs, or any food safety program is difficult to assess because of its apparent complex structure. These programs are best

assessed through systematic audits of conformance to specified criteria. For purposes of establishing links between bacteriological quality and these sanitation programs, one suggestion is to focus on key elements such as control of source quality or operational treatment procedures rather than the whole program. Also, another problem identified was the low response rates of sanitation surveys possibly for reasons of confidentiality, trade secrets, non-cooperation, or possible lack of understanding of the questions presented in a survey.

Based on the limited responses experienced from the survey one valuable information generated was the importance of conducting a formal cognitive assessment of questionnaires before performing an actual survey. In this study, it was not possible to perform this assessment because the target population is very limited and there was no planned site visits prior to data collection. Although an informal undocumented peer evaluation of the questionnaire was made, a formal cognitive assessment is more appropriate in order to refine the structure of the questionnaire and elicit better response rates (Presser et al., 2004). But because of this study, we now learned the importance of designing and testing a questionnaire with the assistance of a cognitive specialist and moderator. Despite the fact that this survey is only a secondary objective and the challenges faced from collecting sensitive and confidential company information were high, a pre-testing using focus group discussion (FGD) and thorough review may improve the clarity of the questions and response rates. This would help inform future surveys on dealing with questions involving human individuals from different positions and expertise in a company.

From a preventive and proactive perspective, the author also suggests the application of a HACCP-based monitoring of indicator organisms in drinking water. While *E. coli* is acknowledged as the best biological indicator of health risk in drinking waters, testing for public health significance should be based on pre-emptive, anticipatory approach to safety. The probability of a health risk scenario associated with a contaminated drinking water is mostly a result of inadequate source protection and process management. Hence, before a critical *E. coli* contamination can occur, it is suggested that target and action limits be developed through the use of other non-health risk associated indicators such as HPCs and coliforms. Since the use of non-health risk indicators may also overestimate safety when results are positive, the assessment of drinking water safety should be based on the application of combined indicators, one anticipatory and one critical indicator limits. HACCP technique accomplishes this by the identification of indicator organisms as a hazard and the determination of levels (values) for processing concerns (target limits) and health risk issues (critical limits). Since critical limits can only be reached when target limits are exceeded, warning limits (values between the target and critical limits) are required to enable process adjustments. The processing step where the hazard must be controlled is known as the critical control point (CCP). The succeeding steps that focuses only on the CCPs and consisting of control measures, monitoring, corrective actions, verification, and documentation general builds on the success of these two pre-requisite activities.

5.4 Overall risk from water consumption

In this study, there were generally no significant issues of public health concern except for one tap water sample from one occasion that showed positive *E. coli*

and coliform results and thus did not comply with the Philippine regulatory standards. However, there was no reported outbreak of illness from this site at the time of sampling. From a bacteriological quality standpoint, the occurrence of bacterial growth in both bottled and tap water portions suggests a similar likelihood of bacterial contamination in both water types especially when source water protection and monitoring, treatment methods, and sanitary processing procedures are ineffective. In the context of both water types' intended for use as safe drinking water for human consumption, the results imply that assurance of safety as well as the probability of contamination occurring are fairly similar.

However, there could be a significant difference from a health risk management perspective. Based on the 'single drinking occasion' consumption unit of reference described in the Materials and Methods chapter, the probability of risk from ingesting pathogen contaminated water maybe higher for tap water than in bottled water. The main reason is that the tap water samples were positive for *E. coli* and all other indicator bacteria, whilst the bottled water samples were only positive for HPC. As mentioned, *E. coli* is the most direct biological indicator of pathogens occurring in water, whilst HPC only indicates process management inadequacy. Even if the contaminated portions of the bottled water brand was higher than the household tap, the fact that some strains of *E. coli* are pathogens whilst HPCs are not, strongly supports the higher health risk probability when consuming the contaminated tap water sample.

CHAPTER 6 - CONCLUSIONS AND RECOMMENDATIONS

Drinking-water is a live, not sterile product. However, it should be pathogen free to ensure safety for human consumption. The aim of this study was to assess the bacteriological quality of bottled and tap water in Cebu City, Philippines at the point of sale. Despite its limitations, important conclusions about the quality of the water tested can be made.

The results indicate that the bottled water market in the study area is dominated by manufacturers that apply water treatments and quality systems able to effectively safeguard consumers' health, without the need for tighter controls. However, some smaller manufacturers (such as the manufacturer of Brand 6) require closer monitoring by health authorities, as these products consistently failed the bacteriological standards and did not demonstrate the firm's capacity to independently manage their own quality systems. Furthermore, the results showed there could be significant batch-to-batch variability in the bacteriological quality of the water produced by such manufacturers. This variability should be taken into consideration when sampling strategies are designed for these manufacturers: rather than adhering to the strict testing of five bottles per batch required by regulations, it could be more cost-effective to sample and test a smaller number of bottles from as many batches as possible.

For tap water supplies, most of the samples were bacteriologically negative, indicating effective disinfection systems. However, the finding of *E. coli* may indicate a greater overall risk from consumption of tap, than bottled water. *E. coli* contamination was observed only shortly after a water supply interruption event occurred, reinforcing the need for a close monitoring of such potential hotspots in

the water supply system. Furthermore, these results suggest that modernisation of the reticulation system aimed at decreasing the number of interruptions would reduce the bacteriological risk. Such improvements could increase the consumers' confidence in the quality of the city water supply, thereby reducing the consumption of bottled water, which was shown to have greater environmental impacts than tap water (Fantin et al., 2011; Lena & Pirollo, 2011).

Compared with other studies, the present study used a relatively large sample size and multiple tests in parallel. Limitations included the haphazard collection of bottles from store shelves, which did not take into account the high diversity of bottled water batches sampled. This resulted in the sampling of only one bottle in 45/83 (54%) batches. In the future, it would be advisable to consider the presence of multiple batches on the shelves and take this factor into consideration when developing the sampling strategy. The results suggest that it is not always necessary to apply a rigid scheme of sampling of five bottles per batch, as the sampling of fewer bottles allows testing of more batches per unit of budget. For example, sampling of five bottles per batch would have allowed the testing of only 30 (36%) batches (5 per brand) within budget. Considering the large variability of production batches observed on the shelves and the fact that production batch was a significant variable in the models, sampling of a limited number of batches would have failed to detect contaminated batches. Another interesting result was the finding of a significant effect of the production batch on the HPC counts, but no effect of the sampling occasion. This result suggests that one sampling occasion per production batch could capture most of the variability in the counts.

In summary, this study provided a glimpse on the bacteriological quality of bottled and tap water in the study area and indicated a number of factors associated with bacterial growth in both water types. The results could inform the design of future large scale studies of the factors associated with bacterial contamination in drinking water.

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Appendix 1 – Sampling procedure for bottled water samples

(Based on Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, 22nd ed., 2012, Sample Collection)

1. The pre-identified bottled drinking water brands are purchased from two big supermarkets and two small retail stores in the study area.

2. Five bottles of each bottled water brand is collected at random from the shelves of the stores (refer to sampling schedule on Chapter III Table 3-3). One bottle represents one sample unit. Samples are labelled with date and time of collection, temperature, and source (name of store). Samples will also be checked and noted for appearance and bottle integrity during collection. Only those bottles with satisfactory bottle packaging integrity (no broken seals, bottle cracks, and deformities) are collected. These information including the brand name and batch code is recorded in a controlled logbook.

3. For reference and recommendation purposes of this study, batch codes of additional twenty bottles of the same brand in the store's shelves are also recorded.

4. Samples are transported at 4 - 10°C using sealable, cooling buckets to the microbiology laboratory.

5. The samples are stored in a laboratory chiller (2 - 6°C) and tested for bacteriological analysis on the same day of the sampling, specifically within six hours after collection.

6. Bacteriological analysis to be performed on the samples are *E. coli* count, thermotolerant coliform count, total coliform count, and heterotrophic plate count. Test method's standard operating procedures used are based on SMEWW published by APHA, AWWA, and WEF, 22nd ed., 2012.

7. After analysis, samples are aseptically kept for 3 weeks in laboratory chillers (2 - 5°C) for reference purposes.

8. Sampling and testing ends when all the sampling occasions are completed (refer to sampling schedule on Chapter III table 3-3).

Appendix 2 – Sampling procedure for tap water samples

(Based on Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, 22nd ed., 2012, Sample Collection)

1. Drinking water samples are collected from pre-identified, household tap water sources in villages with the greatest number of local water district concessionaires in the study area.

2. Five samples at 500ml each, from each household drinking water tap is aseptically collected using sterilized, sealable, non-reactive, borosilicate glass bottles. Samples are labelled with date and time of collection, temperature, and source (name of household and village). Samples are checked and noted for product appearance and integrity during collection. These information are recorded in a controlled sampling logbook.

3. Selected tap should be directly connected with the main distribution line, not for example, served from a storage tank. Faucet attachments such as splash guards or screens must be removed and samples must not be taken from leaking taps. Ensure sampling bottle is kept closed before being filled. Filling of the bottle must be done without rinsing.

4. Tap is opened fully and water run to waste for 2 - 3 minutes or for a sufficient time to permit clearing of the service pipeline.

5. Disinfect faucet by applying 70% ethanol and flame. Let water run to waste for additional 2 - 3 minutes. Then reduce the water flow (to allow filling bottle without splashing) and collect the water sample using the designated sampling bottle.

6. Leave enough air space (at least 2.5 cm) to facilitate mixing by shaking prior to each bacteriological analysis in the laboratory. Re-place the cap to the bottle immediately to prevent contamination.

7. Samples are transported at 4 - 10°C using sealable, cooling buckets to the microbiology laboratory.

8. The samples are stored in a laboratory chiller (2 - 6°C) and tested for bacteriological analysis on the same day of the sampling, specifically within six hours after collection.

9. Bacteriological analysis to be performed on the samples are *E. coli* count, thermotolerant coliform count, total coliform count, and heterotrophic plate count. Test method's standard operating procedures used are based on SMEWW published by APHA, AWWA, and WEF, 22nd ed., 2012.

10. After analysis, samples are aseptically kept for 3 weeks in laboratory chillers (2 - 5°C) for reference purposes.

11. Sampling and testing ends when all the sampling occasions re completed (Refer to sampling schedule on Chapter III table 3-3).

Appendix 3 - Standard Operating Procedure for *E. coli*, thermotolerant coliforms, and total coliform count analysis of drinking water

A. Purpose and Scope

The test is carried out as part of the methodology of the research entitled: "A Study of Bacteriological Quality of Bottled and Tap Water in Cebu City, Philippines". The test is performed at the Regional Standards and Testing Laboratory, Department of Science and Technology, Regional Office VII, Cebu City, Philippines (an ISO 17025 accredited laboratory).

B. Definitions

MPN = most probable number

LST/LTB = lauryl sulphate broth/lauryl tryptose broth

BGLB = brilliant green lactose bile broth

C. Analytical Principle

This procedure is used to test the sanitary index bacteria (the coliforms, thermotolerant coliforms, and *E. coli* as a coliform)

D. Equipment and Reagents

a) Equipment for sampling

Water sampling equipment and materials used by the researcher (cooling buckets, etc.)

b) Equipment for sample preparation

c) Equipment for analysis

biosafety cabinet

mixer

incubator, maintained at 35 ± 1^{o}C

autoclave

pH meter

water bath, circulating covered bath, maintained at 45^{o}C

micropipet with tips, 1000 ul, calibrated

glass pipets

dilution bottles

d) Reagents and Culture Media

i. Chemicals and culture media

KH_2PO_4	grade AR
NaOH, 1N	grade AR
BGLB	dehydrated
LST/LTB	dehydrated
EC Broth	dehydrated
Distilled water	

ii. Solutions

Butterfield's phosphate stock solution preparation: 8.5 g KH_2PO_4 in 100 ml distilled

Buffered solution water; adjust to pH 7.2 with 1N NaOH, dilute to 250 ml distilled water; to prepare buffered water for dilutions, dilute 1.25 ml stock solution to 1L boiled and cooled water; autoclave for 15 min at 121°C.

dilution blanks, 90 ± 1 ml Butterfield's phosphate-buffered dilution water, 1 bottle

Lauryl tryptose broth/ 7.1 g LST in 200 ml distilled water; dispense 10 ml portions to tubes containing inverted fermentation tubes; autoclave for 15 min at 121°C; final pH 6.8 ± 0.2 after sterilization; make LTB/LSB of such strength that adding 100-ml or 10-ml portions of sample to medium will not reduce ingredient concentrations below those of the standard medium; prepare in accordance with Table 1.

Brilliant green lactose bile broth 8.0 g BGLB in 200 ml distilled water; dispense 10 ml portions to tubes containing inverted fermentation tubes; autoclave for 15 min at 121°C; final pH 7.2 ± 0.1 after sterilization.

EC broth 7.4 g EC in 200 ml distilled water, dispense 10 ml portions to tubes containing inverted fermentation tubes; autoclave for 15 min at 121°C; final pH 6.9 ± 0.2 after sterilization.

Table Appendix 3-1. Preparation of Lauryl Tryptose Broth.

Inoculum mL	**Amount of medium in tube mL**	**Volume of medium + inoculum mL**	**Dehydrated lauryl tryptose broth required *g/L***
20	10	30	106.8 (triple strength)

E. Analytical Procedure

a) Safety

See User Manuals: biosafety cabinet; incubator; autoclave; pH meter; top-loading balance; circulating covered bath.

b) Sampling

Water samples collected by the researcher. Researcher is advised to follow suggested water sampling procedures available at the laboratory's receiving/releasing section.

c) Storage

Samples are maintained at 0^{o} to 5^{o}C until analysed. Water samples are stored for 2 weeks prior to disposal.

d) Preparation

Arrange five (5) fermentation tubes in a test tube rack.

e) Procedure

1. Prepare appropriate diluents and dilutions (see Table 1 on previous page).

2. Transfer 20 ml of the original sample to each of the 5 tubes of triple strength 10 ml LTB (containing inverted Durham tubes).

3. Incubate inoculated fermentation tubes at 35 + 1oC.

4. After 24 + 2 h shake each tube gently and examine it for gas production and, if no gas has formed and been trapped in the inverted tubes, re-incubate and re-examine at the end of 48 + 3 h. (Note: Blinded experiment is employed, hence reading of results is done by another trained analyst to minimize bias or systematic error in results)

5. Record presence or absence of gas formation. Formation of gas in any amount in the inverted tubes within 48 + 3 h constitutes a positive presumptive reaction. Perform a confirmed test on all presumptive positive (gassing) tubes. Perform step 7 and 8 simultaneously.

6. Perform a positive (+) control from a known pure culture of E. coli (E. coli ATCC 25922).

7. Confirmed test for coliforms. Transfer loopful of each gassing LST tube to tubes of BGLB; incubate BGLB tubes 48 + 3 h at 35 ± 1oC; examine for gas production and record. Formation of gas in any amount in the inverted tubes of the BGLB at any time within 48 + 3 hours constitutes a positive confirmed phase. Perform a completed test (5.5.8 to 5.5.15) on positive confirmed tubes to establish definitively the presence of coliform bacteria and to provide quality control data.

8. Confirmed test for thermotolerant coliforms. Transfer loopful of each gassing LST tube to tubes of EC broth; incubate EC tubes in water bath 44.5 + 1oC h for 24 h; Maintain a sufficient water depth in the water bath incubator to immerse tubes to the upper level of the medium.

9. Gas productions in EC broth fermentation tubes within 24 + 2 h is considered a confirmed positive reaction indicating coliforms of fecal origin. Failure to produce gas (growth sometimes occur) constitutes a negative reaction indicating a source other than the intestinal tract of warm blooded animals.

10. Confirmed test for E. coli. Streak loopful of suspension from each gas-positive tubes to EMB and/or McConkey agar.

11. Incubate plates (inverted) at 35 ± 1°C for 24 ±2 hrs.

12. Examine plates for typical lactose-fermenting colonies on MacConkey which are red, and may be surrounded by an opaque zone of precipitated bile. On EMB plates, examine for dark centred and flat colonies with or without metallic sheen.

13. From each plate pick one or more typical, well-isolated colonies and perform the gram stain and biochemical test.

14. Run a positive (+) control from a known pure culture of E. coli (E. coli ATCC 25922).

15. Perform gram stain; examine all cultures appearing as Gram-negative short rods and perform the following IMViC biochemical activities:

    a. Indole production - inoculate tube of TB and incubate 24 + 2 h at 35 ± 1oC; test indole by adding 0.2-0.3 ml Kovac's reagent; appearance of distinct red colour in the upper layer is positive test.

    b. Methyl red-reactive compounds - inoculate tube of MR-VP broth and incubate 48 + 2 h at 35 ± 1oC; add 5 drops methyl red solution; a distinct red colour is a positive test.

    c. Vogues-Proskauer-reactive - inoculate tube of MR-VP broth and incubate at 48 + 2 h at 35 ± 1oC; add 0.6 ml alpha-naphthol solution and 0.2 ml 40% KOH and shake; test is positive if eosin pink colour develops.

    d. Citrate - lightly inoculate tube of KCM; avoid detectable turbidity; incubate 96 + 2 h at 35 ± 1oC; development of distinct turbidity is positive reaction.

    e. Gas from lactose - inoculate tube of LST broth (single strength) and incubate 48 + 2 h at 35 ± 1oC; displacement of medium from inner tube or effervescence after gentle agitation is positive reaction.

    f. Interpret the reaction results for E. coli as follows. All cultures that (a) ferment lactose with production of gas within 48 h at 35oC, (b) appear as Gram-negative non spore-forming rods, and (c) give IMViC patterns (++--) (biotype 1) or (-+--) (biotype 2) are considered to be *E. coli*.

F. Calculation of the Results

Record the number of positive findings of coliform group organisms (either confirmed or completed) resulting from multiple-portion decimal-dilution plantings as the combination of positives and compute in terms of the Most Probable Number (MPN) (see Table 2, MPN Index when five 20-ml portions are used).

Table Appendix 3-2 MPN Index and 95% Confidence Limits for All Combinations of Positive and Negative Results when five 20-ml Portions are used.

No. of tubes giving positive reaction out of 5 (20ml each)	MPN Index / 100 ml	95% Confidence Limits (Exact)	
		Lower	Upper
0	<1.1	----	3.5
1	1.1	0.051	5.4
2	2.6	0.40	8.4
3	4.6	1.0	13
4	8.0	2.1	23
5	>8.0	3.4	----

Source: APHA, AWWA, and WEF, 2012, page 9-70

G. Documentation

a) Internal

Request form

Sample accompanying form

Controlled log book and result form with blinding control

b) External

Certificate of analysis

H. Result form for E. coli, thermotolerant coliforms, and total coliform count analysis of drinking water

Parameter: *E. coli,* thermotolerant coliform, and total coliform count analysis for drinking water							
Performed by: _____ Time started: _____ Date started: _____ Time of incubation: _____					Results read by: _____ Date/time read: _____		
Results checked by: _____ Date checked: _____ Remarks: _____							
	Sample No.		Sample No.		Positive Control*	Negative Control**	*Remarks*
Presumptive Test	*24 H*	*48H*	*24H*	*48H*			
LTB (triple strength) 1							
2							
3							
4							
5							
MPN							
Confirmed Test for Coliforms BGLB							
MPN							
Confirmed Test for thermotolerant coliforms and E. coli EC Broth, *48H, 44.5°C*							
L-EMB, *24H*							
Gram Staining							
IMViC Biochemical Screening Indole Production, *24H*							
Methyl Red, *48H*							
Vogues-Proskauer, *48H*							
Koser's Citrate Broth, *96H*							
Interpretation							
Classification of culture(s)							

pH distilled water _____ Temp. of incubator _____
pH LTB _____ Incubation time _____
pH BGLB _____ Incubator used _____
pH EC Broth _____ Pipette used _____
pH EMB

*Pure culture of *Escherichia coli* ATCC # 25922 as positive control
**Sterilized media as negative control

Appendix 4 - Standard Operating Procedure for Heterotrophic plate count analysis of drinking water

A. Purpose and Scope

The test is carried out as part of the methodology of the research entitled: “A Study of Bacteriological Quality of Bottled and Tap Water in Cebu City, Philippines”. The test is performed at the Regional Standards and Testing Laboratory, Department of Science and Technology, Regional Office 7, Cebu City, Philippines (an ISO 17025 accredited laboratory).

B. Definitions

HPC = heterotrophic plate count

SPC = standard plate count

PCA = plate count agar

Cfu = colony forming units

C. Analytical Principle

The heterotrophic plate count (HPC), formerly known as the standard plate count (SPC) is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment and distribution or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term “colony-forming units (cfu).

D. Equipment and Reagents

a) Equipment for sampling

Water sampling equipment and materials used by researchers (cooling buckets, etc.)

b) Equipment for sample preparation

c) Equipment for analysis

biosafety cabinet

mixer

incubator, maintained at 35 ± 1^{o}C

autoclave

pHmeter

colony counter

water bath, circulating covered bath, maintained at 45^{o}C

petri dishes

micropipet with tips, 1000 ul,

glass pipets

dilution bottles

d) Reagents and Culture Media

i. Chemicals and culture media

KH_2PO_4	grade AR
NaOH, 1N	grade AR
PCA	dehydrated

Distilled water

ii. Solutions

Butterfield's phosphate	stock solution preparation: 8.5 g KH_2PO_4 in 100 ml distilled water, adjust to pH 7.2 with 1N NaOH, dilute to 250 ml distilled water; to prepare buffered water for dilutions, dilute 1.25 ml stock solution to 1L boiled and cooled water; autoclave for 15 min at 121°C.
Buffered solution	dilution blank, 99 ± 1 ml Butterfield's phosphate-buffered dilution water, 1 bottle
Plate Count Agar	3.375 g PCA in 150 ml distilled water; autoclave for 15 min at 121°C; pH 7.0 ± 0.2 after sterilization.

E. Analytical Procedure

a) Safety

See User Manuals: biosafety cabinet; vortex mixer; incubator; autoclave; circulating covered bath.

b) Sampling

Water samples collected by researchers. Researchers are advised to follow suggested water sampling procedures available at the receiving/releasing section.

c) Storage

Samples are maintained at 0° to 5°C until analysed. Water samples are stored for 2 weeks prior to disposal.

d) Sample Preparation

1. Mark each plate with sample number, dilution, date, and any other necessary information before examination. Prepare duplicate plates for each volume.

2. Thoroughly mix all samples by rapidly making about 25 complete up and down movements. Optionally, use mechanical shaker.

e) Procedure

1. Add 1.0 ml raw water sample to 99 ± 1 ml Butterfield's phosphate-buffered dilution water. This results in a dilution of 10^{-2}. Shake by vortexing for 30 seconds.

2. Plant 1 ml and 0.1 ml undiluted sample and 1 ml and 0.1 ml of the 10^{-2} dilution into appropriately marked petri plates. Prepare at least two replicate plates for each sample dilution used.

3. Add 12-15 ml PCA to each plate. Do not let more than 20 minutes elapse between starting pipetting and pouring plates.

4. Perform fallout pour plates for microbial density of air in working area taken during plating.

5. Immediately mix sample dilution and agar medium thoroughly and uniformly.

6. Let agar solidify, invert petri dishes, and incubate promptly for 48 ± 2 hours at 35 ±1°C. Do not stack plates when pouring agar or when agar is solidifying.

7. After incubation, count colonies in duplicate plates in suitable range (30-300 colonies), record results per dilution plate counted. (**Note: Blinded experiment is employed; hence reading of results must be done by another trained analyst to minimize bias or systematic error in results**)

F. Calculation of the Results

Use and count plates with 30 – 300 colonies. To compute the HPC, divide total number of colonies or average number (if duplicate plates of the same dilution) per plate by the sample volume. Record sample volumes used and number of colonies on each plate counted or estimated. Report counts as "colony-forming units" (CFU) per millilitre. If there is no plate within that range, and one or more plates have more than 300 colonies, use the plate(s) having a count nearest 300 colonies. Compute the count by multiplying average count per plate by the reciprocal of the dilution used and report as "estimated colony-forming units" per millilitre. If plates from all dilutions of any sample have no colonies, report the count as less than one (<1) times the reciprocal of the corresponding lowest dilution. For example, if no colonies develop on the 1:100 dilution, report the count as less than 100 (<100) estimated colony-forming units/ml.

$$\text{HPC (cfu)} = \frac{\text{Total number of colonies}}{\text{Volume of sample in dish, ml}}$$

G. Documentation

a) Internal

- Request form
- Sample accompanying form
- Controlled log book and result form with blinding control

b) External

- Certificate of analysis

H. Result form for Heterotrophic plate count analysis of drinking water

Parameter: Heterotrophic plate count testing for drinking water			Section: Microbiology	
Performed by: ______________Time started: ______________ Date started: ______________ Time of incubation: __________			Results read by: __________________ Date/time read: ___________________	
Results checked by: _________Date checked: ____________			Remarks: ________________________	
Sample Number	Measurement		Calculation / Result	Remarks
	Dilution	Readings		
	10^{-0}			
	10^{-1}			
	10^{-2}			
	10^{-3}			
	10^{-0}			
	10^{-1}			
	10^{-2}			
	10^{-3}			
	10^{-0}			
	10^{-1}			
	10^{-2}			
	10^{-3}			
	10^{-0}			
	10^{-1}			
	10^{-2}			
	10^{-3}			
	10^{-0}			
	10^{-1}			
	10^{-2}			
	10^{-3}			
	10^{-0}			
	10^{-1}			
	10^{-2}			
	10^{-3}			

pH water used ______________ Temp. of incubator _______________
pH diluent ______________ Incubation time _______________
pH PCA ______________ Incubator used _______________
Pipette used _______________

Sterility Control:

Media/plate/air____________

Pipette/tips/diluent ___________

Appendix 5 - Simple assessment of differences between proportions of bacteriologically positive samples between bottled and tap water using two-tailed fisher's exact tests

19/05/2014 4:10:30 p.m. 1

Difference of Two Proportions Report (using NCSS-PASS software)

Counts Summary

X11	X21	X12	X22	N1 (X11+X12)	N2 (X21+X22)	M1 (X11+X21)	M2 (X12+X22)	N (N1+N2)
21	5	129	115	150	120	26	244	270

(Note: In this model, successes means with bacterial counts; non-successes means no bacterial count. Group 1 is bottled water, group 2 is tap water)

Proportions Summary

Group	'Successes'	'Non-Successes'	Sample Size	'Success' Proportion	'Non-Success' Proportion
1	21	129	150	**0.1400**	0.8600
2	5	115	120	**0.0417**	0.9583
1 & 2	26	244	270	0.0963	0.9037

Differences, Ratios, and Odds Ratios Summary

------ Differences ------		-------- Ratios --------		---------- Odds Ratios ----------	
p1 - p2	p2 - p1	p1/p2	p2/p1	Odds1/Odds2	Odds2/Odds1
0.0983	-0.0983	3.3600	0.2976	3.7442	0.2671

Risk Summary

Group 1 Experimental Event Rate p1	Group 2 Control Risk Event Rate p2	Absolute Risk Difference |p1 - p2|	Number Needed 1/|p1 - p2|	Relative Risk Reduction |p1 - p2|/p2	Relative Odds Reduction p1/p2	Risk Ratio Odds1/Odds2
0.1400	0.0417	0.0983	10.1695	2.3600	3.3600	3.7442

Simple Z Confidence Interval of Difference (P1 - P2)

Confidence Interval Method	Sample p1	Sample p2	Difference (p1 - p2)	95% Confidence Interval Lower Limit	Upper Limit
Simple Z	0.1400	0.0417	0.0983	0.0323	0.1644

Two-Sided Z-Test of Two Proportions

H0: P1 = P2 vs. Ha: P1 ≠ P2

Distribution of Test Statistic: Normal

Test Name	p1	p2	p1 - p2	Z-Statistic	Prob Level	Reject H0 at α = 0.05?
Z-Test	0.1400	0.0417	0.0983	2.722	0.0065	Yes

Fisher's Exact Two-Sided Test of Two Proportions

H0: P1 = P2 vs. Ha: P1 ≠ P2

Distribution of Test Statistic: Hypergeometric

Test Name	p1	p2	p1 - p2	Prob Level	Reject H0 at α = 0.05?
Fisher's Exact	0.1400	0.0417	0.0983	0.0067	Yes

Appendix 6 - Survey questionnaire for bottled water manufacturers

MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

SURVEY QUESTIONNAIRE FOR BOTTLED WATER MANUFACTURERS

Research Title: A Study of the Bacteriological Quality of Bottled and Tap Water in Cebu City, Philippines

Thank you very much for taking the time to complete this survey! Participation to this survey is voluntary. Completion and return of this questionnaire implies consent. You have the right to decline to answer any particular question. Instructions on returning the completed questionnaire to the researcher can be found at the last page of this document.

Student Researcher Name: Bryan B. Ybanez

I. Contact information
1. Company name:
2. Physical address:
3. Phone: Fax:
4. Email:
5. How many brands of water do you bottle?
6. What are the brands that you sell?
7. How long have you been operating the current business?
8. Do you run any other business from the same premise as water bottling?
Yes No
If Yes, please specify

II. Source
1. What is the source of the bottled water:

Stream	Yes	No	Town Water Supply	Yes	No
Aquifer	Yes		No	If Yes, which town	
Bore	Yes	No	If Yes, is the bore artesian	Yes	No

Others (please specify)
2. Location of the source:
3. Location of the water bottling plant:
4. Are there backflow devices in place to prevent contamination of the water supply being tapped into? Yes No
If Yes, what type of backflow device has been installed?
5. How do you ensure, that backflow devices are maintained and inspected?

III. Collection and transportation
1. How is the source water collected?

Pump	Yes	No	Gravity	Yes	No

2. How is the source water transported from the source to the water bottling facility?

Pipes	Yes	No

If yes, are the pipes stainless steel/PVC/other (encircle one)
(specify other)

Containers	Yes	No

If yes, are the containers stainless steel/PVC/other (encircle one)?
(specify other)

Are the containers sterile?	Yes	No

IV. Water treatment

1. Is the water treated prior to bottling?	Yes	No

If yes, what is the treatment (tick all that apply)

Filtration	Yes	No	UV	Yes	No
Distillation	Yes	No	Chlorination	Yes	No
Ozonation		Yes		No	

Others (please describe in detail or attach protocols if possible?

V. Bottles

1. Are the bottles:	Plastic	Yes	No	Glass	Yes	No

2. What is the volume: 250ml / 300ml / 500ml / 600 ml / 750ml / 800ml/ 1L / 1.5L / 2L

5L / 10L / 20L (encircle all that apply)
Other
3. Do the bottles have plastic screw-on cap / pump top / metal screw - on top?
(Encircle all that apply)
Others
4. How many bottled water do you produce per year?
5. Where are the bottles purchased from?

6. Are the bottles sterilized by the supplier?	Yes	No

If yes, how:	Autoclaving	Yes	No	
Gamma Radiation		Yes	No	

Other

VI. Bottling process
1. What is the water bottling process that you use?

Hand bottling	Yes	No	Machine bottling	Yes	No

2. Is the water chilled prior to bottling?	Yes	No
3. Is the water chilled after bottling?	Yes	No

4. What cleaning materials are used for the cleaning of pipework/tankers/bottling equipment?

5. Do you have a batch tracing system?	Yes	No

If yes, please describe:

VII. Routine testing

1. Do you have a food safety programme in place?	Yes	No
2. Do you test microbiological quality of the water that you bottle?	Yes	No

If yes, what tests are routinely undertaken?

Heterotrophic plate count	Yes	No
Total coliform count	Yes	No
Fecal coliform count	Yes	No
E. coli count	Yes	No

Others:

VIII. Staff

1. How many staff do you have?
2. What are their qualifications?
3. What training is provided for the staff?
4. What are their responsibilities?

IX. Procedures. Do you have written procedures regarding the following?

1. Sourcing of water	Yes	No
2. Transporting water	Yes	No
3. Bottling water	Yes	No
4. Delivery and dispatch of water	Yes	No
5. Water protection against contamination at the source	Yes	No
6. Water protection against contamination during transportation	Yes	No
7. Water protection against contamination during bottling	Yes	No
8. Cleaning of pipework/tanks	Yes	No
9. Cleaning of bottling equipment	Yes	No
10. Staff health?	Yes	No

If Yes to any of the above, we would appreciate a copy of all written procedures.

X. Utilities	(Material inputs and emissions)	Year:

Please indicate values per unit/time

1. Energy consumption	1a. Electricity:
1b. Diesel:	
1c. LPG:	
1d. Others (please specify):	
2. Water consumption	
3. Chemicals and material inputs	
4. Emissions to	4a. Air:
4b. Water:	
4c. Soil:	
5. Wastes	5a. Packaging wastes:

5b. Other wastes:
Others:

XI. Others.
1. Have you encountered any problems that you wish to bring up?
With your operation Yes No
Comments
With environmental health issues Yes No
Comments
2. If you have any concerns that you wish to discuss, please use the 'Comments' section below.

XII. Comments
Thank you very much! When you completed this form, please submit to the researcher via:
email at: B.Ybanez@massey.ac.nz or fax at: (032) 254-7049
or provide us with your address and we will be happy to visit you and pick-up the form.

Appendix 7 - Survey questionnaire for water service provider

MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

SURVEY QUESTIONNAIRE FOR WATER SERVICE PROVIDER

Research Title: A Study of the Bacteriological Quality of Bottled and Tap Water in Cebu City, Philippines

Thank you very much for taking the time to complete this survey! Participation to this survey is voluntary. Completion and return of this questionnaire implies consent. You have the right to decline to answer any particular question. Instructions on returning the completed questionnaire to the researcher can be found at the last page of this document.

Student Researcher Name: Bryan B. Ybanez

I. Contact information
1. Company name:
2. Physical address:
3. Phone: Fax:
4. Email:
5. What is the total volume of drinking water do you deliver to consumers for the latest year? (please state the year and units)

year:_____ ave. per day:_____

6. How many drinking water consumers do you currently serve?
Please provide us with a list of your areas (barangays) served, number of concessionaires, location of source or sub source, and type of source. This will assist in the preparation of sampling plan, sampling stratification, and sample size. Thank you.
7. How long have you been operating the current business?
8. Do you run any other business from the same premise besides providing drinking water
Yes No
If Yes, please specify

II. Source
1. What is the source of the drinking water:

Stream	Yes		No		Others (please specify)	
Aquifer		Yes		No		
Bore	Yes	No	If Yes, is the bore artesian	Yes		No

2. Location of the source:
3. Location of the drinking water plant:
4. Are there backflow devices in place to prevent contamination of the water supply being tapped into? Yes No
If Yes, what type of backflow device has been installed?
5. How do you ensure, that backflow devices are maintained and inspected?

III. Collection and transportation

1. How is the source water collected?

Pump	Yes	No	Gravity	Yes	No

2. How is the source water delivered from the source to the consumers?

Pipes	Yes	No
If yes, are the pipes stainless steel/PVC/other (encircle one)		
(specify other)		
Containers	Yes	No
If yes, are the containers stainless steel/PVC/other (encircle one)?		
(specify other)		
Are the containers sterile?	Yes	No
Water Transport Vehicles	Yes	No
If yes, is the vehicle cleaned and sanitized?	Yes	No

IV. Water treatment

1. Is the water treated prior to delivery to distribution pipes?	Yes	No

If yes, what is the treatment (tick all that apply)

Filtration	Yes	No	UV	Yes	No
Distillation	Yes	No	Chlorination	Yes	No
Ozonation		Yes		No	

Others (please describe in detail or attach protocols if possible?

V. Sanitation and Maintenance

1. What cleaning materials are used for the cleaning of pipework/tankers/bottling equipment?

2. Do you have a batch tracing system?	Yes	No

If yes, please describe:

VI. Routine testing

1. Do you have a food safety programme in place?	Yes	No
2. Do you test microbiological quality of the water that you bottle?	Yes	No

If yes, what tests are routinely undertaken?

Heterotrophic plate count	Yes	No
Total coliform count	Yes	No
Fecal coliform count	Yes	No
E. coli count	Yes	No

Others:

VII. Staff

1. How many staff do you have?
2. What are their qualifications?
3. What training is provided for the staff?

What are their responsibilities?

VIII. Procedures. Do you have written procedures regarding the following?

1. Sourcing of water	Yes	No
2. Transporting water	Yes	No
3. Bottling water	Yes	No
4. Delivery and dispatch of water	Yes	No
5. Water protection against contamination at the source	Yes	No
6. Water protection against contamination during transportation	Yes	No
7. Water protection against contamination during processing	Yes	No
8. Cleaning of pipework/tanks	Yes	No
9. Cleaning of equipment and materials	Yes	No
10. Staff health?	Yes	No

If Yes to any of the above, we would appreciate a copy of all written procedures.

IX. Utilities (Material inputs and emissions) Year:

Please indicate values per unit/time

1. Energy consumption 1a. Electricity:
1b. Diesel:
1c. LPG:
1d. Others (please specify):
2. Water consumption
3. Chemicals and material inputs
4. Emissions to 4a. Air:
4b. Water:
4c. Soil:
5. Wastes 5a. Packaging wastes:
5b. Other wastes:
Others:

X. Others.

1. Have you encountered any problems that you wish to bring up?

With your operation Yes No
Comments
With environmental health issues Yes No
Comments

2. If you have any concerns that you wish to discuss, please use the 'Comments' section below.

XII. Comments

Thank you very much! When you completed this form, please submit to the researcher via:

email at: B.Ybanez@massey.ac.nz or fax at: (032) 254-7049

or provide us with your address and we will be happy to visit you and pick-up the form.

Appendix 8 - Human ethics approval letter

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

22 May 2013

COPY FOR YOUR INFORMATION

Bryan Ybanez
DOST 7
Lahug Science and Technology Complex
Sudlon, Lahug
Cebu City
PHILIPPINES

Dear Bryan

Re: HEC: Southern A Application – 13/21
Bacteriological quality and life cycle assessment of drinking water from bottled and deep well tapped sources in Cebu City, Philippines

Thank you for your letter dated 20 May 2013.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

Please note that travel undertaken by students must be approved by the supervisor and the relevant Pro Vice-Chancellor and be in accordance with the Policy and Procedures for Course-Related Student Travel Overseas. In addition, the supervisor must advise the University's Insurance Officer.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Dr Brian Finch, Chair
Massey University Human Ethics Committee: Southern A

cc Dr Alex Grinberg
IVABS
PN412

Dr Sarah McLaren & Dr Ranvir Singh
Institute of Agriculture & Environment
PN433

Prof Frazer Allan, HoI
IVABS
PN412

Prof Peter Kemp, HoI
Institute of Agriculture & Environment
PN433

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E humanethics@massey.ac.nz animalethics@massey.ac.nz gtc@massey.ac.nz **www.massey.ac.nz**

Appendix 9 - Bacteriological test results of bottled and tap drinking water samples

Water sample unit identification and description					**Bacteriological test results**			
Sample No.	Brand for Bottled Water or Village name for Tap Water	Store name for Bottled water or Household No. for Tap Water	Occasion (fiscal month period)	Bottle or Container No.	**HPC** cfu/ ml	**CC** MPN /100ml	**TTCC** MPN /100ml	**ECC** MPN /100ml
1	Brand 1	Metro Ayala	1	1	<1	<1.1	<1.1	<1.1
2	Brand 1	Metro Ayala	1	2	<1	<1.1	<1.1	<1.1
3	Brand 1	Metro Ayala	1	3	<1	<1.1	<1.1	<1.1
4	Brand 1	Metro Ayala	1	4	<1	<1.1	<1.1	<1.1
5	Brand 1	Metro Ayala	1	5	<1	<1.1	<1.1	<1.1
6	Brand 2	Metro Ayala	1	6	<1	<1.1	<1.1	<1.1
7	Brand 2	Metro Ayala	1	7	<1	<1.1	<1.1	<1.1
8	Brand 2	Metro Ayala	1	8	<1	<1.1	<1.1	<1.1
9	Brand 2	Metro Ayala	1	9	<1	<1.1	<1.1	<1.1
10	Brand 2	Metro Ayala	1	10	<1	<1.1	<1.1	<1.1
11	Brand 3	Metro Ayala	1	11	<1	<1.1	<1.1	<1.1
12	Brand 3	Metro Ayala	1	12	<1	<1.1	<1.1	<1.1
13	Brand 3	Metro Ayala	1	13	<1	<1.1	<1.1	<1.1
14	Brand 3	Metro Ayala	1	14	<1	<1.1	<1.1	<1.1
15	Brand 3	Metro Ayala	1	15	<1	<1.1	<1.1	<1.1
16	Guadalupe	H1	1	16	<1	<1.1	<1.1	<1.1
17	Guadalupe	H1	1	17	<1	<1.1	<1.1	<1.1
18	Guadalupe	H1	1	18	<1	<1.1	<1.1	<1.1
19	Guadalupe	H1	1	19	<1	<1.1	<1.1	<1.1
20	Guadalupe	H1	1	20	<1	<1.1	<1.1	<1.1
21	Lahug	H2	1	21	<1	<1.1	<1.1	<1.1
22	Lahug	H2	1	22	<1	<1.1	<1.1	<1.1
23	Lahug	H2	1	23	<1	<1.1	<1.1	<1.1
24	Lahug	H2	1	24	<1	<1.1	<1.1	<1.1
25	Lahug	H2	1	25	<1	<1.1	<1.1	<1.1
26	Brand 4	Metro Ayala	1	26	<1	<1.1	<1.1	<1.1
27	Brand 4	Metro Ayala	1	27	<1	<1.1	<1.1	<1.1
28	Brand 4	Metro Ayala	1	28	<1	<1.1	<1.1	<1.1
29	Brand 4	Metro Ayala	1	29	<1	<1.1	<1.1	<1.1

30	Brand 4	Metro Ayala	1	30	<1	<1.1	<1.1	<1.1
31	Brand 5	Metro Ayala	1	31	<1	<1.1	<1.1	<1.1
32	Brand 5	Metro Ayala	1	32	<1	<1.1	<1.1	<1.1
33	Brand 5	Metro Ayala	1	33	<1	<1.1	<1.1	<1.1
34	Brand 5	Metro Ayala	1	34	<1	<1.1	<1.1	<1.1
35	Brand 5	Metro Ayala	1	35	<1	<1.1	<1.1	<1.1
36	Brand 6	Gaisano Grand	1	36	620	<1.1	<1.1	<1.1
37	Brand 6	Gaisano Grand	1	37	970	<1.1	<1.1	<1.1
38	Brand 6	Gaisano Grand	1	38	750	<1.1	<1.1	<1.1
39	Brand 6	Gaisano Grand	1	39	2600	<1.1	<1.1	<1.1
40	Brand 6	Gaisano Grand	1	40	1600	<1.1	<1.1	<1.1
41	Punta Princesa	H3	1	41	<1	<1.1	<1.1	<1.1
42	Punta Princesa	H3	1	42	<1	<1.1	<1.1	<1.1
43	Punta Princesa	H3	1	43	<1	<1.1	<1.1	<1.1
44	Punta Princesa	H3	1	44	<1	<1.1	<1.1	<1.1
45	Punta Princesa	H3	1	45	<1	<1.1	<1.1	<1.1
46	Mabolo	H4	1	46	<1	<1.1	<1.1	<1.1
47	Mabolo	H4	1	47	<1	<1.1	<1.1	<1.1
48	Mabolo	H4	1	48	<1	<1.1	<1.1	<1.1
49	Mabolo	H4	1	49	<1	<1.1	<1.1	<1.1
50	Mabolo	H4	1	50	<1	<1.1	<1.1	<1.1
51	Brand 1	Lucky 7 Supermart	1	51	<1	<1.1	<1.1	<1.1
52	Brand 1	Lucky 7 Supermart	1	52	<1	<1.1	<1.1	<1.1
53	Brand 1	Lucky 7 Supermart	1	53	<1	<1.1	<1.1	<1.1
54	Brand 1	Lucky 7 Supermart	1	54	<1	<1.1	<1.1	<1.1
55	Brand 1	Lucky 7 Supermart	1	55	<1	<1.1	<1.1	<1.1
56	Brand 2	Lucky 7 Supermart	1	56	<1	<1.1	<1.1	<1.1
57	Brand 2	Lucky 7 Supermart	1	57	<1	<1.1	<1.1	<1.1
58	Brand 2	Lucky 7 Supermart	1	58	<1	<1.1	<1.1	<1.1
59	Brand 2	Lucky 7 Supermart	1	59	<1	<1.1	<1.1	<1.1
60	Brand 2	Lucky 7 Supermart	1	60	<1	<1.1	<1.1	<1.1
61	Brand 3	Lucky 7 Supermart	1	61	<1	<1.1	<1.1	<1.1

62	Brand 3	Lucky 7 Supermart	1	62	<1	<1.1	<1.1	<1.1
63	Brand 3	Lucky 7 Supermart	1	63	<1	<1.1	<1.1	<1.1
64	Brand 3	Lucky 7 Supermart	1	64	<1	<1.1	<1.1	<1.1
65	Brand 3	Lucky 7 Supermart	1	65	<1	<1.1	<1.1	<1.1
66	Guadalupe	H5	1	66	<1	<1.1	<1.1	<1.1
67	Guadalupe	H5	1	67	<1	<1.1	<1.1	<1.1
68	Guadalupe	H5	1	68	<1	<1.1	<1.1	<1.1
69	Guadalupe	H5	1	69	<1	<1.1	<1.1	<1.1
70	Guadalupe	H5	1	70	<1	<1.1	<1.1	<1.1
71	Lahug	H6	1	71	<1	<1.1	<1.1	<1.1
72	Lahug	H6	1	72	<1	<1.1	<1.1	<1.1
73	Lahug	H6	1	73	<1	<1.1	<1.1	<1.1
74	Lahug	H6	1	74	<1	<1.1	<1.1	<1.1
75	Lahug	H6	1	75	<1	<1.1	<1.1	<1.1
76	Brand 4	Lucky 7 Supermart	1	76	<1	<1.1	<1.1	<1.1
77	Brand 4	Lucky 7 Supermart	1	77	<1	<1.1	<1.1	<1.1
78	Brand 4	Lucky 7 Supermart	1	78	<1	<1.1	<1.1	<1.1
79	Brand 4	Lucky 7 Supermart	1	79	<1	<1.1	<1.1	<1.1
80	Brand 4	Lucky 7 Supermart	1	80	<1	<1.1	<1.1	<1.1
81	Brand 5	Lucky 7 Supermart	1	81	<1	<1.1	<1.1	<1.1
82	Brand 5	Lucky 7 Supermart	1	82	<1	<1.1	<1.1	<1.1
83	Brand 5	Lucky 7 Supermart	1	83	<1	<1.1	<1.1	<1.1
84	Brand 5	Lucky 7 Supermart	1	84	<1	<1.1	<1.1	<1.1
85	Brand 5	Lucky 7 Supermart	1	85	<1	<1.1	<1.1	<1.1
86	Brand 6	Cristy Sari-Sari Store	1	86	130	<1.1	<1.1	<1.1
87	Brand 6	Cristy Sari-Sari Store	1	87	99	<1.1	<1.1	<1.1
88	Brand 6	Cristy Sari-Sari Store	1	88	40	<1.1	<1.1	<1.1
89	Brand 6	Cristy Sari-Sari Store	1	89	40	<1.1	<1.1	<1.1
90	Brand 6	Cristy Sari-Sari Store	1	90	60	<1.1	<1.1	<1.1
91	Punta Princesa	H7	1	91	<1	<1.1	<1.1	<1.1
92	Punta Princesa	H7	1	92	<1	<1.1	<1.1	<1.1

93	Punta Princesa	H7	1	93	<1	<1.1	<1.1	<1.1
94	Punta Princesa	H7	1	94	<1	<1.1	<1.1	<1.1
95	Punta Princesa	H7	1	95	<1	<1.1	<1.1	<1.1
96	Mabolo	H8	1	96	<1	<1.1	<1.1	<1.1
97	Mabolo	H8	1	97	<1	<1.1	<1.1	<1.1
98	Mabolo	H8	1	98	<1	<1.1	<1.1	<1.1
99	Mabolo	H8	1	99	<1	<1.1	<1.1	<1.1
100	Mabolo	H8	1	100	<1	<1.1	<1.1	<1.1
101	Brand 1	Metro Ayala	2	101	<1	<1.1	<1.1	<1.1
102	Brand 1	Metro Ayala	2	102	<1	<1.1	<1.1	<1.1
103	Brand 1	Metro Ayala	2	103	<1	<1.1	<1.1	<1.1
104	Brand 1	Metro Ayala	2	104	<1	<1.1	<1.1	<1.1
105	Brand 1	Metro Ayala	2	105	<1	<1.1	<1.1	<1.1
106	Brand 2	Metro Ayala	2	106	<1	<1.1	<1.1	<1.1
107	Brand 2	Metro Ayala	2	107	<1	<1.1	<1.1	<1.1
108	Brand 2	Metro Ayala	2	108	<1	<1.1	<1.1	<1.1
109	Brand 2	Metro Ayala	2	109	<1	<1.1	<1.1	<1.1
110	Brand 2	Metro Ayala	2	110	<1	<1.1	<1.1	<1.1
111	Brand 3	Metro Ayala	2	111	<1	<1.1	<1.1	<1.1
112	Brand 3	Metro Ayala	2	112	<1	<1.1	<1.1	<1.1
113	Brand 3	Metro Ayala	2	113	<1	<1.1	<1.1	<1.1
114	Brand 3	Metro Ayala	2	114	<1	<1.1	<1.1	<1.1
115	Brand 3	Metro Ayala	2	115	<1	<1.1	<1.1	<1.1
116	Guadalupe	H1	2	116	270	1.1	<1.1	<1.1
117	Guadalupe	H1	2	117	280	1.1	<1.1	<1.1
118	Guadalupe	H1	2	118	160	1.1	<1.1	<1.1
119	Guadalupe	H1	2	119	160	2.6	1.1	<1.1
120	Guadalupe	H1	2	120	270	2.6	2.6	2.6
121	Lahug	H2	2	121	<1	<1.1	<1.1	<1.1
122	Lahug	H2	2	122	<1	<1.1	<1.1	<1.1
123	Lahug	H2	2	123	<1	<1.1	<1.1	<1.1
124	Lahug	H2	2	124	<1	<1.1	<1.1	<1.1
125	Lahug	H2	2	125	<1	<1.1	<1.1	<1.1
126	Brand 4	Metro Ayala	2	126	<1	<1.1	<1.1	<1.1
127	Brand 4	Metro Ayala	2	127	<1	<1.1	<1.1	<1.1
128	Brand 4	Metro Ayala	2	128	<1	<1.1	<1.1	<1.1
129	Brand 4	Metro Ayala	2	129	<1	<1.1	<1.1	<1.1

130	Brand 4	Metro Ayala	2	130	<1	<1.1	<1.1	<1.1
131	Brand 5	Metro Ayala	2	131	<1	<1.1	<1.1	<1.1
132	Brand 5	Metro Ayala	2	132	<1	<1.1	<1.1	<1.1
133	Brand 5	Metro Ayala	2	133	<1	<1.1	<1.1	<1.1
134	Brand 5	Metro Ayala	2	134	<1	<1.1	<1.1	<1.1
135	Brand 5	Metro Ayala	2	135	4500	<1.1	<1.1	<1.1
136	Brand 6	Gaisano Grand	2	136	1500	<1.1	<1.1	<1.1
137	Brand 6	Gaisano Grand	2	137	2800	<1.1	<1.1	<1.1
138	Brand 6	Gaisano Grand	2	138	1500	<1.1	<1.1	<1.1
139	Brand 6	Gaisano Grand	2	139	1500	<1.1	<1.1	<1.1
140	Brand 6	Gaisano Grand	2	140	1600	<1.1	<1.1	<1.1
141	Punta Princesa	H3	2	141	<1	<1.1	<1.1	<1.1
142	Punta Princesa	H3	2	142	<1	<1.1	<1.1	<1.1
143	Punta Princesa	H3	2	143	<1	<1.1	<1.1	<1.1
144	Punta Princesa	H3	2	144	<1	<1.1	<1.1	<1.1
145	Punta Princesa	H3	2	145	<1	<1.1	<1.1	<1.1
146	Mabolo	H4	2	146	<1	<1.1	<1.1	<1.1
147	Mabolo	H4	2	147	<1	<1.1	<1.1	<1.1
148	Mabolo	H4	2	148	<1	<1.1	<1.1	<1.1
149	Mabolo	H4	2	149	<1	<1.1	<1.1	<1.1
150	Mabolo	H4	2	150	<1	<1.1	<1.1	<1.1
151	Brand 1	Lucky 7 Supermart	2	151	<1	<1.1	<1.1	<1.1
152	Brand 1	Lucky 7 Supermart	2	152	<1	<1.1	<1.1	<1.1
153	Brand 1	Lucky 7 Supermart	2	153	<1	<1.1	<1.1	<1.1
154	Brand 1	Lucky 7 Supermart	2	154	<1	<1.1	<1.1	<1.1
155	Brand 1	Lucky 7 Supermart	2	155	<1	<1.1	<1.1	<1.1
156	Brand 2	Lucky 7 Supermart	2	156	<1	<1.1	<1.1	<1.1
157	Brand 2	Lucky 7 Supermart	2	157	<1	<1.1	<1.1	<1.1
158	Brand 2	Lucky 7 Supermart	2	158	<1	<1.1	<1.1	<1.1
159	Brand 2	Lucky 7 Supermart	2	159	<1	<1.1	<1.1	<1.1
160	Brand 2	Lucky 7 Supermart	2	160	<1	<1.1	<1.1	<1.1

161	Brand 3	Lucky 7 Supermart	2	161	<1	<1.1	<1.1	<1.1
162	Brand 3	Lucky 7 Supermart	2	162	<1	<1.1	<1.1	<1.1
163	Brand 3	Lucky 7 Supermart	2	163	<1	<1.1	<1.1	<1.1
164	Brand 3	Lucky 7 Supermart	2	164	<1	<1.1	<1.1	<1.1
165	Brand 3	Lucky 7 Supermart	2	165	<1	<1.1	<1.1	<1.1
166	Guadalupe	H5	2	166	<1	<1.1	<1.1	<1.1
167	Guadalupe	H5	2	167	<1	<1.1	<1.1	<1.1
168	Guadalupe	H5	2	168	<1	<1.1	<1.1	<1.1
169	Guadalupe	H5	2	169	<1	<1.1	<1.1	<1.1
170	Guadalupe	H5	2	170	<1	<1.1	<1.1	<1.1
171	Lahug	H6	2	171	<1	<1.1	<1.1	<1.1
172	Lahug	H6	2	172	<1	<1.1	<1.1	<1.1
173	Lahug	H6	2	173	<1	<1.1	<1.1	<1.1
174	Lahug	H6	2	174	<1	<1.1	<1.1	<1.1
175	Lahug	H6	2	175	<1	<1.1	<1.1	<1.1
176	Brand 4	Lucky 7 Supermart	2	176	<1	<1.1	<1.1	<1.1
177	Brand 4	Lucky 7 Supermart	2	177	<1	<1.1	<1.1	<1.1
178	Brand 4	Lucky 7 Supermart	2	178	<1	<1.1	<1.1	<1.1
179	Brand 4	Lucky 7 Supermart	2	179	<1	<1.1	<1.1	<1.1
180	Brand 4	Lucky 7 Supermart	2	180	<1	<1.1	<1.1	<1.1
181	Brand 5	Lucky 7 Supermart	2	181	<1	<1.1	<1.1	<1.1
182	Brand 5	Lucky 7 Supermart	2	182	<1	<1.1	<1.1	<1.1
183	Brand 5	Lucky 7 Supermart	2	183	<1	<1.1	<1.1	<1.1
184	Brand 5	Lucky 7 Supermart	2	184	<1	<1.1	<1.1	<1.1
185	Brand 5	Lucky 7 Supermart	2	185	<1	<1.1	<1.1	<1.1
186	Brand 6	Cristy Sari-Sari Store	2	186	380	<1.1	<1.1	<1.1
187	Brand 6	Cristy Sari-Sari Store	2	187	110	<1.1	<1.1	<1.1
188	Brand 6	Cristy Sari-Sari Store	2	188	190	<1.1	<1.1	<1.1
189	Brand 6	Cristy Sari-Sari Store	2	189	190	<1.1	<1.1	<1.1
190	Brand 6	Cristy Sari-Sari Store	2	190	410	<1.1	<1.1	<1.1
191	Punta Princesa	H7	2	191	<1	<1.1	<1.1	<1.1

192	Punta Princesa	H7	2	192	<1	<1.1	<1.1	<1.1
193	Punta Princesa	H7	2	193	<1	<1.1	<1.1	<1.1
194	Punta Princesa	H7	2	194	<1	<1.1	<1.1	<1.1
195	Punta Princesa	H7	2	195	<1	<1.1	<1.1	<1.1
196	Mabolo	H8	2	196	<1	<1.1	<1.1	<1.1
197	Mabolo	H8	2	197	<1	<1.1	<1.1	<1.1
198	Mabolo	H8	2	198	<1	<1.1	<1.1	<1.1
199	Mabolo	H8	2	199	<1	<1.1	<1.1	<1.1
200	Mabolo	H8	2	200	<1	<1.1	<1.1	<1.1
201	Brand 1	Metro Ayala	3	201	<1	<1.1	<1.1	<1.1
202	Brand 1	Metro Ayala	3	202	<1	<1.1	<1.1	<1.1
203	Brand 1	Metro Ayala	3	203	<1	<1.1	<1.1	<1.1
204	Brand 1	Metro Ayala	3	204	<1	<1.1	<1.1	<1.1
205	Brand 1	Metro Ayala	3	205	<1	<1.1	<1.1	<1.1
206	Brand 2	Metro Ayala	3	206	<1	<1.1	<1.1	<1.1
207	Brand 2	Metro Ayala	3	207	<1	<1.1	<1.1	<1.1
208	Brand 2	Metro Ayala	3	208	<1	<1.1	<1.1	<1.1
209	Brand 2	Metro Ayala	3	209	<1	<1.1	<1.1	<1.1
210	Brand 2	Metro Ayala	3	210	<1	<1.1	<1.1	<1.1
211	Brand 3	Metro Ayala	3	211	<1	<1.1	<1.1	<1.1
212	Brand 3	Metro Ayala	3	212	<1	<1.1	<1.1	<1.1
213	Brand 3	Metro Ayala	3	213	<1	<1.1	<1.1	<1.1
214	Brand 3	Metro Ayala	3	214	<1	<1.1	<1.1	<1.1
215	Brand 3	Metro Ayala	3	215	<1	<1.1	<1.1	<1.1
216	Guadalupe	H1	3	216	<1	<1.1	<1.1	<1.1
217	Guadalupe	H1	3	217	<1	<1.1	<1.1	<1.1
218	Guadalupe	H1	3	218	<1	<1.1	<1.1	<1.1
219	Guadalupe	H1	3	219	<1	<1.1	<1.1	<1.1
220	Guadalupe	H1	3	220	<1	<1.1	<1.1	<1.1
221	Lahug	H2	3	221	<1	<1.1	<1.1	<1.1
222	Lahug	H2	3	222	<1	<1.1	<1.1	<1.1
223	Lahug	H2	3	223	<1	<1.1	<1.1	<1.1
224	Lahug	H2	3	224	<1	<1.1	<1.1	<1.1
225	Lahug	H2	3	225	<1	<1.1	<1.1	<1.1
226	Brand 1	Lucky 7 Supermart	3	226	<1	<1.1	<1.1	<1.1
227	Brand 1	Lucky 7 Supermart	3	227	<1	<1.1	<1.1	<1.1

228	Brand 1	Lucky 7 Supermart	3	228	<1	<1.1	<1.1	<1.1
229	Brand 1	Lucky 7 Supermart	3	229	<1	<1.1	<1.1	<1.1
230	Brand 1	Lucky 7 Supermart	3	230	<1	<1.1	<1.1	<1.1
231	Brand 2	Lucky 7 Supermart	3	231	<1	<1.1	<1.1	<1.1
232	Brand 2	Lucky 7 Supermart	3	232	<1	<1.1	<1.1	<1.1
233	Brand 2	Lucky 7 Supermart	3	233	<1	<1.1	<1.1	<1.1
234	Brand 2	Lucky 7 Supermart	3	234	<1	<1.1	<1.1	<1.1
235	Brand 2	Lucky 7 Supermart	3	235	<1	<1.1	<1.1	<1.1
236	Brand 3	Lucky 7 Supermart	3	236	<1	<1.1	<1.1	<1.1
237	Brand 3	Lucky 7 Supermart	3	237	<1	<1.1	<1.1	<1.1
238	Brand 3	Lucky 7 Supermart	3	238	<1	<1.1	<1.1	<1.1
239	Brand 3	Lucky 7 Supermart	3	239	<1	<1.1	<1.1	<1.1
240	Brand 3	Lucky 7 Supermart	3	240	<1	<1.1	<1.1	<1.1
241	Punta Princesa	H3	3	241	<1	<1.1	<1.1	<1.1
242	Punta Princesa	H3	3	242	<1	<1.1	<1.1	<1.1
243	Punta Princesa	H3	3	243	<1	<1.1	<1.1	<1.1
244	Punta Princesa	H3	3	244	<1	<1.1	<1.1	<1.1
245	Punta Princesa	H3	3	245	<1	<1.1	<1.1	<1.1
246	Mabolo	H4	3	246	<1	<1.1	<1.1	<1.1
247	Mabolo	H4	3	247	<1	<1.1	<1.1	<1.1
248	Mabolo	H4	3	248	<1	<1.1	<1.1	<1.1
249	Mabolo	H4	3	249	<1	<1.1	<1.1	<1.1
250	Mabolo	H4	3	250	<1	<1.1	<1.1	<1.1
251	Guadalupe	H5	3	251	<1	<1.1	<1.1	<1.1
252	Guadalupe	H5	3	252	<1	<1.1	<1.1	<1.1
253	Guadalupe	H5	3	253	<1	<1.1	<1.1	<1.1
254	Guadalupe	H5	3	254	<1	<1.1	<1.1	<1.1
255	Guadalupe	H5	3	255	<1	<1.1	<1.1	<1.1
256	Lahug	H6	3	256	<1	<1.1	<1.1	<1.1
257	Lahug	H6	3	257	<1	<1.1	<1.1	<1.1
258	Lahug	H6	3	258	<1	<1.1	<1.1	<1.1
259	Lahug	H6	3	259	<1	<1.1	<1.1	<1.1

260	Lahug	H6	3	260	<1	<1.1	<1.1	<1.1
261	Punta Princesa	H7	3	261	<1	<1.1	<1.1	<1.1
262	Punta Princesa	H7	3	262	<1	<1.1	<1.1	<1.1
263	Punta Princesa	H7	3	263	<1	<1.1	<1.1	<1.1
264	Punta Princesa	H7	3	264	<1	<1.1	<1.1	<1.1
265	Punta Princesa	H7	3	265	<1	<1.1	<1.1	<1.1
266	Mabolo	H8	3	266	<1	<1.1	<1.1	<1.1
267	Mabolo	H8	3	267	<1	<1.1	<1.1	<1.1
268	Mabolo	H8	3	268	<1	<1.1	<1.1	<1.1
269	Mabolo	H8	3	269	<1	<1.1	<1.1	<1.1
270	Mabolo	H8	3	270	<1	<1.1	<1.1	<1.1

Legend:

HPC – Heterotrophic plate count

CC – Total coliform count

TTCC – Thermotolerant coliform count (previously known as faecal coliform count)

ECC – E. coli count

Cfu – Colony-forming units

MPN – Most probable number

Appendix 10 - Statistical modelling of the contaminated bottled water brand through multivariable Poisson regression analysis using R-studio software

Output from the R-studio computer software

```
> #### read datafile
> countdata<-read.table(file="hpc.csv",sep=",",header=TRUE)
> countdata <- countdata[,-10] # Remove trial from the hpc dataset
> # countdata<-read.table(file="tcc.csv",sep=",",header=TRUE) # only
found in tap water, therefore don't use this dataset in the analysis
> # countdata<-read.table(file="thermotc.csv",sep=",",header=TRUE) #
only found in tap water, therefore don't use this dataset in the
analysis
> # countdata<-read.table(file="ecoli.csv",sep=",",header=TRUE) # only
found in tap water, therefore don't use this dataset in the analysis
## Subset the data for only bottled water of brand B5
> countdata_bottle<-subset(countdata,
countdata$Type_delivery=="bottled")
> countdata_bottle_Brand<-subset(countdata,
countdata$Brand_Village_Code=="B6")
> mydata <- countdata_bottle_Brand[,c(3,5,6,7,8,9,10)]
> # Resetting the levels of each factor to only include the levels
seen in the subset of data
> mydata$Batch_Code <- as.character(mydata$Batch_Code)
> mydata$Batch_Code <- as.factor(mydata$Batch_Code)
> mydata$Store_House_hold_Code <-
as.character(mydata$Store_House_hold_Code)
> mydata$Store_House_hold_Code <-
as.factor(mydata$Store_House_hold_Code)
> mydata$Brand_Village_Code <- as.character(mydata$Brand_Village_Code)
> mydata$Brand_Village_Code <- as.factor(mydata$Brand_Village_Code)
> mydata$Bottle_Tap_Code <- as.character(mydata$Bottle_Tap_Code)
> mydata$Bottle_Tap_Code <- as.factor(mydata$Bottle_Tap_Code)
> mydata$Occasion <- as.factor(mydata$Occasion)
>
> names(mydata) <- c("Bottle", "Brand", "Store", "Occasion", "Batch",
"Counts", "sample_vol")
> ## Load the libraries needed to do the analysis
> library(MASS)
> library(lme4)

> ## Analysis using only Brand B6
 > ## Model with no covariates in them
> mod1 <- glmer(Counts ~ 1 + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)  #random intercept for bottle
> summary(mod1)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ 1 + (1 | Bottle)
   Data: mydata
      AIC       BIC    logLik  deviance
 788.4498  794.2751 -392.2249  784.4498

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 1.804    1.343
Number of obs: 136, groups: Bottle, 20
```

```
Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   5.9820     0.3008   19.89   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> ## Models with a covariate in them

> mod2a <- glmer(Counts ~ Batch + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)
> summary(mod2a)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ Batch + (1 | Bottle)
   Data: mydata

      AIC       BIC    logLik  deviance
 749.2809  763.8441 -369.6404  739.2809

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 0.1829   0.4276
Number of obs: 136, groups: Bottle, 20

Fixed effects:
                        Estimate Std. Error z value Pr(>|z|)
(Intercept)               7.1110     0.1765   40.28  < 2e-16 ***
Batch123115               0.1692     0.2787    0.61    0.544
BatchB6NOBATCH:15092013  -1.7198     0.2628   -6.55 5.94e-11 ***
BatchB6NOBATCH:18082013  -2.9318     0.2631  -11.14  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
             (Intr) B12311 BB6NOBATCH:15
Batch123115   -0.633
BB6NOBATCH:15 -0.672  0.426
BB6NOBATCH:18 -0.671  0.425  0.451

## Interpretation of the model:
## At least one of the batches is significantly different to the
baseline batch. Occasion is not significant (model 3a) so has been
removed.

> mod2b <- glmer(Counts ~ Occasion + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
> summary(mod2b)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ Occasion + (1 | Bottle)
   Data: mydata

      AIC       BIC    logLik  deviance
 788.9050  797.6430 -391.4525  782.9050

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 1.67     1.292
Number of obs: 136, groups: Bottle, 20
```

```
Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   5.6153     0.4094  13.716   <2e-16 ***
Occasion2     0.7334     0.5789   1.267    0.205
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
          (Intr)
Occasion2 -0.707
>
> mod2c <- glmer(Counts ~ Store + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)
> summary(mod2c)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ Store + (1 | Bottle)
   Data: mydata

      AIC       BIC    logLik  deviance
 759.1600  767.8979 -376.5800  753.1600

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 0.3691   0.6075
Number of obs: 136, groups: Bottle, 20

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   7.1772     0.1931   37.17   <2e-16 ***
StoreS4      -2.3909     0.2737   -8.74   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
        (Intr)
StoreS4 -0.706

## There are too many combinations of covariates to try models with
them in all combinations
## A model with Batch and Store in it will not fit because there are
no batches that occurred in more than 1 store

> table(mydata$Batch, mydata$Store)

                     S2 S4
  123114             48  0
  123115             32  0
  B6NOBATCH:15092013  0 40
  B6NOBATCH:18082013  0 40

## Models with 2 covariates

> mod3a <- glmer(Counts ~ Batch + Occasion + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
> summary(mod3a)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ Batch + Occasion + (1 | Bottle)
   Data: mydata
```

```
      AIC       BIC    logLik  deviance
 750.8799  768.3558 -369.4400  738.8799

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 0.1792   0.4233
Number of obs: 136, groups: Bottle, 20

Fixed effects:
                        Estimate Std. Error z value Pr(>|z|)
(Intercept)              7.06114    0.19159   36.85  < 2e-16 ***
Batch123115             -0.07856    0.47712   -0.16    0.869
BatchB6NOBATCH:15092013 -1.96781    0.46820   -4.20 2.64e-05 ***
BatchB6NOBATCH:18082013 -2.88177    0.27213  -10.59  < 2e-16 ***
Occasion2                0.29762    0.46773    0.64    0.525
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
             (Intr) B12311 BB6NOBATCH:15 BB6NOBATCH:18
Batch123115   0.000
BB6NOBATCH:15  0.000  0.815
BB6NOBATCH:18 -0.704  0.000  0.000
Occasion2     -0.410 -0.816 -0.831         0.288

## Batch should go in the model first because the bottle are sorted
into batches before you decide to purchase them on the occasions.
Otherwise, batch can only explain the variation not already explained
by Occasion.

> mod3b <- glmer(Counts ~ Occasion + Batch + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
> summary(mod3b)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ Occasion + Batch + (1 | Bottle)
   Data: mydata

      AIC       BIC    logLik  deviance
 750.8799  768.3558 -369.4400  738.8799

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 0.1792   0.4233
Number of obs: 136, groups: Bottle, 20

Fixed effects:
                        Estimate Std. Error z value Pr(>|z|)
(Intercept)              7.06114    0.19159   36.85  < 2e-16 ***
Occasion2                0.29762    0.46773    0.64    0.525
Batch123115             -0.07856    0.47712   -0.16    0.869
BatchB6NOBATCH:15092013 -1.96781    0.46820   -4.20 2.64e-05 ***
BatchB6NOBATCH:18082013 -2.88177    0.27213  -10.59  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
             (Intr) Occsn2 B12311 BB6NOBATCH:15
Occasion2     -0.410
Batch123115    0.000 -0.816
BB6NOBATCH:15  0.000 -0.831  0.815
```

```
BB6NOBATCH:18 -0.704  0.288  0.000  0.000

## Occasion is not significant in either model with Batch (or in the
models on its own).

> mod3c <- glmer(Counts ~ Occasion + Store + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
> summary(mod3c)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ Occasion + Store + (1 | Bottle)
   Data: mydata

      AIC       BIC    logLik  deviance
 752.5240  764.1746 -372.2620  744.5240

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 0.2387   0.4886
Number of obs: 136, groups: Bottle, 20

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   6.8158     0.1911   35.67  < 2e-16 ***
Occasion2     0.7227     0.2209    3.27  0.00107 **
StoreS4      -2.3913     0.2209  -10.82  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
          (Intr) Occsn2
Occasion2 -0.580
StoreS4   -0.574  0.000
>
> mod3d <- glmer(Counts ~ Store + Occasion + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
> summary(mod3d)
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Counts ~ Store + Occasion + (1 | Bottle)
   Data: mydata

      AIC       BIC    logLik  deviance
 752.5240  764.1746 -372.2620  744.5240

Random effects:
 Groups Name        Variance Std.Dev.
 Bottle (Intercept) 0.2387   0.4886
Number of obs: 136, groups: Bottle, 20

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   6.8158     0.1911   35.67  < 2e-16 ***
StoreS4      -2.3913     0.2209  -10.82  < 2e-16 ***
Occasion2     0.7227     0.2209    3.27  0.00107 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
          (Intr) StorS4
StoreS4   -0.574
```

```
Occasion2 -0.580  0.000

## When batch is not included in the model, store and occasion
together give the batch info (ie for one store and one occasion, there
is approximately is one batch).

> table(mydata$Batch, mydata$Occasion, mydata$Store)
, ,  = S2


                     1  2
  123114            40  8
  123115             0 32
  B6NOBATCH:15092013  0  0
  B6NOBATCH:18082013  0  0

, ,  = S4


                     1  2
  123114             0  0
  123115             0  0
  B6NOBATCH:15092013  0 40
  B6NOBATCH:18082013 40  0
```

The anova's show that model 2a is best (Counts ~ Batch + (1 | Bottle)); model 3a is not significantly better than model 2a; and when batch is only included in the third model (comparing models 2b and 3b), the 3rd model is significant, indicating that Occasion does not need to be in the model.

```
> anova(mod1, mod2a, mod3a)
Data: mydata
Models:
mod1: Counts ~ 1 + (1 | Bottle)
mod2a: Counts ~ Batch + (1 | Bottle)
mod3a: Counts ~ Batch + Occasion + (1 | Bottle)
      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
mod1   2 788.45 794.28 -392.22   784.45
mod2a  5 749.28 763.84 -369.64   739.28 45.169      3  8.519e-10 ***
mod3a  6 750.88 768.36 -369.44   738.88  0.401      1     0.5266
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> anova(mod1, mod2b, mod3a)
Data: mydata
Models:
mod1: Counts ~ 1 + (1 | Bottle)
mod2b: Counts ~ Occasion + (1 | Bottle)
mod3a: Counts ~ Batch + Occasion + (1 | Bottle)
      Df    AIC    BIC  logLik deviance   Chisq Chi Df Pr(>Chisq)
mod1   2 788.45 794.28 -392.22   784.45
mod2b  3 788.91 797.64 -391.45   782.91  1.5447      1     0.2139
mod3a  6 750.88 768.36 -369.44   738.88 44.0251      3  1.491e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
-----------------------------------------------------------------------
----

Computer commands to load the statistical models using R-studio

#### Set working directory###
setwd("C:/Documents and Settings/bybanez/My Documents/bryan
2013/massey u notes 2013 THESIS/microbiology protocols")
#### read datafile
countdata<-read.table(file="hpc.csv",sep=",",header=TRUE)
countdata <- countdata[,-10] # Remove trial from the hpc dataset
# countdata<-read.table(file="tcc.csv",sep=",",header=TRUE) # only
found in tap water, therefore don't use this dataset in the analysis
# countdata<-read.table(file="thermotc.csv",sep=",",header=TRUE) #
only found in tap water, therefore don't use this dataset in the
analysis
# countdata<-read.table(file="ecoli.csv",sep=",",header=TRUE) # only
found in tap water, therefore don't use this dataset in the analysis
head(countdata)
names(countdata)
summary(countdata)
# countdata[which(countdata[,9]>0),] # use to find the rows with
positive counts
countdata_bottle<-subset(countdata,
countdata$Type_delivery=="bottled")
countdata_bottle_Brand<-subset(countdata,
countdata$Brand_Village_Code=="B6")
mydata <- countdata_bottle_Brand[,c(3,5,6,7,8,9,10)]
# Resetting the levels of each factor to only include the levels seen
in the subset of data
mydata$Batch_Code <- as.character(mydata$Batch_Code)
mydata$Batch_Code <- as.factor(mydata$Batch_Code)
mydata$Store_House_hold_Code <-
as.character(mydata$Store_House_hold_Code)
mydata$Store_House_hold_Code <-
as.factor(mydata$Store_House_hold_Code)
mydata$Brand_Village_Code <- as.character(mydata$Brand_Village_Code)
mydata$Brand_Village_Code <- as.factor(mydata$Brand_Village_Code)
mydata$Bottle_Tap_Code <- as.character(mydata$Bottle_Tap_Code)
mydata$Bottle_Tap_Code <- as.factor(mydata$Bottle_Tap_Code)
mydata$Occasion <- as.factor(mydata$Occasion)

names(mydata) <- c("Bottle", "Brand", "Store", "Occasion", "Batch",
"Counts", "sample_vol")
summary(mydata)
str(mydata)
head(mydata)

library(MASS)
library(lme4)

## Analysis using only Brand B6
## remove factor levels not present in this data set
# mydata1 <- subset(mydata, mydata$Brand=="B6")
# mydata1$Brand <- as.character(mydata1$Brand)
# mydata1$Brand <- as.factor(mydata1$Brand)
# mydata1$Store <- as.character(mydata1$Store)
# mydata1$Store <- as.factor(mydata1$Store)
```

```
# mydata1$Batch <- as.character(mydata1$Batch)
# mydata1$Batch <- as.factor(mydata1$Batch)

summary(mydata)
str(mydata)
head(mydata)

mod1 <- glmer(Counts ~ 1 + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)  #random intercept for bottle
summary(mod1)
##
mod2a <- glmer(Counts ~ Batch + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)
summary(mod2a)

mod2b <- glmer(Counts ~ Occasion + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)
summary(mod2b)

mod2c <- glmer(Counts ~ Store + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)
summary(mod2c)

##
## Cant put batch and store into the same model as no batches were
found at 2 stores

mod3a <- glmer(Counts ~ Batch + Occasion + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
summary(mod3a)

mod3b <- glmer(Counts ~ Occasion + Batch + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
summary(mod3b)

mod3c <- glmer(Counts ~ Occasion + Store + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
summary(mod3c)

mod3d <- glmer(Counts ~ Store + Occasion + (1|Bottle),
offset=log(sample_vol), family=poisson, data=mydata)
summary(mod3d)

# Batch is significant, occasion is not
anova(mod1, mod2a, mod3a)
anova(mod1, mod2b, mod3a)


## Final Model

mod2a <- glmer(Counts ~ Batch + (1|Bottle), offset=log(sample_vol),
family=poisson, data=mydata)
summary(mod2a)

table(mydata$Batch, mydata$Occasion, mydata$Store)
```