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A STUDY OF THE MICROBIOLOGICAL QUALITY OF BOTTLED WATER SOLD IN NEW ZEALAND

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF
PHILOSOPHY



"Forget lemonade. The real money's in bottled water."

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ABSTRACT

The aim of this study was to determine if retailed bottled water in New Zealand complied with the Australia and New Zealand Food Standards (ANZFS) Code (2002) and the New Zealand Microbiological Reference Criteria (1995).

The New Zealand Microbiological Reference Criteria for Packaged Waters include Total Coliforms, *Escherichia coli*, Enterococci (Group D streptococci) and *Pseudomonas aeruginosa*. Standard 1.6.1 of the ANZFS includes Total Coliforms, *Escherichia coli*, Enterococci (Group D streptococci), *Pseudomonas aeruginosa* and Total Viable Count (TVC).

In this study five samples of randomly selected 38 brands of different types of domestic and imported bottled waters were purchased from local retail stores in Wellington region. The samples were tested for Total Coliforms, *Escherichia coli*, Enterococci (Group D streptococci), *Pseudomonas aeruginosa*, TVC, Yeasts and Moulds and *Campylobacter spp.*

Three domestic brands did not comply with both of the above criteria for Total Coliforms.

Seventeen brands did not comply with TVC criteria of ANZFS Code (100 CFU/ml) with nine out of 17 being domestic New Zealand brands.

Twenty one brands displayed the growth of colonies on Sabouraud dextrose agar plates. Half of them displayed the growth of moulds.

Due to high incidence of campylobacteriosis in New Zealand composite samples of brands with TVC counts equal or higher than 100 CFU/ml were tested for *Campylobacter spp.* All samples were negative for *Campylobacter spp.*

A survey questionnaire was used to assess the impact of manufacturing procedures on bottled water quality. The aim of the survey was to investigate possible significant public health links between the source water quality, type of abstraction, pipework materials, bottling process, staff training, policies and procedures. All four manufacturers, which responded to the questionnaire and represented 11 bottled water brands, bottled at least one brand that did not comply with the ANZFS Code for TVC.

Ten years after the initial study was performed by Hasell and Capill in 1999 microbiological contamination in bottled waters in New Zealand was still being detected. We demonstrated that monitoring bottled water microbiological quality was essential and that the presence of manufacturers' procedures for ensuring satisfactory bottled water microbiological quality did not always guarantee it.

PREFACE

“Everything comes from water!

And everything is kept alive by water!”

J.W. von Goethe, *Faust II*, 1833

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DEDICATION

I dedicate my work to everyone who believed in me.

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CHAPTER ONE- INTRODUCTION

Water is essential for the majority of the body functions, to maintain a healthy lifestyle and is especially important for thermoregulation (EFSA, 2010). In addition to thermoregulation, water also protects and cushions our vital organs and is required for breathing and transporting nutrients and oxygen throughout the body (www.bottledwater.org.au). Water is the main constituent of the human body, comprising around 60% of body weight in adult males, 50 to 55% in females and 75% in newborn infants is water. However this varies depending on body constitution (EFSA, 2010). Our bodies obtain water from a variety of sources, such as drinking water (tap and bottled water), beverages, moisture content of foods and water produced by oxidative processes in the body (EFSA, 2010).

It is estimated that approximately two litres of water per day should be consumed by a 60 kg person and one litre per day for a 10 kg child (WHO, 2000). However, it is dependant upon climate, physical activity and culture.

Good health is dependent upon clean, potable (drinkable) water. This means that water must be free of pathogens, dissolved toxins, and disagreeable turbidity, odour, colour and taste (Talaro, 1999). If this is not ensured, then outbreaks can occur. Two examples of waterborne outbreaks were an epidemic of cholera, where thousands of people were killed due to the consumption of water contaminated with *Vibrio cholerae* bacteria in South America (Blake et al., 1974) and an outbreak of *Cryptosporidium spp.* in Wisconsin, USA, which affected 370 000 people (Talaro, 1999). The latter outbreak was traced to a contaminated community drinking water supply.

United Nations Conference on Environment and Development (UNCED) in 1992 recommended nominating an international day to celebrate freshwater. Since 1993 the International World Water Day is held every year on 22 March. The purpose of this day is to raise the awareness of freshwater and to advocate the sustainable management of freshwater resources. Every year the World

Water Day raises awareness of a specific aspect of freshwater. (<http://www.unwater.org/worldwaterday/about.html>).

Drinking water can be sourced from surface water and from ground water. Surface water includes rivers, lakes, springs and reservoirs. Ground water is pumped from wells or bores that are drilled into aquifers (www.excelwater.com).

Drinking Water Standards for New Zealand 2005 (DWSNZ) state that a fundamental requirement for public health is safe drinking water available to everyone. Confidence in the public health safety of water is increased if multiple barriers to contamination are in place (DWSNZ). These barriers include protection of source waters to minimise the number of pollutants of public health significance entering the water source. Any pollutants of the source water must then be dealt with by the complex staged treatment processes, for example filtration to remove particulate matter, disinfection to inactivate any pathogenic organisms present and protection of treated water from subsequent contamination.

Concerns about pollution and presence of pathogenic bacteria in drinking water have prompted many people to turn to bottled water as a substitute for ordinary tap water (<http://www.articlesbase.com>). What first started as a trend is now a profit making worldwide industry.

1.1. Bottled Water Quality

The quality of bottled and packaged waters may vary considerably since bottled waters are not subjected to extensive quality standards, unlike municipal water supplies. A variety of organisms have been recovered from bottled water. Recovery of *Staphylococcus aureus* and *Aeromonas hydrophila* from bottled water has caused concerns about its safety over the last thirty years (Guo-Jane Tsai & Shou-Chin Yu, 1997). Beuret et al. (2002) described the detection of Ribonucleic acid (RNA) with nucleotide sequences specific for “Norwalk-like viruses” in European bottled mineral water.

Bottled water is drinking water that meets all relevant standards, is sealed in a container and sold for human consumption. Bottled water is consumed by people of all age groups and various occupations. Generally bottled water consumers may be perceived as being more health conscious, contemporary and socially aware. Some people choose to drink bottled water, because they want to avoid chemicals used in the treatment of public water supplies, others do it purely for convenience and some people choose to buy bottled water because of its taste. Bottled water may be distributed in emergency relief operations, such as Asian Tsunami, cyclones in Queensland and other emergencies that have interrupted the delivery of safe drinking water. Bottled water is supplied to communities that lack safe and clean potable water around the world, such as in the events of earthquakes, flooding or military missions and operations.

Grant (1997) stated that consumption of bottled water was steadily increasing as a result of public concerns about palatability and microbial and chemical contaminants in tap water. European consumption of bottled water has increased by 200% between 1987 and 1997. Developing nations, such as China and Indonesia were projected to increase bottled water demand by 30% in the next 5 years following the research published in 1997 (Grant, 1997). Even though bottled water is often perceived as a sterile product, water obtained even from a deep aquifer (a water bearing underground layer of rock or sand) may contain microorganisms at levels as high as 10^7 CFU/ml (Grant, 1997). The microflora in source water may also increase after bottling, typically reaching maximum levels after one week. Personnel hygiene practices in bottling plants have also been shown to contribute to contamination of bottled water (Grant, 1997). Grant (1997) stated, that out of 104 brands of retail bottled water from 10 countries (container sizes ranged from 296 ml to 3, 785 ml; 101 containers were plastic and 3 glass) tested, 36 different brands contained presumptive coliforms.

According to the most recently available International Bottled Water Association statistical data (Table 1-1) that had been obtained from the Beverage Marketing

Corporation, the consumption of bottled water worldwide in 2002-2007 had increased by 7.6% (www.bottledwater.org).

Table 1- 1. Global Bottled Water Market - Leading Countries' Consumption and Compound Annual Growth Rates 2002 – 2007.

2007 rank	Countries	Millions of gallons		CAGR*
		2002	2007	2002/07
1	United States	5,795.6	8,823.0	8.8%
2	Mexico	3,898.6	5,885.2	8.6%
3	China	2,138.4	4,787.8	17.5%
4	Brazil	2,541.8	3,621.1	7.3%
5	Italy	2,558.2	3,100.9	3.9%
6	Germany	2,291.5	2,743.2	3.7%
7	Indonesia	1,622.5	2,400.6	8.2%
8	France	2,225.6	2,283.2	0.5%
9	Thailand	1,277.0	1,533.1	3.7%
10	Spain	1,191.4	1,284.0	1.5%
	Top 10 Subtotal	25,540.7	36,462.2	7.4%
	All Others	9,054.2	13,407.3	8.2%
	World total	34,594.9	49,869.6	7.6%

* Compound annual growth rate.

The Beverage Marketing Corporation (www.bottledwater.org) also compared the annual bottled water consumption per person in gallons in leading countries (Table 1-2).

Table 1-2. Global Bottled Water Market - Per Capita Consumption by Leading Countries.

2007 rank	Countries	Gallons per capita	
		2002	2007
1	United Arab Emirates	35.2	68.6
2	Mexico	37.7	54.1
3	Italy	44.2	53.3
4	Belgium-Luxembourg	32.7	39.5
5	France	37.1	35.8
6	Germany	27.8	33.3
7	Spain	29.7	31.7
8	Lebanon	24.9	29.3
9	United States	20.1	29.0
10	Hungary	13.5	28.5
11	Switzerland	24.2	28.2
12	Slovenia	18.8	25.2
13	Austria	20.9	25.0
14	Czech Republic	21.1	24.6
15	Croatia	14.9	24.3
16	Saudi Arabia	23.8	24.1
17	Cyprus	21.4	24.0
18	Thailand	20.1	23.6
19	Israel	12.4	23.2
20	Portugal	19.9	22.4
	Global average	5.6	7.6

While the term *bottled water* is widely used, the term *packaged water* is perhaps more accurate as water sold for human consumption can come in cans, laminated boxes and even plastic bags. However, bottled water is most commonly sold in glass or disposable plastic bottles. Bottled water also comes

in various sizes from single servings to large carboys holding up to 80 litres (WHO, 2000).

All bottled waters sold for drinking in the UK are safe to consume. However, in order to make an informed choice about what it is that you are drinking, it is useful to understand the differences between the various categories of bottled water, which are described in the Table 1-3 below.

Table 1-3. Main Types of Bottled Water.

Type of bottled water	Description*
Spring water	<p>Must be derived from an underground source from which water flows naturally to the surface of the earth.</p> <p>Spring water must be collected only at the spring or through a borehole that taps into the aquifer feeding the spring. Spring water collected with the use of an external force must be from the same underground stratum as the spring and must have all the physical properties before treatment.</p> <p>The properties of the water drawn from the bore hole must be the same as that of the water in the spring. It must then be bottled at source and be microbiologically safe without treatment.</p> <p>In the UK certain treatments are permitted for spring waters.</p> <p>Treatments may include the removal of certain minerals as defined by the European Union Scientific Committee for Food to allow for the removal of undesirable substances.</p>
Purified Water	<p>It is produced through distillation, deionization, reverse osmosis or some other water treatment process. This water originates as either tap water or groundwater. Depending on the water treatment process used, other acceptable names include <i>distilled water</i>, <i>purified drinking water</i>, <i>distilled drinking water</i> and <i>deionized water</i>.</p>
Natural Mineral	<p>It must come from an identified and protected source, usually</p>

Water	<p>from a spring and has the minerals found only in the water as it flows from the ground. It is guaranteed to be consistent in its composition and naturally wholesome without any treatment, except in some cases with the addition of carbon dioxide to make the water sparkle.</p> <p>To be granted a 'Natural Mineral Water status' the water must be proven to be free from pollution and have a characteristic stable composition. If the product is not labelled <i>natural</i>, it means some minerals may have been added or removed.</p>
Mineral Water	<p>It contains more than 250 parts per million (ppm) of total dissolved solids (TDS) that are present at the point of coming out from the source. Minerals cannot be added to this water and it cannot be drawn from a municipal source.</p> <p>The International Bottled Water Association (IBWA) defines mineral water as bottled water that contains not less than 500 parts per million total dissolved solids.</p> <p>In Europe only a recognized spring water with minerals can be called mineral water.</p>
Sparkling/Carbonated Bottled Water	<p>This water contains the same amount of carbon dioxide that it contained when it was drawn from the source.</p> <p>Sparkling bottled waters may be labelled as <i>sparkling drinking water</i>, <i>sparkling mineral water</i> or <i>sparkling spring water</i>.</p>
Artesian Water/Artesian Well Water	<p>It is drawn from a well that taps into a confined aquifer in which the water level stands at some height above the top of the aquifer.</p>
Well Water	<p>This water is comes from a hole bored or drilled that taps the water of an aquifer. It must be pumped to the surface.</p>
Tap water	<p>Municipal water piped into buildings.</p>
Table water	<p>Bottled filtered tap water.</p>

* Information from (www.bottleyourbrand.com, www.britishbottledwater.org, www.myspringwater.com, www.norlandintl.com, www.premiumrefreshment.com)

1.1.1. Worldwide Literature Review

A number of studies have shown that bacteria isolated from waterfowl droppings, such as *Campylobacter*, *Salmonella*, *Escherichia* and *Aeromonas*, have the potential for human pathogenicity which can lead to infection and disease, such as diarrhoea or gastroenteritis. (Gould et al., 1978; Jones et al., 1978; Hussong et al., 1979; Levesque et al., 1993; Hatch, 1996; Clarke et al., 1998). With regard to protozoal pathogens, studies by Graczyk et al. (1996; 1998) provided clear evidence that waterfowl can distribute *Giardia* cysts and *Cryptosporidium* oocysts in the environment and that these protozoa in water may have epidemiological implications. Outbreaks due to the consumption of contaminated water containing *E.coli* 0157:H7 have occurred in USA (Swerdlow et al., 1992; Keene et al., 1994), South Africa, Swaziland (Isaacson et al., 1993) and Scotland (Dev et al., 1991). While many of these outbreaks were related to the consumption of contaminated surface waters, currently there is an increasing concern that the entry of this microorganism into groundwater supplies may pose risks in relation to the consumption of bottled waters (Kerr et al., 1999).

Bottled water has been known to be a source of *Vibrio cholerae* (Blake et al., 1974), *Salmonella spp* (Palmera-Suárez et al., 2007) and Norovirus (Beuret et al., 2002). Blake et al. (1974) described transmission of *Vibrio cholerae* by bottled mineral water, where the organism was isolated from two springs which supplied mineral water to a spa and to a commercial water bottling plant. Palmera-Suárez et al. (2007) described the first published outbreak of *Salmonella Kottbus* that was associated with commercial bottled water in Spain and Europe. The latter study found out that pigeons frequently visited the water reservoirs that supplied the local factory. *Salmonella Kottbus* was detected in bottles randomly selected from markets and in the local bottling factory in the island of Gran Canaria. *Salmonella spp.* was detected in the pigeons. Both studies were case-control studies that studied non-carbonated waters. Blake et al. (1974) demonstrated that bacteriologically confirmed cholera cases had a history of consuming bottled non-carbonated water. The results of the second study (Palmera-Suárez et al., 2007), led to the inspections by the Public Health

authorities, which then resulted in the closure of the bottling factory and recall of bottled water. In another study 11 brands of European mineral waters were found to contain nucleotide sequences specific for “Norwalk-like viruses” (currently called Norovirus) that causes more than 90% cases of acute viral gastroenteritis worldwide (Beuret et al., 2002) each year.

Warburton et al. (1986) examined 114 samples that represented five lots of domestic and imported mineral water brands. Although they did not detect any faecal coliforms or *E.coli*, it was observed that if mineral water was governed by the aerobic colony count standards for bottled water, five lots domestic and imported mineral water examined in this study (a total of 114 samples) would have been found to be unsatisfactory.

Bottled natural mineral waters are not as microbiologically ‘pure’ as some suppliers seem to claim (Hunter et al., 1987). The study carried out by Hunter et al. (1987) demonstrated, that carbonated waters were found to be of food quality, and surmised that this was most likely due to carbon dioxide’s antibacterial activities. On the other hand, the research in Taiwan (Guo-Jane Tsai & Shou-Chin Yu, 1997) was carried out on uncarbonated mineral waters. In this study 88 domestic and 48 imported samples were tested. While coliforms and faecal streptococci were not detected in any of the samples tested, two of domestic samples were found to be contaminated with *Aeromonas hydrophila* and four with *Pseudomonas aeruginosa*. The legally permitted level of Heterotrophic Plate Count (HPC) in Taiwan is 200 CFU/ml. The study found that 51.1% of domestic samples and 60.4% of imported samples failed to comply with this limit. There is no information available whether any changes to the legislation were implemented after this finding. The other species of bacteria isolated in the bottled water samples in this study were *Pseudomonas*, *Aeromonas*, *Flavobacterium*, *Pasteurella*, *Xanthomonas*, and *Staphylococcus*. In this study moulds were also detected in both, domestic (38.6 %) and imported (18.8%) samples. Sefcová H. (1998) found that limits of psychrophilic microorganisms were higher in still table water compared with carbonated water.

During 1995-2003 Venieri et al. (2006) studied the microbiological quality of 1,527 samples of bottled non-carbonated ('still') mineral water that represented 10 manufacturing companies in Greece. The samples were tested for coliforms, *Escherichia coli*, *Enterococcus spp.*, *Pseudomonas aeruginosa* and HPC (at 22°C and 37°C). Venieri et al. (2006) study found that 13.95% of tested bottled water samples did not comply with the Greek bottled water regulations. In addition to *P. aeruginosa*, other bacteria, such as *Pseudomonas spp.*, *Aeromonas spp.*, *Pasteurella spp.*, *Citrobacter spp.*, *Flavobacterium spp.*, *Providencia spp.* and *Enterococcus spp.* were isolated.

A number of studies had demonstrated that many factors, such as material of bottles, colour of bottles and the length of storage influence the microbiological quality of bottled water. Fewtrell et al. (1997) reported lower colony counts from glass bottles compared to plastic bottles. They also noted that the colour of containers affected the total colony counts. *Pseudomonas aeruginosa* was the most frequently detected microorganism in samples tested by Fewtrell et al. (1997). Mavridou (1992) findings were consistent with Fewtrell et al. (1997) and her study demonstrated that during storage larger numbers of bacteria grew in PVC rather than glass bottles. The largest number of bacteria grew in PVC bottles filled by hand. Massa et al. (1997) examined heterotrophic plate counts in 31 glass and 40 plastic (PVC) bottled mineral waters.

Several researchers studied the effects of storage in plastic and glass bottles on the microbiological quality of bottled waters. A quantitative study of bacterial populations in mineral water was carried out by Gonzalez et al. (1987). This study demonstrated that bacterial counts in samples collected from the spring source in sterile glass flasks and from the bottling factory in conventional plastic and glass containers after 3 days storage were much higher than those found in water obtained directly from the spring source.

Bischofberger et al. (1990) found that after 1 week of storage at 20°C higher numbers of colony counts were found in plastic bottles than glass bottles. Raj (2005) also examined the effects of time and storage temperature on bacterial growth in bottled waters. His study found that the bacterial counts in bottled

waters increased dramatically if bottles were stored for more than 48 hours at 37°C. Bischofberger et al. (1990) thought that the growth promotion by dissolved organic substances in the plastic bottles played only a minor role. Their study concluded that the difference of bacterial proliferation between the two types of bottles was caused by an inhibition of growth due to residues of cleaning detergents in the glass bottles. Raj (2005) found that bacterial growth was reduced under refrigeration compared with room temperature. While Bischofberger et al. (1990) and Raj (2005) did not describe the genera of bacteria isolated in their studies, the most frequently isolated genus in Gonzalez et al. (1987) study was *Caulobacter*, followed by *Sphaerotilus-Leptothrix*, *Acinetobacter calcoaceticus* and *Pseudomonas fluorescens*. *Pseudomonas putida*, *Arthrobacter spp.*, *Aeromonas hydrophilia*, and *Corynebacterium spp.* were isolated less frequently.

E.coli (Lal & Kaur, 2006; Bharath et al., 2003), coliforms (Olayemi, 1999; Zamberlan da Silva et al., 2008; Kassenga, 2007; Lal & Kaur, 2006; Bharath et al., 2003; Jeena et al., 2006), *P. aeruginosa* (Ogan 1992; Venieri et al., 2006), Enterococci (Venieri et al., 2006), HPC (Kassenga, 2007; Venieri et al., 2006; Bharath et al., 2003; Croci et al., 2001; Jeena et al., 2006; Saleh et al., 2008) and fungi (Ribeiro et al., 2006; Criado et al., 2004; Cabral 2002; Papapetropoulou et al., 1997; Lal and Kaur, 2006) have been detected in bottled waters in many countries around the world. Bottled water has been found to contain bacteria that are resistant to antibiotics (Jeena et al., 2006) and toxin producing fungi (Criado et al., 2004).

Olayemi (1999) examined spring waters packaged and hawked in cellophane in Nigeria. The study found that the majority of brands tested positive for the presence of coliform bacteria and concluded that 40% of the hawked water did not meet drinking water quality standards. The Trinidad study (Bharath et al., 2003) found that 5% of bottled water sold in Trinidad was unfit for human consumption. Those brands, all domestic, were found to contain coliforms and 1.5% of samples contained *E.coli*. Bottled waters sold in India were tested by Lal and Kaur (2006). They demonstrated that out of the 23 brands examined one brand of bottled water contained the presumptive coliforms and one brand

was positive for *E.coli* and therefore was unfit for human consumption due to the presence of *E.coli*. Kassenga (2007) detected total coliforms in 4.6% of brands and faecal coliform bacteria in 3.6% of brands tested in Tanzania. Grant (1998) analysed 104 brands of bottled water originating from 10 countries and detected presumptive coliform colonies in 5.8% of the bottled water samples tested. The presumptive coliforms had not been confirmed as true coliforms in subsequent analysis.

Another Indian study (Jeena et al., 2006) demonstrated a linear relationship between HPC and coliform bacteria. In this study out of 150 samples that represented 30 brands Jeena et al. (2006) found that 44% of the samples that displayed HPC counts between 100 and 1000 CFU/ml also tested positive for coliforms. 14% of samples examined in this study were positive for coliforms.

Some researchers compared bottled water quality with tap water quality. Breuer et al. (1990) concluded that bottled waters sold in Iowa were of the same microbiological quality standard as the typical drinking water from public drinking water supplies in the state. Zamberlan da Silva et al. (2008) compared bacteriological quality of municipal tap water with that of 20-L bottles of mineral water collected from water dispensers. This study found that 36.4% of the tap water samples from municipal water systems and 76.6% of the 20-L bottles of mineral water from water dispensers in Brazil were contaminated by at least one coliform per milliliter. While Breuer et al. (1990) cautioned that bottled waters may not be tested as frequently and for as many contaminants as public water supplies, the results of the Brazilian study are alarming.

Pseudomonas spp. were isolated in bottled water brands sold in several countries, such as Spain (Rivilla & Gonzalez (1988), Nigeria (Olayemi, 1999), Trinidad (Bharath et al., 2003), India (Lal and Kaur , 2006) and Greece (Venieri et al., 2006). 7.6% of samples tested in Trinidad by Bharath et al. (2003) contained *Pseudomonas spp.* and 13% of samples tested in the Indian study (Lal and Kaur, 2006) also tested positive for *Pseudomonas spp.* *Pseudomonas aeruginosa*, some of which are antibiotic resistant, have been isolated in bottled waters. *P. aeruginosa* was isolated in Nigeria (Ogan, 1992), Greece (Venieri et

al., 2006) and Spain (Rivilla & Gonzalez, 1988). Venieri et al. (2006) found that *P. aeruginosa* was the most frequently isolated microorganism in bottled non-carbonated ('still') mineral waters tested and the Nigerian study isolated antibiotic resistant *P. aeruginosa* from two brands of bottled water. Rivilla & Gonzalez (1988) found that all samples tested in their study did not comply with the European Economic Community (EEC), Food and Agriculture Organization of the United Nations (FAO), World Health Organisation (WHO) or the Spanish normative requirements. Therefore they recommended using testing for *Pseudomonas aeruginosa* as one of the parameters for determining bottled water quality.

The growth of heterotrophic bacteria has also been observed in bottled water brands. The difficulty with heterotrophic plate count (HPC) in bottled water is that this microbiological criterion is not regulated in some countries, such as USA. Contrary to USA, HPC values in bottled waters are governed in India. The study by Jeena et al. (2006) found that approximately 40% of the samples tested in her study exceeded the limit of 100 CFU/ml set by the Department of Health and by the Bureau of Indian Standards (BIS), Government of India.

Saleh et al. (2008) found that four out of the 35 brands of the bottled water samples analysed in Texas were found to be contaminated with heterotrophic bacteria. Massa et al. (1997) detected HPC in 31 glass and 40 plastic (PVC) Italian bottled mineral waters. Breuer et al. (1990) stressed that the presence of HPC of 100-500 CFU/ml, in 5 out of the 31 samples they tested, indicated a problem with the bacterial cleanliness of the samples. Breuer et al. (1990) thought that the lack of microbiological information about the source water, the treatment methods and the containers being used could have affected the interpretation of HPC results. This is one of the reasons why in my study I have chosen to look in more detail at the source water quality, treatment, transportation, materials of bottles, the status of Food Safety Programmes, policies, procedures and training (Section 2.3 and Chapter 4).

Several studies recommended that bottled water should not be consumed by immunocompromised individuals. Croci et al. (2001) stated that the presence of

high densities of *Aeromonas hydrophila* in bottled mineral water can constitute a risk. While *A. hydrophila* naturally occurs in mineral waters, the level of mineral content, temperature, length of storage, and, in some cases, the type of container used may favor the growth of *A. hydrophila*, which is not desirable for immunocompromised individuals. Jeena et al. (2006) concluded that high levels of HPC bacteria with multiple drug resistance (to ampicillin, nalidixic acid, novobiocin and oxytetracycline) posed a significant health hazard to the consumers, especially to immunocompromised individuals. Papapetropoulou et al. (1997) also stressed that when bottled water is going to be consumed by immunocompromised patients, the environmental mycobacteria counts in bottled water was a useful guide of the hygienic quality.

Olayemi (1999) recovered *Staphylococcus aureus* in spring waters packaged and hawked in cellophane in Nigeria. Bharath et al. (2003) isolated aerobic bacteria in 33.6% of domestic brands and in 14.8% of imported brands in Trinidad. Kassenga (2007) detected heterotrophic bacteria in 92% of the 13 bottled water brands tested in Tanzania. Venieri et al. (2006) tested still mineral waters in Greece and isolated *Pseudomonas spp*, *Aeromonas spp*, *Pasteurella spp*, *Citrobacter spp*, *Flavobacterium spp*, *Providencia spp* and *Enterococcus spp*. In bottled waters sold in Puerto Rico *P. fluorescens*, *Corynebacterium sp. J-K*, *S. paucimobilis*, *P. versicularis*, *A. baumannii*, *P. chlororaphis*, *F. indologenes*, *A. faecalis* and *P. cepacia* were isolated by Reyes et al. (2008).

Breuer et al. (1990) recommended using HPC as a quality control check or as a regulatory check of the source water quality immediately after the bottling process. They recommended using a control value in line with the order of the European standard of approximately 100 CFU/mL. The study also concluded that additional quality control checks for containers to prevent bacterial growth during storage and transit should be carried out.

Inspecting water bottling plants adds a new dimension to the multifacet approach of ongoing research of bottled water in general. As researchers have no legal powers to enter the bottled water plants, it is very difficult for them to gain entry into those plants and to achieve the cooperation of the manufacturer. The to date available research that involves the inspections of bottled water

plants has been described by Mavridou et al. (1994) and Defives et al. (1999). Defives et al. (1999) found that while the initial levels of bacteria in some French mineral waters were low, the counts were higher after the bottling process. This indicated that contamination could have occurred during the bottling process. Mavridou et al. (1994) collected microbiological data by inspecting thirty eight bottling water plants in Greece between 1987 and 1992. The data was collected using two methods: 26 factories were monitored monthly according to the legislation and 12 factories were inspected after complaints from consumers were received or within the routine work of health officers. The researchers tested the collected bottled water brands for total coliforms, faecal coliforms, *E.coli*, total streptococci, *Pseudomonas aeruginosa* and *Clostridium perfringens* in accordance with the International Standards Organization (ISO) techniques (ISO 1993). Mavridou et al. (1994) found that 31.3% of samples tested were unsuitable for consumption according to the corresponding Greek legislation. In 1997, Papapetropoulou and colleagues, isolated environmental mycobacteria in 15.3% of the bottled water samples tested that were bottled by the Greek factories. Following the studies by Mavridou et al. (1994), Defives et al. (1999) and Papapetropoulou et al. (1997), the researchers made recommendations. As the researchers do not have legal powers, from their respective articles I was unable to ascertain whether the recommendations, for example not to consume certain brands or batches of bottled water or withdraw it from sales, had been followed or implemented.

To date there have been four studies (Cabral, 2002; Criado et al., 2004; Riberio et al., 2006; Papapetropoulou et al., 1997) that have investigated the presence of fungi in bottled water. While there is no legal requirement under the government regulations for testing for fungi with respect to bottled water quality, the presence of fungi in water may indicate poor process control. Papapetropoulou et al. (1997) isolated environmental mycobacteria in 23 of the 150 tested bottled water samples bottled by Greek factories. The environmental mycobacteria detected were *Mycobacterium chelonae*, *Mycobacterium phlei*, *Mycobacterium gordonae* and *Mycobacterium flavescens*. In 2002 Cabral reported detecting 5 different fungal isolates, namely *Penicillium* (46%), *Cladosporium* (32%), *Rhizopus* (8%), *Aspergillus* (3%) and *Phoma* (3%), from

samples of eight commercial mineral water brands in Argentina. Riberio and colleagues (2006) performed an one year long fungal survey located at one bottling plant. The purpose of the survey was to evaluate the incidence and fluctuations of the mycobiota (Riberio et al., 2006). The dominant fungal genera in order of highest numbers isolated were found to be *Penicillium*, *Cladosporium* and *Trichoderma*. The samples were collected during the warmer months, particularly during May and June. Fungal strains were isolated from the water filter and were also detected elsewhere in the factory. This highlighted the need to change filters more often. As a result of this survey a HACCP programme was implemented and Best Practice Guidelines introduced in this factory. Although the perceived public health risk posed by filamentous fungi in water is thought to be negligible, some fungi, such as *Penicillium citrinum*, that have been isolated from mineral water may be toxigenic.

Criado et al. (2004) studied the influence of different storage conditions, such as temperature, illumination, brand of mineral water and storage time on growth of mould spores. Mineral and mineralised waters packaged in polyethylene terephthalate (PET) bottles were inoculated with *Alternaria alternata*, *Penicillium citrinum* and *Cladosporium cladosporioides*. Storage time was the parameter that had the most important influence on mould growth. Spores grew into visible colonies after 5 months of incubation in bottles just filled, and in one month in bottles that had been stored for 5 months. *A. alternata* and *P. citrinum* strains were toxicologically characterised and both strains produced mycotoxins in vitro. *P. citrinum* also produced citrinin, a toxigenic substance, in mineral water.

Several researchers (Defives et al., 1999; Ribeiro et al., 2006; Kokkinakis et al., 2007; Zamberlan da Silva et al., 2008) have highlighted the importance of Good Manufacturing Practice (GMP). Defives et al. (1999) also recommended Best Practice Guidelines. Kokkinakis et al. (2007) recommended improvement of Hazard Analysis of Critical Control Points (HACCP) based systems. Zamberlan da Silva et al. (2008) highlighted the need for an improved surveillance system for the bottled water industry. Ribeiro et al. (2006) highlighted the need to change filters in the factories more often during periods of high fungal

contamination, such as warmer months of the year. As a result of Ribeiro et al. (2006) study HACCP programme was implemented and Best Practice Guidelines were introduced in one water bottling factory.

Defives et al. (1999) also pointed towards the need of more stringent legal requirements to be introduced. Kokkinakis et al. (2007) highlighted the importance of continuous monitoring of the source water quality, implementing the correct storage conditions, hygiene procedures and customer training at supermarkets. This was a confirmation for inclusion of HACCP related questions in my survey (Appendix I).

According to the European Community Directive (1980), natural mineral water in Europe cannot be treated. The safety is maintained by strict controls at the source. In Spain and France legislation does not permit the treatment of mineral water, because the water is considered to have 'therapeutic' properties. The presence of naturally occurring bacteria is seen as unavoidable, even indicative that the mineral water has not been sterilized and therefore its therapeutic characteristics have been conserved (Gonzalez et al., 1987).

In the European Union (EU) natural mineral waters are subject to an authorisation procedure, which is carried out by the competent authorities of the EU member states or by European Economic Area (EEA) countries (<http://ec.europa.eu>). Natural mineral waters in EU and EEA must to come from an approved source. Any possible treatments are strictly regulated.

1.1.1.2. United Kingdom Situation

Demand for bottled water In the United Kingdom has increased significantly since 1970s. In the 1980s more and more brands appeared on the UK market due to the segmentation of the market and stylish advertising, which resulted in increased demand for bottled water (CIEH, 22/02/2008). Producing bottled water can be viewed as controversial as the production of bottles involves the use of oil, with empty bottles adding to the amount of waste worldwide. To add to this there is a moral argument- how it could be right for the developed world

to spend so much money on buying bottled water, when 6 000 children die every day from consumption of poor quality drinking water. And then there is a cost. Tap water is 141 times cheaper than Evian mineral water, which is the bestselling bottled water. When bought in a supermarket, Evian costs 31p a litre (www.which.co.uk), while the cost of tap water is 0.22p per litre.

Environmental Health News, weekly magazine of the Chartered Institute of Environmental Health (22/02/2008) gave an example of manufacturing one particular brand of bottled water. The water is extracted in Fiji, where a third of the population does not have access to safe water and typhoid is still common. Then the water is transported to 'rich', western countries, where it is sold to 'wealthy' people in nice bottles labelled with catchy marketing messages.

In the developed world water from taps that is safe to drink is available. Tests demonstrated that the tap water in the UK is very safe to consume. Generally unless the bottled water is carbonated, people would not be able to tell the difference between still bottled water and tap water. In blind taste testings tap water has come out better than bottled water many times (CIEH, 22/02/2008).

In the UK drinking water is regulated by the Drinking Water Inspectorate (DWI). Tap water is regularly tested by the water companies and the DWI publishes the results in their Annual Chief Inspector's report on the DWI website in July each year. The DWI and the Consumer Council for Water jointly host a public meeting each year to present the Chief Inspector's Annual Report on drinking water quality in every Consumer Council for Water region in England and Wales. Every year water companies sample drinking water in their respective regions to test for compliance with the standards outlined in the Water Supply (Water Quality) Regulations 2001. The compliance rates are very high. Out of the 4.5 million samples tested in both 2006, 2007 and 2008, 99.96% met the standards set down in the above mentioned regulations. In 2009 the overall good quality of drinking water was demonstrated with compliance levels achieving 99.5%, only marginally lower than in previous three years (<http://www.dwi.gov.uk>). Results for 2010 will be published on DWI website in July 2011.

According to the Consumer Council for Water UK (www.ccwater.org.uk) with drinking water being of such a high standard and no empty bottles to throw away, tap water is more environmentally friendly than bottled water. It is also much cheaper as there are no transportation or packaging costs.

It costs less than £1 a year for one person to drink eight glasses of tap water a day, compared to £500 for the same amount of bottled water. Tap water is priced at about £1.00 per m³ and equivalent volume of the cheapest bottled water is £91 per m³, with the most expensive being £26,600 per m³! In 2008 the global bottled water market was worth £25 billion and in the UK the market for bottled water in 2008 was worth £2 billion.

1.1.1.3. New Zealand Context

Water quality is one good indicator of environmental health. There is no single measure of water quality: 'good' or "poor" water quality depends on the uses and values of the water (Ministry of Health, 1995).

In the Maori holistic view of health, the environment, which includes water, is linked to and is inseparable from the spiritual, mental, physical and social well-being of individuals and people as a collective. In their relationship with the environment the well-being of individuals and groups are seen in the context of te taha wairua (spiritual), te taha hinengaro (mental and emotional), te taha tinana (physical) and te taha whanua (family) and in overall relation to the environment (Ministry of Health, 1995).

Having had worked as a Health Protection Officer in Public Health Units in New Zealand, travelled substantially and currently working as a Senior Environmental Health Officer in one of the most prestigious and advanced Local Authorities in the UK I observed that generally most consumers in New Zealand as well as globally perceive bottled water to be safer than municipal water even though the latter is subjected to far more extensive and stringent testing and legal requirements.

In New Zealand there has been a dramatic increase since the mid-1980s in locally bottled water brands available for retail sale (Hasell & Capill, 2000). In the early 1990s concerns about the rapidly expanding industry resulted in a survey undertaken by the Consumer's Institute in association with the Department of Health (Hasell & Capill, 2000). In 1999 a survey was undertaken by the ESR for the New Zealand Ministry of Health. Samples of still and carbonated bottled water were purchased in the supermarkets throughout New Zealand. The survey tested 23 brands of local and imported water. Five samples of each brand were tested. None of the samples tested displayed detectable levels of coliforms, *E.coli*, faecal streptococci or *Pseudomonas aeruginosa*. It would appear that an earlier Australian National Health and Medical Research Council survey in 1987, which found that out of 43 brands tested in Australia these bacteria were detected in two brands, must have inspired the Ministry of Health to pursue the above mentioned project in 1999.

Hasell & Capill (2000) undertook a microbiological survey of bottled water for the New Zealand Ministry of Health. From January to March 1999, 1000 batches of bottled water were purchased. The samples were tested for coliforms, *E.coli*, faecal streptococci, *Pseudomonas spp.* and fungi. The testing results did not identify any major public health issues. The results of some batches did show that the bottlers of three brands did not have the bacteriological quality of the water fully under control, for example the bottlers were not carrying out microbiological monitoring of their bottled waters. Fungi were found in five brands, all originating from New Zealand bottling plants. This could have originated from poor quality control of either the water or the containers. Faecal streptococci were found in one brand, which suggested the inadequacy of the source. One bottle had a high coliform count, which suggested hygiene failure. One brand had a persistent low level of *Pseudomonas spp.*, which could be due to poor plant hygiene or a source water quality issue.

The results of this survey did not identify any issues of public health concern, but they did indicate that the potential for a poor water quality product to enter

the retail market exists. To ensure that this does not become a public health problem, Hasell& Capill (2000) proposed that water bottlers need to use a range of microbiological tests to monitor the safety and efficacy of source water quality and plant and container hygiene. Ideally this testing should be frequent and a part of a HACCP based product safety programme (Hasell& Capill, 2000).

Campylobacteriosis is the leading cause of reported gastrointestinal disease in New Zealand (Appendix II) and its incidence is ten times higher than salmonellosis. The number of campylobacteriosis cases reported to Public Health Units in New Zealand is growing each year (www.esr.cr.nz). While this may partially be attributed to the development of more advanced *Campylobacter spp.* detection methods, it is evident that the numbers of reported campylobacteriosis cases are increasing each year. For this reason in my study we tested the composite samples of brands that displayed HPC counts of 100 CFU/ml or higher for presence of *Campylobacter spp*

1.2. Bottled Water Quality Monitoring- Legislative Responsibilities

1.2.1. Introduction

There have been several guidelines published by the World Health Organization (WHO) and the most recent edition is dated 2008. Many countries use the WHO Guidelines for Drinking-water Quality as the basis to establish their own national standards. In these Guidelines a scientific assessment of the risks to health from biological and chemical determinands of drinking-water and of the effectiveness of control measures is described. When adapting the Guideline values to national standards WHO recommends using a risk-benefit approach and to take into account social, economic and environmental factors. As the WHO Guidelines for the Drinking Water Quality are meant to be the base for the development of standards, including bottled water, the actual standards will sometimes vary from the Guidelines. WHO also raised the importance of microbiological quality of ice designated for human consumption. What they meant was that the ice should be of drinking water standard. The equipment where the ice is made and stored must be of satisfactory standard of

cleanliness. The same principles should apply for water in large glass bottles that are protected by basketwork or wooden boxes. WHO, one of the co-sponsors of the Codex Alimentarius Commission (CAC), has advocated the use of the Guidelines for Drinking Water Quality as the basis for derivation of standards for all bottled waters (www.who.int).

In many European countries consumers believe that natural mineral waters have medicinal properties or offer some health benefits. These waters generally are high in mineral content and, in some cases, notably above the concentrations that are generally accepted in drinking water. Such waters have a long tradition of use and are often accepted on the basis that they are considered foods rather than drinking water. In some countries bottled waters with very low mineral content, such as distilled or demineralised waters, are available for sale.

CAC is the intergovernmental body for the development of internationally recognized standards for food. The CAC has developed a Codex Standard for Natural Mineral Waters and an associated code of practice. The Codex Standard describes the product and its labelling, compositional and quality factors, including limits for certain chemicals, hygiene, packaging and labelling. The Codex Code of Practice for Collecting, Processing and Marketing of Natural Mineral Waters provides guidance to the industry on a range of matters of good manufacturing practices. While CAC standards and recommendations are not strictly mandatory, Codex health and safety requirements are recognized by the World Trade Organization as representing the international consensus for consumer protection. Any deviation from Codex recommendations may require a scientifically based justification.

A draft of a Codex Standard for Bottled and Packaged Waters to cover drinking water other than natural mineral waters is being developed. Under the existing Codex Standard and Code of Practice, natural mineral waters must comply with strict requirements regarding, for example, the direct collection and bottling of water without any further treatment from a natural source, such as a spring or a well. The draft Codex Standard for Bottled and Packaged Waters has been proposed to include waters from other sources in addition to springs and wells.

It also proposed to include the treatment to improve the safety and quality of bottled water. The distinctions between these standards are especially relevant in regions where natural mineral waters have a long cultural history. Within the CAC, the Codex Committee for Natural Mineral Waters, which is hosted by Switzerland, is responsible for the development of draft Codex Standards and Codes of Practice in consultation with other relevant Codex Committees, especially the Codex Committees on Food Additives and Contaminants and Food Hygiene.

CAC and WHO do not certify bottled or mineral water products. Many countries have national standards for bottled waters and some countries have national certification schemes. At present there is no universally accepted international certification scheme for bottled and mineral waters.

The European Union European Directive 80/777/EEC, modified by Directive 96/70/EC deals with the marketing and development of natural mineral waters in the European Union. The Australasian Bottled Water Institute Inc. (ABWI) is an Australian bottled water industry lobby group. It is a regional member of the International Council of Bottled Water Associations (ICBWA). The regions covered are Australia, New Zealand and Oceania.

1.2.2. New Zealand Requirements for the Microbiological Compliance of Bottled Water (New Zealand Ministry of Health)

New Zealand legal requirements for bottled water are outlined in the Microbiological Reference Criteria for Food (Food Administration Manual S. 11: Microbiological Criteria Version 2.0) October 1995.

1.2.2.1. General

In the Microbiological Reference Criteria for Food there are two sets of criteria referred to, namely standards and reference criteria.

Microbiological Standards were part of the New Zealand Food Regulations 1984, which clearly established a microbiological content or level that it was unlawful to exceed. They were legislative and mandatory. As such they were identified separately to reference criteria.

Microbiological Reference Criteria are not part of a New Zealand law. They should be used where no standard exists in law to monitor the microbiological safety of a manufacturing process or the safety of a particular food. They may be used as supplements to existing standards where public health concerns dictate.

The Microbiological Reference Criteria can be of prime importance in deciding if a food is unsound or in reinforcing other observations and providing reasons to suspect that a food item may not meet sound public health practices. If the bacteriological quality is outside these reference criteria, an audit of the company's food safety programme will almost inevitably reveal unsatisfactory practices.

1.2.2.2. Sampling and Interpretation

The reference criteria are expressed in the format prepared by the International Commission on Microbiological Specifications for Foods (ICMSF). The ICMSF Scheme assists with the practical difficulties of representative sampling and interpretation of data provided by the laboratory. It permits some degree of tolerance to compensate for the difficulties of statistical sampling, and non-uniformity of bacterial load. The following terms listed below are used by the ICMSF in these reference criteria:

n = The number of sample units which must be examined from a lot of food to satisfy the requirements of a particular sampling plan.

c = The maximum allowable number of defective sample units. When more than this number is found, the lot is rejected by the sampling plan.

m = Represents an acceptable level and values above it are marginally acceptable or unacceptable in the terms of the sampling plan.

M = A microbiological criterion which separates marginally acceptable quality from defective quality. Values above M are unacceptable in the terms of the sampling plan and detection of one or more samples exceeding this level would be cause for rejection of the lot.

1.2.2.2.1. Sampling

At random 10% or 20 units or individual packages, depending on whichever is the less, from a lot or consignment (production batch or shipment) should be selected. Where a consignment is made up of a variety of component units, a minimum of 5 units from each variety is randomly selected. This may result in more than 20 units. Wherever possible, unit samples of a product are submitted to the laboratory in the original unopened packaging, maintained in their physical state as at the time of sampling.

When establishing the overall standard of a variety of foods in assessing an individual food-processing premise, for example takeaway food or a restaurant one grab sample of each individual selected food may be appropriate. The M value can be applied to one sample but c, n and m only apply when taking five samples. M values are useful for a broad-brush approach, but should be used under specific circumstances for premises or batches of product. It is important to know the sampling objective when deciding which sampling plan to adopt.

It should be recognized that a sampling plan of $n = 5$ is a minimum, applicable often by a regulatory authority as a cost governed expediency for surveillance purposes, the stringency of such a sampling programme being governed by the value applied to m.

Other sampling plans have been formulated by ICMSF which are responsive to given prevailing circumstances related to risk, or identified levels of concern for pathogenic microorganisms.

Such sampling plans should only be applied after consultation, taking into account perceived risks and other specific factors pertaining to the food in question.

1.2.2.2.2. Interpretation

With the exception in some instances of nil tolerance, where the non-compliance of 1 unit from a lot or consignment constitutes rejection, the following assessment is generally applied.

Where 5 or more units of the same variety from a lot or consignment are analysed ($n = 5$), no more than 2 units ($c = 2$) should exceed the maximum tolerance limits (m) for microbiological levels stated in the reference criteria. No one unit should exceed the stated level for the maximum tolerance (M). In some cases c may have a different value, e.g., $c = 3$ (Table 1-4).

Table 1- 4. Microbiological Reference Criteria for Foods Packaged Waters (Including Mineral Waters and Those Bottled from Natural Underground Sources)

Microorganism	N	C	m	M
Coliform (/100 ml)	$n = 5$	$c = 1$	$m = 10$	$M = 10^2$
<i>Escherichia coli</i> (/100 ml)	$n = 5$	$c = 0$	$m = 0$	
Group D streptococci (/100 ml)	$n = 5$	$c = 1$	$m = 10$	$M = 10^2$
<i>Pseudomonas aeruginosa</i> (/100 ml)	$n = 5$	$c = 0$	$m = 0$	

1.2.3. Australia and New Zealand Requirements for the Microbiological Compliance of Bottled Water under the Food Standards Code

1.2.3.1. Background

In adopting the new Australia New Zealand Food Standards Code in November 2000 (www.foodstandards.gov.au), the Ministerial Council agreed to a two-year transition period. After this, the new Code replaced both the old Code and the New Zealand regulations (New Zealand Food Regulations 1984).

During this two-year phase-in period, foods in Australia could have complied with either the old Code or the new Code, but not a combination of these. In New Zealand foods could have complied with the old Code or the new Code or the New Zealand regulations, but not a combination of these.

After this, the old Code and New Zealand regulations were repealed and all food sold in Australia and New Zealand now has to comply with the new Code. The new Code meant changes in the way manufacturers and retailers make and present food for sale.

In consultation with the Australian and New Zealand governments and industry representatives the Australia New Zealand Food Authority (ANZFA) developed a user guide. The purpose of this guide was to help manufacturers and retailers interpret and apply Standard 1.6.1. Microbiological Limits for Food in the new Code. The guide may also be used by food officers to help interpret food standards in the new Code. The user guide, unlike the standard itself, is not legally binding and if there is any doubt about interpreting the standards, legal advice should be sought.

As well as complying with food standards requirements, manufacturers and retailers must also continue to comply with other legislation. In Australia, this legislation includes the *Trade Practices Act 1974*, the *Imported Food Control*

Act 1992, and State and Territory Fair Trading Acts and Food Acts. In New Zealand, this legislation includes the *Food Act 1981* and *Fair Trading Act 1986*.

The overall purpose of the guide was to provide information to help retailers, caterers, manufacturers, including bottled water manufacturers, and food officers interpret and apply Standard 1.6.1. Microbiological Limits for Food.

1.2.3.2. Microbiological standards and guideline criteria

Standard 1.6.1 specifies microbiological standards for nominated foods or classes of foods. Foods listed in the standard must meet the prescribed microbiological limits at any stage of their manufacture or sale.

Food Standards Australia New Zealand (ANZFA) developed microbiological guideline criteria for various foods that are additional to the standard but not mandatory. These guideline criteria act as an identification point for unacceptable levels of microbial contamination in foods. When these levels are exceeded it generally indicates a failure in the food production process or hygiene procedures. It means that action should be taken to identify and remedy the problem.

Reliance on these microbiological guideline criteria alone does not assure safe food production and handling procedures. The Safe Food Australia –A Food Standards Australia New Zealand (2001) has more specific information regarding safe food production and handling.

1.2.3.3. Food description

The Food Standards define bottled water under the terms of mineral water or packaged water. The differences between these two terms are described below:

Mineral water (or spring water) - refers to ground water obtained from subterranean water-bearing strata that, in its natural state, contains soluble matter. This standard applies to both packaged mineral water and water sampled at source.

Packaged water - is any potable water that is manufactured, distributed or offered for sale in food-grade bottles or other containers and is intended for human consumption.

1.2.3.4. Sampling plans

The schedule to Standard 1.6.1 sets out the microbiological criteria for the acceptance or rejection of sample lots. It sets out:

- a) the food which must comply with the microbiological limits set in relation to that food;
- b) the micro-organism or group of micro-organisms of concern;
- c) the number of sample units to be taken and tested;
- d) the level of micro-organisms considered acceptable, marginally acceptable or critical, depending on the sampling plan specified; and
- e) the number of samples that should conform to these limits.

The following terms, as used by the International Commission on Microbiological Specifications for Foods (ICMSF), are defined and used in Standard 1.6.1.

n = the number of sample units which must be examined from a lot of food. Most sampling plans specify taking five sample units. However, when the risk has been assessed as relatively high, a greater number of sample units is specified. This is the case for *Salmonella spp.* in coconut, cereal-based foods for infants and infant formula where 10 sample units should be examined.

c = the maximum allowable number of defective sample units. This is the number of sample units, which may exceed the microbiological limit specified by

'm'. These are considered marginal results, but are acceptable providing they do not exceed the limit specified by 'M'.

For example, the standard for coagulase-positive staphylococci in cooked Crustacea allows for two samples ($c=2$) to exceed the acceptable microbiological level of 10^2 ('m'=10²), providing no sample exceeds a level of 10^3 ('M'=10³). In many cases $c=0$ which means no sample may exceed the specified limit 'm'.

m = the acceptable microbiological level in a sample unit. Sampling plans in which $m=0$ and $c=0$ are equivalent to 'absent' or 'not detected' reporting for the stated analytical unit size. In most cases this is 25 g, e.g. not detected in 25 g.

M = the level which, when exceeded in one or more samples, would cause the lot to be rejected.

A lot means a quantity of food, which is prepared or packed under essentially the same conditions, usually either from a particular preparation or packing unit and during a particular time ordinarily not exceeding 24 hours. This is described in more detail in Standard 1.1.1. A lot of food would not comply with the standard if the number of defective sampled units is greater than c or the level of a particular micro-organism in a food in any one of the sample units exceeds M .

Sampling plans are presented in the format used by ICMSF. More detailed information on their use can be found in the ICMSF publication *Micro-organisms in Foods, Sampling for Microbiological Analysis; Principles and Specific Applications (1986)*.

1.2.3.5. Methods

Standard 1.6.1 prescribes the use of the Australian/New Zealand Standard Methods for Food.

Microbiology AS/NZS 1766 is to determine whether a food has exceeded the maximum permissible levels of food-borne microorganisms. Alternative methods may be used when their equivalence to the prescribed method has been validated by the protocol provided in AS/NZS 4659. For analyses on packaged water, packaged ice and mineral water, methods from the Australian/New Zealand Standard AS/NZS 4276: *Water Microbiology* are the reference methods required (Table 1.5).

The Microbiological Limits for Food of Standard 1.6.1 also include *E. coli* standards for mineral water, packaged water and packaged ice (Table 1-5).

Table 1- 5. Australian/New Zealand Microbiological Standard for Mineral Water and Packaged Water and Ice.

	Food microorganism	N	C	m	M
Mineral water	Coliforms (/250 ml)	n = 5	c = 0	m = 0	
	<i>Pseudomonas aeruginosa</i> (/250 ml)	n = 5	c = 0	m = 0	
Packaged water and ice	Coliforms (/250 ml)	n = 5	c = 0	m = 0	
	<i>Pseudomonas aeruginosa</i> (/250 ml)	n = 5	c = 0	m = 0	
	Standard plate count (SPC)/ml Total viable count, TVC	n = 5	c = 0	m = 10 ²	

The Standard plate count (SPC) in Table 1-5, is also referred to as the Heterotrophic Plate Count (HPC), Aerobic Plate Count (APC) or the Total Viable Count (TVC), and is one of the most common tests applied to indicate the microbiological quality of food. The significance of HPC, however, varies noticeably according to the type of food product and the processing it has received. When HPC testing is applied on a regular basis it can be a useful means of observing trends by comparing HPC results over time. Three levels of HPC are listed in Table 1-5 based on food type and the processing/handling the food has undergone.

Level 1 - applies to ready-to-eat foods in which all components of the food have been cooked in the manufacturing process or preparation of the final food product and, as such, microbial counts should be low (Table 1-5) with Satisfactory level of $<10^4$, Marginal $<10^5$ and Unsatisfactory $\geq 10^5$ (CFU/gram).

Level 2 - applies to ready-to-eat foods that contain some components that have been cooked and then further handled, either stored, sliced or mixed prior to preparation of the final food or where no cooking process has been used (Table 1-5). Satisfactory level is $<10^6$, Marginal $<10^7$ and Unsatisfactory $\geq 10^7$ (CFU/gram).

Level 3 - SPCs not applicable. This applies to foods such as fresh fruits and vegetables, including salad vegetables, fermented foods and foods incorporating these in other foods, such as sandwiches and filled rolls. It would be expected that these foods would have an inherent high plate count because of the normal microbial flora present.

It is important that examination of the microbiological quality of a food item should not be based solely on SPC or HPC counts. The significance of high or unsatisfactory SPC or HPC counts can not truly be made without identifying the microorganisms that outweigh or without other microbiological testing.

1.2.3.6. Categories of microbiological quality

Four categories of microbiological quality levels have been assigned based on standard plate counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous (Table 1-6).

a) Satisfactory- results indicate good microbiological quality. No action is required if the food sample is satisfactory.

b) Marginal- results are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food. Resampling and testing of the food may be required. Premises that regularly demonstrate borderline results would and should have their food handling controls investigated.

c) Unsatisfactory- results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices. Further sampling, including the sampling of other foods from the food premise may be required and an investigation undertaken to determine whether food handling controls and hygiene practices are adequate.

d) Potentially Hazardous- the levels in this range may cause food borne illness and immediate remedial action should be initiated. Consideration should be given to the withdrawal of any of the food still available for sale or distribution and, if applicable, recall action may be indicated. An investigation of food production or handling practices should be instigated to determine the source/cause of the problem so that remedial actions can commence.

Table 1- 6. Guideline Levels for Determining the Microbiological Quality of Ready-To-Eat Foods *

Test	Test microbiological quality (CFU per gram)			
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Standard Plate Count				
Level 1	<10 ⁴	<10 ⁵	≥10 ⁵	
Level 2	<10 ⁶	<10 ⁷	≥10 ⁷	
Level 3	N/A	N/A	N/A	
Indicators				
<i>Enterobacteriaceae</i> *	<10 ²	10 ² -10 ⁴	≥10 ⁴	
<i>Escherichia coli</i>	<3	3-100	≥100 **	
Pathogens				
Coagulase +ve staphylococci	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
<i>Clostridium perfringens</i>	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
<i>Bacillus cereus</i> and other pathogenic <i>Bacillus</i> spp.	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
<i>Vibrio parahaemolyticus</i> #	<3	<3 -10 ²	10 ² -10 ⁴	≥10 ⁴
<i>Campylobacter</i> spp	not detected in 25g			Detected
<i>Salmonella</i> spp	not detected in 25g			Detected
<i>Listeria monocytogenes</i>	not detected in 25g	detected but <10 ²		≥10 ² ##

**Enterobacteriaceae* testing is not applicable to fresh fruits and vegetables or foods containing these.

**Pathogenic strains of *E. coli* should be absent.

V. parahaemolyticus should not be present in seafoods that have been cooked. For ready-to-eat seafoods that are raw, a higher satisfactory level may be applied (<102 cfu/g).

The potentially hazardous level of *V. parahaemolyticus* relates to Kanagawa-positive strains. Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g.

The detection of *L. monocytogenes* in ready-to-eat foods prepared specifically for .at risk population groups (the elderly, immunocompromised and infants) should also be considered as potentially hazardous.

N/A .SPC testing not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these (such as sandwiches and filled rolls).

The table 1-6 was taken out of Food Standards Australia New Zealand.

Document accessed via <http://www.foodstandards.gov.au>.

1.3. Characteristics of Indicator Organisms and Rationale for Detecting and Enumerating Indicator Organisms

Through ordinary exposure to air, soil and effluents along with harmless microorganisms, pathogenic contaminants may also be present in water. Prominent water borne pathogens of recent time are the Protozoa, such as *Giardia* and *Cryptosporidium*, the bacteria *Campylobacter spp.*, *Salmonella spp.*, *Shigella spp.*, *Vibrio spp.* and *Mycobacterium spp.*, hepatitis A virus and Norovirus (Talaro, 1999). Some of these agents, especially encysted Protozoa, can survive in natural waters for long periods without a human host. Others are present only transiently and are rapidly lost. The microbial content of drinking water must be continuously monitored to ensure that the water is free of infectious agents. Attempting to survey water for specific pathogens can be very difficult, not practical and time consuming. Therefore, most water purity evaluations are more focused on detecting faecal contamination. High faecal levels can mean that water contains pathogens and is consequently unsafe to drink (Talaro, 1999).

Many diseases are spread by the 'faecal to oral' route of transmission, in which a pathogen is normally shed in human or animal faeces. Then it usually contaminates the water and is subsequently ingested. Examples of such diseases are typhoid fever and cholera, caused by bacteria that are shed only in human faeces (Tortora et al., 1997).

The tests for water purity today are aimed at detecting particular **indicator organisms** (Tortora et al., 1997). The usual indicator organisms are the coliform group bacteria. Many standards for food and water specify the identification of faecal coliforms. This is because some coliforms are not solely enteric bacteria, but are more commonly found in plant and soil samples. The predominant faecal coliform is *Escherichia coli*, which constitutes a large proportion of the human intestinal population. There are specialised tests available to distinguish between faecal coliforms and non-faecal coliforms. Under the normal conditions coliforms themselves are not pathogenic, although certain strains can cause diarrhoea and opportunistic urinary tract infections. However, the presence of *E.coli* indicates that there may be a health risk from other pathogenic (disease-causing) organisms such as *Salmonella spp.*, *Campylobacter spp.*, *Giardia spp.* or *Cryptosporidium spp.*

1.4. Objectives and Outline of my Research

The **first objective** of this research was to establish compliance of randomly purchased bottled water brands with the New Zealand Requirements for the Microbiological Compliance of Bottled Water, New Zealand Ministry of Health and Australia and New Zealand Requirements for the Microbiological Compliance of Bottled Water under the Food Standards Code.

The samples were purchased in random retail outlets in Wellington City and the Greater Wellington Region. Then they were tested in the Microbiology Laboratory at the Massey University in Wellington.

The **second objective** was to assess the impact of manufacturing procedures on bottled water microbiological quality by investigating the relationships of the source water quality, type of abstraction, pipework materials, bottling process, staff training, policies and procedures and the microbiological quality of the final product. This objective is unique as nobody in New Zealand had previously surveyed the manufacturers in this respect.

Chapter 2 outlines the materials and methods, sample collection, microbiological and statistical analysis and description of methods used for the microbiological analysis of the samples. It also describes how the survey of water bottling plants was carried out and contains sections on ethical issues, quality control and limitations.

In **Chapter 3** the microbiological results of the 38 tested bottled water brands are presented. The results are followed by the analysis and discussion of the status of compliance with New Zealand Microbiological Reference Criteria for Food and with the Australia and New Zealand Food Standards Code.

Chapter 4 outlines the outcome of the manufacturer's survey.

Chapter 5 outlines the conclusions, recommendations and possible future implications with regards to the microbiological results of my project and the survey of water bottling plants.

CHAPTER TWO – MATERIALS AND METHODS

2.1. Collection and Description of Samples

For the purpose of this research a total of thirty eight brands of bottled water were purchased from retail outlets in Wellington and its region. A variety of brands was sought and there was no preference given to any brands or retail outlets. Whether the brands were domestic or imported was defined by the manufacturers contact details on the label at the time of purchase.

Five bottles of each bottled water brand from the same batch were purchased at different retail outlets. Samples were then taken to the Microbiology laboratory at Massey University in Wellington for microbiological analysis and analysed in duplicates within 6 hours of purchasing. Thirty eight bottled water brands were tested in 2003 to establish microbiological compliance with the New Zealand Microbiological Criteria for Food and Australian and New Zealand Food Standards Code.

The 38 brands tested are listed in Appendix IV and a full description of all 38 brands is in Chapter 3 (Table 3-1).

2.2 Analysis of Samples

2.2.1. *E. coli* Detection and Enumeration

2.2.1.1. *General*

Defined Substrate Technology™ (IDEXX Laboratories USA) is a commercially available test system that detects bacteria with specific enzyme substrates. IDEXX's Colilert™ test was the first system to receive USEPA approval for the detection of total coliforms (1989) and *Esherichia coli* (1992) (Abbott, 2002).

The Colilert system uses specific indicator substrates ortho-nitrophenyl- β D-galactopyronoside (ONPG) and 4-methylumbelliferyl- β -D-glucoronide (MUG) for the target microbes, specifically for total coliforms and *Escherichia coli*. Bottled water samples were incubated with the Colilert reagent for 24 hours. Should a coliform be present, the indicator substrate would be hydrolysed by the enzyme of the microorganism, namely β -galactosidase and would release the indicator portion ortho-nitrophenyl from ONPG. The free indicator then imparts a yellow colour to the solution. *E.coli* possesses an additional constitutive enzyme glucoronidase that hydrolyses the second indicator substrate MUG. As a result of this hydrolysis MUG is cleaved into a substrate portion (glucoronide) that is metabolised and an indicator portion methylumbelliferone that fluoresces under ultraviolet light (Abbott, 2002).

To interpret Colilert testing results Colilert MPN tables were used depending on the volumes of the samples used and whether a 51- well or 97- well Quanti-Tray was used. The 97 well Quanti-Tray MPN table does not show the 95 % confidence limits but these are available on an IDEXX version 3 Windows software programme, (Abbott, 2002). Quantified MPN Colilert results were also determined by using an IDEXX Quanti-TrayTM, which is a sterile disposable tray with either 51 or 97 separate wells. Both types of trays had been used in this research. Sealing the sample – filled tray was accomplished by the use of a specially designed Quanti-Tray Sealer which automatically evenly distributes a 100 ml sample amongst the wells and seals it. (Abbott, 2002).

In my research I used IDEXX Quanti-Trays, which were sterile and disposable. 51-well trays designed for bacterial enumeration using Colilert[®] and EnterolertTM systems were used to analyse samples 6 to 20 (inclusive) and 31 to 34 (inclusive). To analyse samples from 1 to 5 (inclusive), from 21 to 30 (inclusive) and from 35 to 38 (inclusive) I used IDEXX Quanti-Trays/2000, which were sterile disposable, 97-well trays designed for bacterial enumeration using Colilert[®] and EnterolertTM.

The results of the testing were obtained from MPN tables that were enclosed to the trays.

2.2.1.2. Detection and Enumeration of Total coliforms and *Escherichia coli*

2.2.1.2.1. Materials

- Bottled water samples
- *Escherichia coli* (NZRM 916 - nutrient agar culture)
- *Klebsiella pneumoniae* (NZRM 482 - nutrient agar culture)
- *Pseudomonas aeruginosa* (NZRM 981 - nutrient agar culture)
- 97-well Quanti-Trays (IDEXX Laboratories)
- Colilert reagents (IDEXX Laboratories)
- 35°C Incubator
- 365 nm wavelength ultraviolet light with a 6 Watt bulb (De Saga)
- Sterile 250 ml bottles
- Bottles of 5.0 ml sterile distilled water
- IDEXX version 3.0 MPN table (IDEXX Laboratories)

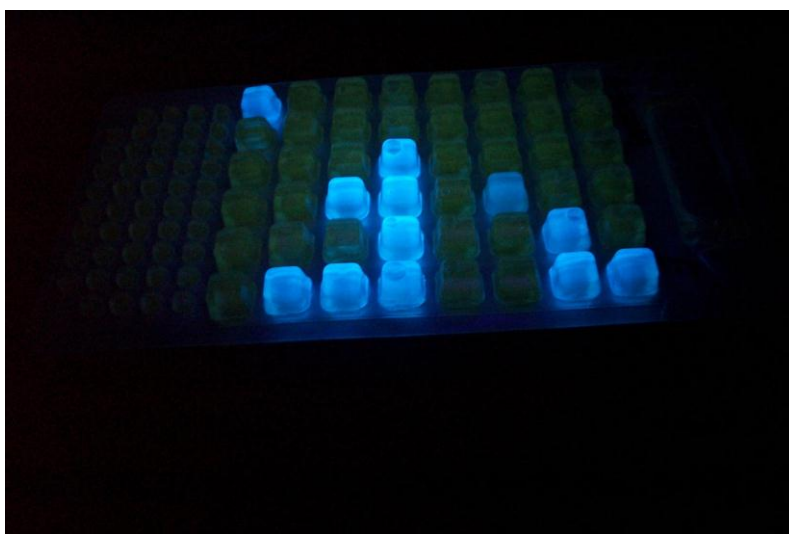
2.2.1.2.2. Method

The Colilert procedure was performed according to the manufacturer's instructions using aseptic techniques. Quanti-Trays and sterile 250 bottles were labelled with the collection time, date and sample ID numbers. Well-mixed 100 ml bottled water samples were made by adding one packet of the powdered Colilert reagent. After shaking the sample to dissolve the powder, the mixture was poured into a sterile 97-well Quanti-Tray. The trays were then mechanically sealed in a Quanti-Tray sealer, which simultaneously distributed the mixture evenly into the wells. The trays then were incubated for 24 hours at 35°C ± 0.5°C. After incubation the tray was viewed firstly for yellow wells. This was considered as a positive reaction for that well and indicated the presence of

Total coliforms. The tray was then viewed in a darkened room by placing it under and within 12 cm of a 365 nm wavelength ultraviolet light. Blue fluorescence in a well was considered a positive reaction for that well and indicated the presence of *Escherichia coli* (Figure 2-1). The number of *Total coliforms* and *Escherichia coli* per 100 ml, based on the number of positive wells counted, was determined by referring to a 97-well MPN table (IDEXX). Wells showing no yellow colour were considered negative for *Total coliforms* and wells showing no fluorescence were considered negative for *Escherichia coli*.

Control cultures were put up at regular intervals throughout the study. A nutrient agar culture of *Klebsiella pneumoniae* was used as a partial positive control (yellow wells but no fluorescence) and a nutrient agar culture of *Escherichia coli* as a complete positive control (yellow and blue fluorescent wells). A nutrient agar culture *Pseudomonas aeruginosa* will be used as the negative control (no yellow wells and no fluorescence). A colony of each bacterial strain was touched with a sterile inoculating loop, transferred to 5.0 ml of sterile distilled water and thoroughly mixed. A 1.0 µl loop full of this mixture was then used to inoculate 100 ml of sterile distilled water. One packet of the powdered Colilert-18 reagent was added to this 100 ml mixture and after shaking to dissolve the powder. The mixture was then poured into a sterile 97-well Quanti-Tray. The rest of the procedure was carried out as described above for the test samples.

Figure 2-1. Colilert Control Positive Reaction*.



* The blue fluorescent wells show positive reaction for *E.coli* under the 365 nm wavelength UV light.

2.2.2. Enterococci Detection and Enumeration

2.2.2.1. Materials

- Bottled water samples
- *Enterococcus faecalis* (NZRM 1106 - blood agar culture)
- *Enterococcus faecium* (NZRM 1236 - blood agar culture)
- *Serratia marcescens* (NZRM 3505 - nutrient agar culture)
- 97-well Quanti-Trays (IDEXX Laboratories)
- Enterolert reagents (IDEXX Laboratories)
- 41°C Incubator
- 365 nm wavelength ultraviolet light with a 6 Watt bulb (De Saga)
- Bottles of 5.0 ml sterile distilled water
- Sterile 250ml bottles
- IDEXX version 3.0 MPN table (IDEXX Laboratories)

2.2.2.2. Method

The Enterolert procedure was performed according to the manufacturer's instructions using aseptic techniques. The Quanti-Trays and the sterile 250 ml bottles with sample ID number, time, date and location of purchase were labelled. 100ml of the well mixed water sample was aseptically poured into the sterile 250ml bottle.

All the Colilert and Enterolert reagents were provided to Massey University by the Environmental Diagnostics in Auckland.

One package of the powdered Enterolert reagent was added to the bottle. After shaking to dissolve the powder the mixture was poured into a sterile 97-well Quanti –Tray. The tray was then mechanically sealed in a Quanti-Tray sealer, which simultaneously distributed the mixture into the wells, then incubated for 24 hours at 41°C \pm 0.5°C. After incubation the tray was viewed in a darkened room by placing it under and within 12 cm of a 365nm wavelength ultraviolet

light with a 6-Watt bulb (De Saga, Heidelberg, Germany). Blue fluorescence in a well was considered as a positive reaction for that well and indicated the presence of *Enterococci*. The number of *Enterococci* per 100 ml was determined, based on the number of positive wells counted, by referring to a 97-well MPN table. Wells showing no fluorescence were considered negative for *Enterococci*.

Control cultures were included at regular intervals throughout the study. Blood agar cultures of *Enterococcus faecalis* and *Enterococcus faecium* were used as positive controls and a nutrient agar culture of *Serratia marcescens* as the negative control. A colony of each bacterial strain were touched with a sterile inoculating loop, transferred to 5.0ml of sterile distilled water and thoroughly mixed. A 1.0 µl loop full of this mixture was then used to inoculate 100 ml of sterile distilled water. One package of powdered Enterolert reagent was then added to this 100 ml mixture and after shaking to dissolve the powder, the mixture was poured into a sterile 97-well Quanti-Tray. The rest of the procedure was carried out as above for the test samples.

The control cultures that were used in detection and enumeration of total coliforms and *E.coli* were *E.coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These control cultures were purchased from an approved culture reference supplier or laboratory. *Quanti-Cult* is one such quality control pack, and is available from Colilert and Enterolert suppliers. It contains freeze-dried ready-to-use low-level dilutions of bacteria, which should result in the following test reactions listed in the Table 2-1 below.

Table 2-1. Reactions of Colilert and Enterolert *Quanti-Cult* Pack.

Microorganism	Normal light	UV light	Control
E.coli	Colour change	Fluorescent blue	Positive E.coli
K. pneumoniae	Colour change	No fluorescence	Positive coliform
P. aeruginosa	No colour change	No fluorescence	Negative

(Table taken from Whyte R.J. *Protocol for Drinking Water monitoring Programmes based on Rapid Test Systems Such as Colilert/Colisure*. A Report for the Ministry of Health. 1996).

Each sample was given a unique reference number. All sample analysis records were traceable by the use of this unique sample reference number. The records of results were being kept until the completion of the thesis. The actual worksheets, including date of purchase, sample identification, analysis date and test result were being kept. As I purchased and analysed all samples myself, I did not need to record the name of the purchaser and the name of the person performing analysis.

2.2.3. Total Viable Count Method, *Pseudomonas aeruginosa* Detection and Enumeration, Detection and Enumeration of Yeasts and Moulds

2.2.3.1. Materials

- Bottled water samples
- 90ml sterile isotonic saline
- 9ml sterile isotonic saline
- Canisters of sterile 10ml pipettes
- Canisters of sterile 1ml pipettes
- Canisters of sterile Pasteur pipettes
- Nutrient agar plates (Difco/ Pecton Dickinson, MD, USA)
- Sabouraud dextrose agar plates (Difco/ Pecton Dickinson, MD, USA)
- MPA agar plates (Fort Richard Laboratories)
- Petri dishes (Fort Richard Laboratories)

2.2.3.2. Method for Detection of Heterotrophic Plate Count (HPC)/ Aerobic Plate Count (APC)/Total Viable Count (TVC)

Five samples of each batch of the same brand of bottled waters were tested as per protocol outlined in the NZMRC and following the laboratory procedures. The samples were tested in duplicates following the steps listed below.

1. Nutrient agar plates were labelled with the sample ID number, brand code, sample number and indicated whether that was a duplicate.
2. A volume of 0.2 ml of each sample from every batch (in duplicates) was dispensed into each plate.
3. A required number of spreaders were made using Pasteur pipettes.
4. Each dilution was spread with a glass spreader evenly over the surface of the entire plate.
5. The plates were incubated at 37°C for 24-48 hours.
6. The results were read after incubation for 24-48 hours and immediately recorded in my Laboratory Book and transferred onto a Microsoft Excel sheet. The results were recorded in 'Colonies per Plate', which then was converted into 'Number of Organisms in Sample' (CFU/ml).

2.2.3.3. *Pseudomonas aeruginosa* enumeration

1. MPA agar plates were marked with a sample ID.
2. Using the MPA agar plates, steps 3 to 5 as above (Section 2.2.3.2.) were repeated.
3. A volume of 0.2 ml of each sample from every batch in duplicates was dispensed into each plate.
4. A required number of spreaders were made using Pasteur pipettes.

5. Each dilution was spread with the spreader evenly over the surface of the entire plate.
6. The plates were incubated at $41.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 72 hours.
7. The results were read after 72 hours and recorded in the Microbiology Laboratory Results Book and then transferred onto the Excel sheet. The results were recorded in 'Colonies per Plate', which then was converted into 'Number of Organisms in Sample' (CFU/ml).

2.2.3.4. Enumeration of Yeasts and Moulds

1. Sabouraud dextrose agar plates were marked with a sample ID.
2. A volume of 0.2 ml of each sample from every batch in duplicates was dispensed into each plate.
3. A required number of spreaders were made using Pasteur pipettes.
4. Each dilution was spread with the spreader evenly over the surface of the entire plate.
5. The plates were incubated at $25^{\circ}\text{C} - 30^{\circ}\text{C}$ for 7 days.
6. The results were read after 7 days and recorded in the Microbiology Laboratory Results Book and then transferred onto the Excel sheet. The results were recorded in 'Colonies per Plate', which then was converted into 'Number of Organisms in Sample' (CFU/ml).

2.2.4. Detection and Enumeration of *Campylobacter* spp.

Composite samples of each bottled water brand that demonstrated TVC counts of 100 CFU/ml or higher were tested for the presence of *Campylobacter spp.* bacteria.

Samples were tested at the International Accreditation Environmental Laboratory Services Ltd. (ELS), Seaview, Lower Hutt, New Zealand. ELS is an International Accreditation New Zealand (IANZ), Laboratory Approval Scheme (LAS) and Ministry of Health approved testing laboratory that offers a broad range of microbiological and inorganic chemistry analyses, covering a large range of sample matrices. ELS Microbiology laboratory currently serves many export meat premises, domestic food companies, dairy premises as well as many water clients from around New Zealand (<http://www.els.co.nz>)

The *Campylobacter spp.* isolation method used by ELS is found in Appendix III.

2.3. Survey of Water Bottling Plants

A survey questionnaire (Appendix I) was compiled to gather more information on the bottled water brands that were tested (Appendix IV). Information was sought on the sources of bottled water, treatment in place, pipework materials, bottling process, sterilisation of bottles, transportation, procedures and protocols, training of staff and safe working practices. The purpose of the questionnaire was to further establish the significance of the above mentioned criteria to the microbiological quality of the final product.

This questionnaire was accompanied by a cover letter (Appendix V). The survey questionnaire was sent out to all bottled water manufacturers and importers in New Zealand and abroad.

2.4. Statistical Analysis of Microbiological Testing Results

The counts of *Escherichia coli*, Total Coliforms, *Pseudomonas aeruginosa* and Enterococci (group D streptococci) obtained in this study were compared with

the values detailed in New Zealand Microbiological Reference Criteria for Food and Australia and New Zealand Food Standards Code (2001). Yeast and fungal spore counts were used as a measure of the overall microbiological quality of the samples selected.

The data was analysed using ratios and correlations using Microsoft Excel and EpiInfo.

Epi-Info™ is public domain statistical software developed by the Centers for Disease Control and Prevention (CDC), headquartered in Atlanta, Georgia (USA). Epi-Info has been in existence for over 20 years and is currently available for Microsoft Windows. This software package allows people to track, report and study emerging epidemics as well as past outbreaks of disease. The introduction of Epi-Info™ to the toolbox of epidemiologists around the world was a significant development in this field, allowing people to collect useful real-time data which could be used to figure out how an epidemic started, and potentially to determine how it could be stopped.

There are several facets to Epi-Info™. In the first sense, it allows epidemiologists to collect and analyse data, using a variety of statistical tools such as analysis of variance (ANOVA). By organizing data about an epidemic, epidemiologists can start to think about how the epidemic developed, looking for patterns which might lead to answers. These statistics can also be transmitted to other workers in the field, allowing people to connect multiple epidemics quickly and trace them to a common source, such as an untreated tuberculosis patient or contaminated food associated with a food premise.

The software also generates surveys, which can be used to help gather statistics about an epidemic. By creating forms and questionnaires epidemiologists can ensure that the same data is collected from every involved patient, generating reams of data which can be collated and studied collectively. Forms can also be generated for medical personnel, friends and family of patients creating a complete picture of an emerging epidemic.

When an outbreak is developing, Epi-Info™ can be extremely valuable. This tool has helped Health Departments around the world, quickly identifying related epidemics through a shared network, and helping people get to the root cause quickly. One of the examples is that by using Epi-Info epidemiologists can realize that people in several different locations all ate the same food. Therefore an immediate recall of contaminated food can be issued to prevent an epidemic. Information shared through Epi-Info™ can also speed up the treatment by identifying what type of treatment would be most likely to work.

This tool can also be used to study epidemics after the fact. By entering data into Epi-Info™ epidemiologists can create data which may be studied later. New ways to handle such epidemics could be identified and Epi-Info™ can be used to connect past and future epidemics to each other. This would potentially establish a historical link between outbreaks of similar illnesses which could be used to gather more information about them. (<http://www.wisegeek.com/what-is-epi-infotrade.htm>).

The software is available in the public domain free of charge. It and can be downloaded from <http://www.cdc.gov/epiinfo>. Limited technical support is available.

Epi-Info was used to analyse the results of the microbiological testing of the thirty-eight bottled water brands purchased in New Zealand retail outlets. The results of the microbiological tests recorded in the Laboratory Book were transferred into the Microsoft Excel Workbook for storage. The average microbiological counts were calculated where appropriate. Consequently the data from Microsoft Excel workbook was transferred into the Epi-Info, where the data was then analysed. Epi-Info was used for the analysis of microbiological testing results and for the analysis of the data obtained from the questionnaire.

2.5. Reliability, Validity and Quality Control

The reliability of a measurement refers to the consistency, the reproducibility and the repeatability of the instrument or measurement procedure (Minichiello

et al., 1999). The question of the degree of reliability required of a measurement technique relates more to the degree of precision required of the instrument (Minichiello et al., 1999).

Validity refers to the 'degree to which a test actually measures what it purports to measure' (Minichiello et al., 1999). Validity also 'refers to the appropriateness, meaningfulness, and usefulness of the specific inferences made from test scores. All assessments of validity are ultimately concerned with the relationship between performance on the test and on other observable facts (Minichiello et al., 1999).

During the course of this study I ensured reliability and validity by purchasing the samples of known brands at known locations myself. Then I transported the samples to the Massey University and analysed them within a required timeframe. I performed the testing at the Massey University Microbiology Laboratory. The samples were tested using the standard methods. The results were then compared with the legal requirements (ANZFS Code) and guidance values (NZMRC).

Names of brands were recorded. By purchasing all the samples myself, I ensured that samples were purchased at the specific stores and that five samples of each brand of the same batch were purchased. I maintained a methods' file and relevant notes. The sampling procedures outlined in the NZMRC were followed. Five bottles of each brand of the same batch in duplicates were tested. Results of quality control practices were documented. The programme of laboratory internal quality control controlled all factors, from sample collection through to data collection.

The IDEXX guaranteed the accuracy of detection of Colilert and Enterolert systems. The Colilert *E.coli*, Enterolert group D streptococci, *Pseudomonas aeruginosa*, TVC, yeasts and moulds results were checked by my immediate supervisor in order to eliminate any possible errors. The tests were performed in the Microbiology Laboratory at Massey University in Wellington. Water samples were tested using aseptic techniques. Work area was cleaned and

hands were washed regularly according to quality control and accreditation specifications. All equipment was regularly maintained. The test media for the TVC and yeasts and moulds tests were made on-site with the assistance of experienced Laboratory Technicians (Nutrient Agar plates and SDA plates respectively). The test media for *Pseudomonas aeruginosa* were purchased from the approved supplier (MPA agar plates). Enterolert and Colilert Quanti-Trays and reagents were supplied by the Environmental Diagnostics in Auckland. The test media, trays and reagents were stored and used in accordance with the manufacturer's instructions. Internal media quality checks were regularly carried out.

Equipment and materials required for collection and analysis of samples were provided by the Institute of Food, Nutrition and Human Health, Massey University at Wellington, New Zealand. The equipment used such as UV light and pipettors, had been calibrated by Massey University staff as per manufacturer's instructions at the required time intervals and in accordance with the quality control protocol.

The bacterial cultures that were used for positive and negative controls were maintained according to the supplier's instructions and as per Massey University quality control procedure. The results of Colilert *E.coli*, Enterolert group D streptococci, *Pseudomonas aeruginosa*, TVC and yeasts and moulds were checked by my immediate supervisor in order to eliminate any possible error.

The data obtained were analysed with the assistance of an experienced statistician.

Results of quality control practices were documented. The programme of laboratory internal quality control controlled all factors, from sample collection through to data collection.

2.6. Limitations

In order to generate results for establishing compliance with New Zealand Microbiological Reference Criteria for Food and Australia/ New Zealand Requirements for the Microbiological Compliance of Bottled Water under the Food Standards Code five samples, which is a minimum number of samples, of each brand were tested in duplicates. The above number of samples may have not been sufficient for the accurate and detailed assessment of the bottled water microbiological quality.

Not every manufacturer of the 38 bottled water brands tested responded to the survey questionnaire.

2.7. Ethical Issues

As no participants were involved in this study, this research was of a 'low risk'. Nevertheless, an Ethic's Committee approval was obtained (Appendix VI).

2.8. Legal Issues

All results obtained in the study were for research purposes only and were not for the use of the public, Local Authorities or Public Health Services unless there would have appeared to have been a major public health risk from the findings of this study.

CHAPTER THREE- MICROBIOLOGICAL TESTING OF BOTTLED WATER RESULTS AND DISCUSSION

This chapter outlines the microbiological quality testing results of the 38 brands of bottled waters tested. All bottled water brands tested are described in Section

3.1 below. Compliance with the New Zealand Microbiological Reference_Criteria for Food and Australia and New Zealand Food Standards Code is discussed in sections 3.2 and 3.3 respectively. In Section 3.4 additional microbiological testing, namely testing for yeasts and moulds (3.4.1) and *Campylobacter spp* (3.4.2) is discussed.

3.1 Description of Bottled Water Brands Sampled During this Study

Out of thirty eight bottled water brands tested 68.4% of the brands (26 brands) were domestic and 31.6% of the brands (12 brands) were imported. 50% of the imported brands originated from Italy, 25% from Australia, 17% from France and 8% from Austria (Table 3-1).

The containers of the bottled water brands purchased varied. 76.3% of containers were plastic and 23.7% were glass. 71% of brands were bottled in clear bottles and 29% of brands in coloured bottles. Out of coloured bottles 18.4% of bottles were dark green, 5.3% were green and 5.2% were dark blue (Table 3-1).

44.7% of brands had been bottled in bottles with a pump cap, 36.8% with a plastic cap and 18.4% with a metal cap. 15.8% of the bottled water brands were bottled in bottles sealed with a thin metallic foil prior to capping (Table 3-1).

Table 3-1. Descriptive Characteristics of Tested Bottled Water Brands.

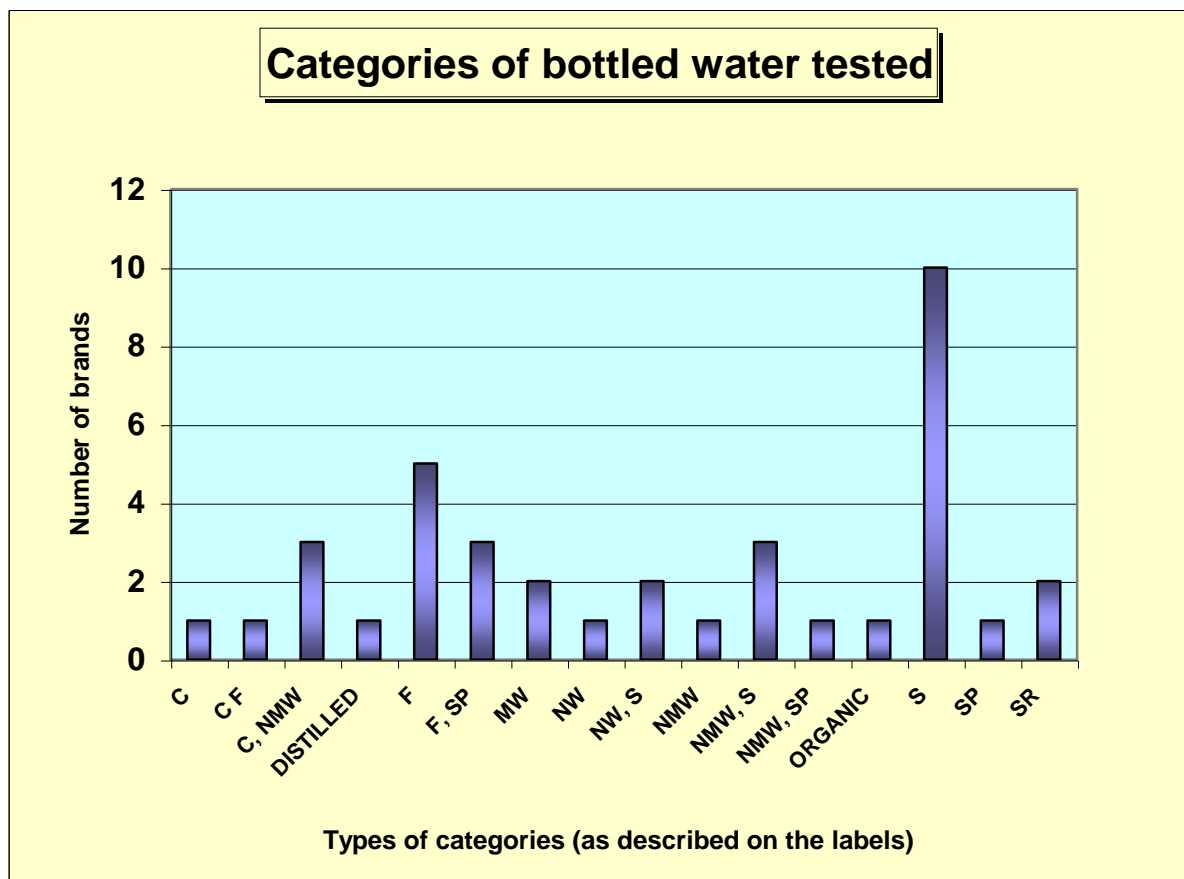
Brand No	Domestic (D) /Imported (I)	Bottles: Glass (G), Plastic (P)	Bottle Colour	Caps: Plastic (P), Pump (PU), Metal (M)	Bottle Sealed (Y/N)	Categories (determined from the label): **
1	D	P	Clear	P	N	S
2	D	P	Clear	P	N	NW
3	D	P	Clear	P	N	SP

4	D	P	Dark green	P	N	MW
5	I (Australia)	P	Clear	PU	Y	F
6	D	P	Clear	PU	N	S
7	D	P	Clear	PU	N	S
8	D	P	Clear	PU	N	S
9	D	P	Clear	PU	N	S
10	I (Italy)	G	Dark green	M	N	C, NMW
11	I (France)	P	Clear	P	N	S
12	D	P	Clear	P	N	SR
13	D	P	Clear	PU	Y	F
14	D	P	Clear	PU	N	S
15	I (Australia)	P	Clear	P	N	S, SW
16	I (Australia)	P	Clear	P	N	Organic
17	D	G	Clear	M, Screw-On	N	F, SP
18	D	P	Clear	PU	N	Distilled
19	D	G	Dark green	M, Screw-On	N	SP, F
20	D	P	Clear	PU	N	MW
21	I (Italy)	G	Dark blue	P	N	C
22	D	P	Clear	PU	N	S
23	I (Italy)	G	Dark blue	P	N	S
24	I (Austria)	P	Clear	P	N	S
25	D	P	Clear	PU	Y	F, SP
26	I (Italy)	G	Dark green	M, Beer	N	NMW, SP
27	I (Italy)	G	Dark green	M, Beer	N	S, NMW
28	I (France)	G	Dark green	M, Screw-On	N	C, F
29	D	P	Dark green	PU	Y	F
30	I (Italy)	G	Dark green	M, Beer	N	C, NMW
31	D	P	Clear	P	N	S
32	D	P	Clear	PU	Y	F
33	D	P	Clear	PU	Y	F
34	D	P	Clear	P	N	S, NMW
35	D	P	Clear	PU	N	SR
36	D	P	Clear	P	N	NMW
37	D	P	Green	PU	N	S, NMW
38	D	P	Clear	P	N	C, NMW

** Carbonated (C), Still (S), Sparkling (SP), Natural Mineral Water (NMW), Flavoured (F), Spring (SR), Natural Water (NW), Mineral Water (MW).

The different categories of the bottled water brands are shown in Figure 3-1 below. The descriptions of categories were obtained from the labels.

Figure 3-1. Categories of Bottled Water Brands as Described on the Labels.



NB: Some brands belong to more than one category due to labelling wording, for example, carbonated natural mineral water, flavoured spring water.

3.2. Compliance of Microbiological Testing Results with New Zealand Microbiological Reference Criteria for Food

3.2.1. Compliance of *Escherichia coli* Counts

All thirty eight bottled water brands tested negative for *Escherichia coli*.

3.2.2. Compliance of Coliforms Counts

Three out of the thirty eight brands tested failed to comply with the Coliform criteria of New Zealand Ministry of Health Microbiological Reference Criteria for Food Guidelines for total coliforms (Table 3-2). The three brands that failed were Brand No. 7, 9 and 17 (Table 3-1). These three brands were domestic New Zealand brands. All imported brands complied with the Coliform criteria of the New Zealand Ministry of Health Microbiological Reference Criteria for Food Guidelines (1995).

Table 3-2. Coliform Counts of Domestic New Zealand Brands that did not Comply with the New Zealand Ministry of Health Microbiological Reference Criteria for Food Guidelines.

Brand Nr	Average count, CFU/ml
17	>200
7	31
9	21

The presence of total coliforms indicated contamination and the possibility of presence of other pathogenic microorganisms. All other tested bottled waters had the total coliforms levels of less than 1 CFU/ml; hence they were compliant with the NZMRC for Packaged Waters.

Brands 7 and 9 that failed to demonstrate compliance with the New Zealand Ministry of Health Microbiological Criteria for Food Guidelines for coliforms were bottled in clear plastic bottles and had pump caps on. Out of thirty eight brands seventeen bottled water brands were bottled with the pump caps on. The third brand (brand 17) was bottled in a clear glass bottle with a metal screw-on top. Out of the remaining thirty five brands that complied for coliforms with the New Zealand Ministry of Health Microbiological Reference Criteria for Food Guidelines, eight were bottled in glass bottles and twenty seven in plastic bottles.

All three domestic brands that failed to comply with the NZMRC were bottled in clear bottles. A total of twenty seven brands were bottled in clear bottles, both glass and plastic. Eleven brands were bottled in dark bottles. All bottled water brands that were bottled in dark bottles complied with the New Zealand Ministry of Health Microbiological Reference Criteria for Food Guidelines for coliforms.

A representative sample of five water bottles per batch, of the brands 17 and 9 were purchased for retesting. More samples of brand 7 could not be purchased as this brand was no longer available in retail outlets. It was thought that that particular brand was a promotional bottled water brand at the time. On retesting the representative samples of the different batches of Brands 17 and 9 for coliforms there were no coliform counts observed.

All bottled water brands that were bottled in bottles sealed with a thin metallic foil prior to capping complied with the coliform criteria of the New Zealand Ministry of Health Microbiological Reference Criteria for Food (1995).

3.2.3. Compliance of *Enterococci* Counts

While in our study all thirty eight brands tested during the course of the study were negative for Enterococci (Group D *Streptococci*), Venieri et al. (2006) isolated Enterococci in Greek bottled waters. In this study the presence of Enterococci indicated possible fecal contamination of Greek bottled waters. We have not detected any Enterococci in the 38 brands tested in our study.

3.2.4. Compliance of *Pseudomonas aeruginosa* Counts

All thirty eight brands of bottled water were tested in duplicates using a spread method on MPA agar media as described in Chapter Two (Section 2.3.4). While we did not detect any *Pseudomonas aeruginosa* in any of the domestic and imported brands that we tested, Reyes et al. (2008) isolated *P. aeruginosa* in

bottled waters sold in Puerto Rico and Venieri et al. (2006) isolated *Pseudomonas aeruginosa* in Greek bottled waters.

3.3. Compliance of Microbiological Testing Results with the Australia and New Zealand Food Standards Code

3.3.1. Overview

Microbiological Compliance Criteria of Mineral Water and Packaged Water & Ice with the Australia and New Zealand Food Standards Code (2002) have been described in detail in Chapter One, Section 1.2.3.

While New Zealand Ministry of Health Microbiological Reference Criteria for Food prescribes microbiological limits for packaged waters, including mineral waters and those bottled from natural underground sources only, Standard 1.6.1 – Microbiological Limits for Food of the Australian New Zealand Food Standards Code (2002) lists separate microbiological limits for mineral water, packaged water and packaged ice.

3.3.2. Compliance of Coliforms Counts

Three out of the thirty eight brands tested failed to comply with the coliform criteria of Standard 1.6.1 – Microbiological Limits for Food, Australian New Zealand Food Standards Code (ANZFSC) for total coliforms. The three brands that failed to comply with the ANZFSC were the same brands that did not comply with the NZMRC (Section 3.2.2.). All imported brands complied with the coliform criteria of this standard.

Representative samples of the brands 17 and 9 were purchased for retesting and after retesting coliform counts in these two brands were not observed. More

samples of brand 7 could not be purchased as this brand was no longer available (Section 3.2.2.).

3.3.3. Compliance of *Pseudomonas aeruginosa* Counts

All thirty eight brands of bottled water were tested in duplicates using a spread method on MPA agar media as described in Chapter Two (Section 2.2.3.3). All samples tested were negative for *Pseudomonas aeruginosa*.

3.3.4. Compliance of Total Viable Counts (TVC)

To fully assess the compliance with the ANZFS Code, the bottled water samples were examined for the Heterotrophic Plate Count (HPC), which can also be referred to as Total Viable Count (TVC) or Standard Plate Count (SPC) (Table 1-6).

All thirty eight brands purchased were tested for the Total Viable Counts (TVC) as described in Section 2.2.3.2. TVC growth was observed in 24 brands tested, 14 domestic brands and 10 imported brands (Table 3-3).

In our study the HPC counts ranged from 5 to too numerous to count (TNTC) CFU/ml. The following 17 brands (45 %) exhibited HPC counts of over 100 CFU/ml and thus failed to comply with the TVC criteria of the Australia and New Zealand Food Standards Code for packaged waters: 7, 9, 10, 11, 12, 14, 15, 19, 21, 22, 23, 26, 27, 28, 29, 34 and 36 (Table 3-3). HPC has been used for the microbiological quality monitoring of bottled waters in other countries (Kassenga, 2007; Venieri et al., 2006; Bharath et al., 2003; Jeena et al., 2006). Jeena et al. (2006) concluded that high levels of HPC bacteria with multiple drug resistance posed a significant health hazard to the immunocompromised consumers. Papapetropoulou et al. (1997) also stressed that when bottled water is going to be consumed by immunocompromised patients the

environmental mycobacteria counts in bottled water is a useful guide of the hygienic quality.

Table 3-3. Average TVC Counts Obtained after Testing the 38 Bottled Water Brands.

	Brand numbers	TVC Average Counts, CFU/ml (Accepted microbiological level is 10 ² CFU/ml)	Manufacturers that responded to the survey
Brands that complied with the ANZFS Code for TVC	38	1	
	35	13	
	37	17	
	24, 30	20	
	17	30	
	8	40	
Brands that did not comply with the ANZFS Code for TVC	36	137	Y
	10	350	
	23	1630	
	12	1740	
	9	2290	Y
	7	2410	Y
	22	2580	
	11	2670	
	34	2950	Y
	19	3090	
	14	3380	
	15	3540	
	21, 26, 27, 28, 29	TNTC	Y (26)

Even though 17 brands were found to exceed the TVC limit prescribed by ANZFA Food Standards Code, the fact that no *E.coli* had been isolated is reassuring. As mentioned previously (Sections 3.2.2. and 3.3.2.), while brands.

7, 9 and 17 displayed coliforms at the time of the initial testing, no coliforms were detected upon retesting.

As a pump cap may be a factor affecting the bottled water microbiological quality, it was interesting to note that out of 14 brands that had pump caps on five brands (brands 7, 8, 14, 22 and 29) grew TVC counts (Table 3-3) of one or more colonies per ml (Figure 3-2). Out of the 24 brands that did not have pump caps, 17 brands (9, 10, 11, 12, 15, 17, 19, 21, 23, 24, 26, 27, 28, 30, 34, 35 and 36) displayed TVC growth of one or more colonies per ml.

Figure 3-2. TVC colonies*.



*- This is a typical example of TVC colonies plate of contaminated bottled water.

It would appear that sealing the bottle with a thin metallic foil prior to capping is a relatively satisfactory measure to control the microbiological quality of bottled water. Out of 38 bottled water brands tested six brands were bottled in the sealed bottles. We observed TVC counts of one or more colonies per ml in only one brand (brand 29) (Table 3-3) out of six brands that were bottled in bottles sealed with a thin metallic foil prior to capping.

Out of 17 brands that failed to comply with ANZFS Microbiological Compliance Criteria for Packaged Water and Ice for TVC, seven brands (brands 10, 23, 19, 21, 26, 27 and 28) were bottled in glass bottles and ten brands (7, 9, 11, 12, 14, 15, 22, 29, 34 and 36) were bottled in plastic bottles.

The brands that did not comply with the New Zealand Microbiological Reference Criteria for Food and the Microbiological Standard for Mineral Water and Packaged Water and Ice of the Australian New Zealand Food Standards Code (brands 9 and 17) for coliforms were repurchased and retested for all microbiological parameters.

While initially the samples of brand 9 displayed the average TVC counts of 2.2×10^3 CFU/ml, after the subsequent retesting of brand 9 the average TVC counts of 5.6×10^3 CFU/ml were displayed. This again did not comply for TVC with the Microbiological Standard for Mineral Water and Packaged Water and Ice of the Australian New Zealand Food Standards Code.

While initially the samples of brand 17 displayed the average TVC counts of 30 CFU/ml, after the subsequent retesting of brand 17 the average TVC counts of 2 CFU/ml were displayed. Both results, those of the original testing and retesting complied with the Microbiological Standard for Mineral Water and Packaged Water and Ice of the Australian New Zealand Food Standards Code for TVC.

Out of the fifteen brands that failed to demonstrate compliance with the Microbiological Compliance Criteria of Packaged Water of the Australia and New Zealand Food Standards Code for TVC, nine were domestic New Zealand brands (brands 7, 9, 12, 14, 19, 22, 29, 34 and 36).

All mineral water brands (brands 4, 10, 20, 26, 27, 29, 34, 36, 37 and 38) complied with Microbiological Compliance Criteria of Mineral Water of the Code Australia and New Zealand Food Standards for TVC as TVC is not amongst the compliance criteria for mineral water.

In Europe mineral waters are sourced from approved sources and are expected to contain TVC bacteria during their shelf life. Typically European mineral bottled waters are subjected to the microbiological examination within 12 hours

after bottling. Therefore it was not unexpected to detect TVC counts in the European mineral bottled water brands 10, 26 and 27.

3.4. Additional testing

Generally the presence of yeasts and moulds in certain foods may be viewed as a quality and safety indicator of the product being tested. For this reason although yeasts and moulds were not amongst the New Zealand Microbiological Reference Criteria for Food or Microbiological Compliance Criteria of Mineral Water, Packaged Water and Ice of the Australia and New Zealand Food Standards Code, all thirty eight brands were tested for yeasts and moulds.

After careful consideration and taking into account current trends of campylobacteriosis incidence in New Zealand (Appendix II), composite samples of brands that displayed TVC counts of 100 or more CFU/ml were tested for *Campylobacter* spp. at the Environmental Laboratory Services Ltd. (ELS).

3.4.1. Yeast and Mould Testing Results

Yeasts and moulds were enumerated using a spread method on Sabouraud dextrose agar plates as described in Section 2.2.3.4 and subsequently incubating the plates at 25°C - 30°C for 7 days.

Twenty four brands (63%) displayed colonies on Sabouraud dextrose agar plates, of which growth of moulds was observed in 21 brands. Interestingly brands 7, 9 and 17, which did not comply with ANZFS Code and NZMRC for total coliforms, displayed fungal growth. This may point towards the presence of the contaminants at the source of water collection.

The fungi detected in this study were not characterised. While testing for yeasts and moulds was not required under the New Zealand Microbiological Reference Criteria for Food (1995) and the Australia New Zealand Food Standards Code

(2002), the presence of yeasts and moulds does give a general indication of process safety and the level of quality control (Hasell and Capill, 2000). Previously fungal contamination arising from multiple sources, such as *Penicillium spp.*, *Cladosporium spp.* and *Trichoderma spp.*, have been isolated in a water bottling plant (Ribeiro et al., 2006). *Aspergillus spp.* and *Paecilomyces spp.* were isolated from Indian bottled waters (Lal and Kaur, 2006). Several foodborne fungi have the ability to produce mycotoxins, which may be responsible for allergic reactions or cause infections, especially in immunocompromised persons, HIV-infected individuals or patients on chemotherapy (USFDA, 2001). Therefore eliminating the presence of fungi is potentially an important public health concern.

While this study was based solely on previously unopened bottled water, work done by Raj (2005) pointed towards the potentially progressive bacterial growth in bottled waters once bottles were opened. Raj (2005) suggested the development of guidelines for refrigeration and expiration time once a bottle is opened.

3.4.2. *Campylobacter spp.* Testing Results

Composite samples of fifteen brands that displayed TVC counts of 100 CFU/ml or higher were tested for *Campylobacter spp.* The following brands were tested for *Campylobacter spp.*: 21, 22, 23, 26, 27, 28, 29, 34, and 36.

Samples were tested at the Environmental Laboratory Services Ltd. (ELS), Seaview, Lower Hutt, New Zealand. ELS is an International Accreditation New Zealand (IANZ), Laboratory Approval Scheme (LAS) and Ministry of Health approved testing laboratory that offers a broad range of microbiological and inorganic chemistry analyses.

The *Campylobacter spp.* isolation method used by ELS is found in Appendix III. Once the tests were performed and testing results obtained, they were then

immediately recorded in the laboratory book. Subsequently the results were transferred into the Microsoft Excel sheet.

Campylobacter spp. was not isolated in any of the bottled water samples tested.

CHAPTER FOUR. RESULTS AND DISCUSSION OF BOTTLING WATER PLANTS SURVEY

A survey questionnaire (Appendix I) was designed and distributed to 32 bottled water manufacturers and importers of the 38 water brands tested in this study. I developed this unique survey using my knowledge, experience and expertise in the fields of health protection, public health and water treatment. I designed the questions in order to obtain information on crucial stages of the bottled water manufacturing, for example source water quality, maintaining hygienic practices during the bottling process, and to establish whether Good Manufacturing Practice (GMP) had been followed by the manufacturers. Some manufacturers were bottling or importing more than one brand and hence a total of 32 bottled water manufacturers were identified. The addresses of the manufacturers were obtained from the labels on the water bottles. The aim of the survey was to investigate any possible significant public health links between the source water quality, type of abstraction, pipework materials, bottling process, staff training, policies and procedures. The data was analysed using EpiInfo analytical package as described in Section 2.4.

Manufacturers representing 29% of the bottled waters tested responded to the survey. The four manufacturers that responded to the questionnaire covered 11 of the bottled water brands that we tested. Out of the four manufacturers that responded to this survey there was one domestic manufacturer and three international manufacturers. Three responses were received by post and one by email.

Out of the four manufacturers that responded to the survey questionnaire one European manufacturer (Manufacturer 3) employed three hundred people and one New Zealand manufacturer (Manufacturer 4) employed three people and was a family owned and run business. The other two international manufacturers in their New Zealand facilities employed eight and nine people respectively

Manufacturer 1 and 2 were international manufacturers (Table 4-1). Manufacturer 1 represented 5 of the 38 brands tested (Brands 2, 6, 8, 12 and 35) and Manufacturer 2 covered 4 of the 38 brands tested (Brands 3, 5, 9 and 34). Manufacturer 3 manufactured the international brand 26 and Manufacturer 4 bottled domestic brand 36. Ten domestic brands were bottled by the four manufacturers. These brands were 2, 6, 8, 12, 35, 5, 3, 9, 34 and 36. One imported brand (brand 26) was manufactured by an international manufacturer. A manufacturer of one domestic brand that did not respond to the survey replied that the information was commercially sensitive and therefore could not be supplied.

One manufacturer of an imported bottled water brand did not fully respond to the survey, but stipulated that their company was producing two types of energy drinks and did not actively produce bottled water. Their head office, which was based in Europe, had used a particular brand of bottled water for the promotional “full moon parties” where the product was free to the bar and not available outside of those promotions. Nevertheless, as a part of this study this particular brand had been purchased in New Zealand.

The responses to the survey revealed that the period of time in which the manufacturers have been in the business of bottling water was rather varied. Manufacturer 1 has been in the manufacturing business for eight years, Manufacturer 2 for seventy five years, the foreign manufacturer that responded to this survey by e-mail (Manufacturer 3), has been in the bottled water manufacturing business for one hundred and ten years and the remaining New Zealand manufacturer (Manufacturer 4) has been in business for seven years.

According to the results of our survey the manufacturers of 5 brands that returned the questionnaire carry out other activities in addition to the bottle water manufacturing. The manufacturers of the remaining 6 brands did not carry out any other activities. The New Zealand manufacturer (Manufacturer 4) grows avocados and Manufacturer 2 makes juices and soft drinks, which constitutes 80% of its activities.

The manufacturers of the eleven brands that responded to the questionnaire specified the sources of water being bottled. The sources of water for these 11 brands were bore water (82%), artesian bore (9%) and municipal water supply (8%) respectively (Table 4-1).

All manufacturers reported that the process line of water abstraction included backflow prevention devices. During the bottling of 10 out of 11 brands, non-return valves (NRV) had been fitted following the abstraction. The manufacturer of one brand did not specify the type of backflow prevention device in place.

Table 4-1 lists the types of abstraction methods used by the manufacturers of the 11 bottled water brands. The bottled water of brand 26 was abstracted by gravity and the domestic brand 36 used a pump. The water for the domestic brands 2, 6, 8, 12 and 35 was abstracted by pump and gravity. Water for brands 3, 9 and 34 was abstracted under pressure. Brand 5 was bottled using town water supply.

The pipework materials play a significant role in inhibiting the growth of bacteria. Some bacteria are capable of using organic compounds as a source of energy and are therefore able to grow and multiply in the PVC pipes. Some species of bacteria, such as *Legionella spp.*, are able to use rust flakes as a source of energy. Other bacteria can form biofilms, which may support the bacterial growth. If the water is not disinfected, these bacteria may contaminate the bottled water.

The manufacturing process for all 11 brands employed stainless steel pipework (Table 4-1). However, one manufacturer (Manufacturer 1) in addition to the

stainless steel pipework (brands 2, 6, 8, 12 and 35) had PVC pipework in place and another manufacturer (Manufacturer 2) had metal pipework in place in addition to the stainless steel pipework (brands 3, 5, 9 and 34).

Out of the five brands that were processed using stainless steel and PVC pipework, brand 12 displayed TVC counts that did not comply with the ANZFS Code (Table 3-3). Brands 8 and 35 displayed TVC counts within the compliance levels.

The findings of the survey indicated that the water used for 10 out of 11 brands was treated with methods ranging from filtration alone, combination of filtration and ozonation to filtration and UV (Table 4-1). The Italian sparkling natural mineral water that was not treated (brand 26) displayed non-compliant levels of TVC (Table 3-3). As it was a mineral water brand and therefore while coming from an approved source, but not treated, we expected to observe higher levels of TVC counts. Ten brands out of eleven bottled treated water, brand 26 being the only brand that did not treat the water prior to bottling.

As none of the manufacturers supplied any details regarding the type of filtration used, it was not possible to establish whether the elimination of Protozoa had been considered and addressed in their manufacturing plants.

In the context of food legislation, bottled water is described as food. Therefore, the manufacturing of bottled water is a food manufacturing process. In Australia and New Zealand, all food businesses are required to have either a Food Safety Programme in place or to register as a food premise with the Local Authority. All food businesses must identify potential hazards within the business and to employ all necessary control measures required to manage them. A 'control of hierarchy' principle, where a hazard should preferably be eliminated and only if that is not possible other remedial measures are to be considered, should be used. This approach of identifying and controlling hazards is called HACCP (Hazard Analysis and Critical Control Points) and the New Zealand Ministry of Agriculture has been advising of its usefulness since 1997 (Hathaway and Cook, 1997).

In the bottled water manufacturing process, one of the Critical Control Points (CCPs) is the sterilisation of bottles. Out of the brands that responded to the questionnaire brand 26 was the only brand bottled in glass bottles. The remaining 10 brands were bottled in plastic bottles. Brand 26 was bottled in dark green glass bottles (Table 3-1). The other 10 brands were bottled in the clear plastic bottles. Only one brand (brand 5) was bottled in a sealed bottle. This brand demonstrated full compliance with all microbiological criteria. The results of the survey indicated that only six (brands 5, 3, 9, 34, 26, 36) out of the 11 brands were bottled in sterilised bottles. The methods of sterilisation used were autoclaving (brand 36), high temperature and caustic washing (brand 26) and rinsing in ozonated water (brands 5, 3, 9, 34) (Table 4-1). The manufacturer of five brands did not specify the method of sterilisation (brands 2, 6, 8, 12, 35).

One brand (brand 36) was bottled by hand and the remaining brands were bottled using an automated process.

Chemicals that were used to clean the equipment included Oxonia active/Savicol/AC 3000 (brands 2, 6, 8, 12, 35), Peracetic acid and specific sanitisers (brands 5, 3, 9, 34), Peracetic acid (brand 26) and Clor-o-gene (brand 36).

Manufacturers of all brands reported that they had batch tracking systems and Food Safety Programmes in place.

All manufacturers responded that they did carry out microbiological monitoring and testing of their final product (Table 4-1); however they did not specify the frequency of monitoring. No manufacturers confirmed whether they carried out water testing at the source. The sources of water for the 11 brands included bore water, artesian bore or municipal water supply (Table 4-1). All four manufacturers carried out testing for *E. coli* and coliforms. Three manufacturers (1, 2 & 4) that represented 10 brands further tested their final product for *P. aeruginosa*. Manufacturers 1 and 3 included testing for yeasts and moulds and manufacturers 1 and 2 carried out testing for Enterococci. In addition to the microbiological testing, manufacturer 2 (brands 3, 5, 9 and 34) daily tested

bottled water for pH and conductivity, annually carried out a full chemical analysis and conducted radiological testing every four years.

In this study, five brands (brands 9, 26, 34, 35 and 36) out of 11 that responded to the survey were tested for *Campylobacter spp.* This was because they displayed TVC counts equal or above 100 CFU/ml. These TVC levels did not comply with the ANZFS Code for TVC for packaged water. Composite samples of all five purchased bottles of each brand were made and *Campylobacter spp.* testing was carried out in the Environmental Laboratory Services Ltd. *Campylobacter spp.* growth was not detected in any of the composite samples.

The results of the survey demonstrated that the manufacturers of brands 2, 6, 8, 12, 35, 26 carried out microbiological tests for yeasts and moulds. While this is not a guideline value under the New Zealand Microbiological Reference Criteria for Food (1995) nor a legal requirement of the Australia and New Zealand Food Standards Code (2002), the presence of yeasts and moulds do give a general indication of process safety and quality. During this study yeasts and moulds were detected in seven brands (brands 6, 8, 9, 12, 26, 34 and 36) that responded to the survey (Table 4-1). One brand (brand 6) was identified as having grown moulds. Detection of yeasts or moulds in these brands indicated that all manufacturers needed to carefully consider their process control at the respective bottling and processing plants.

All manufacturers that responded to the survey stated that they provided staff training. The staff were trained by means of courses, in-house training and using a 'buddy system'. The staff were trained in food safety, maintenance of equipment, the use of machinery and safety at work.

All manufacturers that responded to the survey reported that they had procedures in place for sourcing water, transporting water, bottling water, delivery and dispatch of bottled water, water protection against contamination at the source, water protection against contamination during transportation of bottled water, water protection against contamination during bottling, cleaning of pipework and tanks, cleaning of bottling equipment and staff health. None of the

manufacturers supplied copies of their procedures or indicated encountering any problems with their operation.

The manufacturer of one brand (brand 9) that responded to this survey failed to comply with the Coliform requirements prescribed by the New Zealand Ministry of Health Microbiological Criteria for Food 1995 (Chapter 3). The water that is used to produce this brand was sourced from a bore, treated prior to the bottling and was reported to have been bottled in sterilised bottles.

Out of the 11 brands bottled by the four manufacturers that responded to the survey, five brands (36, 12, 9, 34, and 26) did not comply with the ANZFS Code for TVC (Table 3-3). Out of those five brands only manufacturer of brand 12 did not specify the method of sterilisation. The remaining four bottled water brands were bottled in sterilised bottles, either by rinsing them in ozonated water (brand 9 and 34), subjecting the bottles to high temperature and caustic washing (brand 26) or autoclaving (brand 36) (Table 4-1). We found that all four manufacturers bottled at least one brand that did not comply with the ANZFS Code for TVC (Table 3-3).

No brands in bottles with metal caps complied with the ANZFS Code for TVC criteria. Out of 11 brands that responded to the survey, only bottles of Brand 26 had a metal cap on (Table 3-1). All brands that passed ANZFS Code for TVC criteria were bottled with the plastic caps on.

Having worked as a Health Protection Officer in the Public Health Units in New Zealand and currently working as a Senior Environmental Health Officer in the United Kingdom I found it interesting and to a degree even alarming that none of the manufacturers indicated encountering any problems with their operation or with environmental health issues. It is also interesting to note that none of the manufacturers discussed or raised any concerns in the 'Comments' section provided, which may indicate a lack of interest in improving processes and practices.

There was no clear indication of whether the piping materials, treatment of water, abstraction methods and sterilisation process had any significance in preventing microbiological growth as it was found that all four manufacturers bottled at least one brand (9, 12, 26, 34, and 36) that did not comply with the ANZFS Code for TVC. The brands that grew mould colonies were 6, 8, 9 and 26. Although the manufacturers of brands 6, 8 and 26 tested their bottled waters for yeasts and moulds, we detected moulds in these brands. Our positive result for growth of moulds in the batches of brands 6, 8 and 26 that we examined may mean that either the manufacturers were not carrying out the microbiological monitoring frequently enough or perhaps they did not test all batches of bottled waters that they produced. As the bottled water of brand 9 was not tested for yeasts and moulds by the manufacturer, it was not unexpected to observe mould counts in this brand. Brand 9 also did not comply for total coliforms with the ANZFS Code and NZMRC.

None of the manufacturers supplied any copies of their procedures for the sourcing water, transporting water, bottling water, delivery and dispatch of bottled water, water protection against contamination at the source, water protection against contamination during transportation of bottled water, water protection against contamination during bottling, cleaning the pipework, tanks and bottling equipment. If all procedures for ensuring satisfactory bottled water quality were adhered to then it was interesting to note that 5 brands representing the four manufacturers that responded to the questionnaire did not comply with the ANZFS Code for TVC.

Due to the low response rate of the questionnaire, no clear links could have been established between the microbiological quality of bottled waters and the source water quality, treatment, bottling process and documentation of the manufacturers. However, even from a low response rate it was clear that either the procedures for preventing microbiological contamination in bottled water were not effective in all cases or Good Manufacturing Practice (GMP) and Hazard Analysis of Critical Control Points (HACCP) were not adopted or implemented. The importance of GMP has been previously highlighted by Defives et al. (1999).

CHAPTER FIVE- CONCLUSIONS, RECOMMENDATIONS AND POSSIBLE FUTURE IMPLICATIONS

5.1. Conclusions

Hasell & Capill (2000) study in New Zealand has demonstrated that New Zealanders should not assume that all bottled water sold in New Zealand is of a satisfactory drinking water standard.

Hasell and Capill (2000) identified that 1 brand out of the 23 surveyed in their study did not comply with the New Zealand Microbiological Reference Criteria for Food (1995) for total coliforms. The authors concluded that their study did not identify any issues of public health concern. However, they reasoned that to control the potential risk factors that may affect bottled water microbiological quality regular microbiological testing was required. In our study we demonstrated that the samples of one batch that represented three (brand 7, 9 and 17) out of the 38 brands did not comply with the New Zealand Microbiological Reference Criteria for Food (1995) and Australia and New Zealand Food Standards Code (2002) for total coliforms. While one batch of each of three brands not complying with the NZMRC (1995) for total coliforms on the surface may seem as a relatively small number, it is crucial to remember that should this bottled water be consumed by very young, elderly or immunocompromised individuals, the consequences may be very serious, if not fatal. With the general public's perception that bottled water is safer than tap water bottled water is often consumed in the hospitals or in emergency situations, such as flooding or earthquakes, when safe and potable municipal water is not accessible. The presence of coliforms was an indicator of hygiene failure. The manufacturer of brand 9 did respond to the questionnaire (Appendix I and Chapter 4). This manufacturer did perform microbiological monitoring for *E.coli* and total coliforms. There was however no indication of how regularly the microbiological monitoring occurred in the plant, but evidently the result of not testing regularly enough resulted in an unsatisfactory bottled water quality end product being sold.

All thirty eight domestic and imported bottled water brands complied with the New Zealand Microbiological Reference Criteria for Food (Food Administration Manual S. 11: Microbiological Criteria Version 2.0, October 1995) for *E.coli*, Group D streptococci and *Pseudomonas aeruginosa*.

Out of all the literature researched for my research project, Venieri et al. (2006) study design proved to be the closest to my study. In that study Enterococci were detected in Greek bottled waters, which indicated possible faecal contamination. This was not the case in the New Zealand bottled water brands tested. However, I designed my study prior to Venieri et al. (2006) research was published using my knowledge, skills and experience.

All thirty eight domestic and imported bottled water brands complied with the Food Standards Code (Australia and New Zealand) for *Pseudomonas aeruginosa*.

Seventeen bottled water brands, nine of them New Zealand domestic brands, failed to demonstrate compliance for the TVC with the Packaged Water and Ice Criteria of Australia and New Zealand Food Standards Code (2002). I consider this to be of importance as linear relationship between HPC and coliform bacteria had previously been identified (Jeena et al., 2006). Therefore bottled waters should be routinely and regularly monitored for TVC during or immediately after the bottling process to ensure a satisfactory quality control and adequate plant hygiene.

There was no *Campylobacter spp* detected in any of the thirty eight domestic and imported bottled water brands tested.

While yeasts and moulds are not amongst the compliance criteria of ANZFS Code or guideline values of the NZMRC, if detected in bottled waters they could have originated either from a water source, or contaminated plant or bottles. Hassel and Capill (2000) isolated fungi in 5 out of 23 brands tested in their study. In our study 63% of the brands that we tested contained yeasts and

moulds. Brands 9 and 36 grew fungi. The manufacturers of these two brands stated that they did not test their bottled water for yeasts or moulds, so it was not surprising that brands 9 and 36 tested positive for yeasts and moulds. Although the manufacturers of brands 6, 8, 12 and 26 did test their bottled waters for yeasts and moulds, fungal growth was detected in these brands. Again this suggests that regular microbiological monitoring was not occurring resulting in bottled water of poor microbiological quality being sold.

In this study TVC was examined in the bottled waters, as this is a compliance criterion of the ANZFS Code (2002) for Packaged Water and Ice. Seventeen bottled water brands, nine of them New Zealand domestic brands, failed to demonstrate compliance for TVC. Since high TVC counts may indicate unsatisfactory water quality with possible public health issues, they should be monitored as a matter of quality control. From the information on the treatment processes of brands that responded to the questionnaire it was noted that the brands that had filtration and ozonation in place complied with the ANZFS Code (2002) for HPC. The brands that were subjected to filtration and UV treatment (brands 9 and 24), filtration only (brand 36) or no treatment at all (brand 26) did not comply with the ANZFS Code (2002) for TVC. It was not unexpected to observe non-compliance of TVC in brand 26 as it was a natural mineral water brand and it would not have been subjected to any treatment to remove or destroy microorganisms (Armas & Sutherland 1999).

In addition to subjecting the bottled water samples for microbiological testing, a questionnaire to examine the impact of the manufacturing practices on the microbiological quality of bottled waters tested was performed. There was a low response rate and questionnaires from only four manufacturers representing 11 of the bottled water brands tested were received. Even though most manufacturers (Table 4-1) carried out microbiological monitoring, contaminated bottled water brands were still identified, demonstrating that GMP procedures were not fully effective. These findings support the conclusions made by Zamberlan da Silva et al. (2008) who highlighted the need for an improved surveillance system in the bottled water industry.

Microorganisms were detected in both glass and plastic (PVC) bottles, which is consistent with the findings of the study carried out by Massa et al. (1997). This indicated that the type of bottled water packaging did not influence the prevention of microbial growth.

This Master's research study demonstrated that New Zealanders should not assume that all batches of all bottled water brands sold in New Zealand is of a satisfactory drinking water standard. Some domestic and international brands tested did not comply with the NZMRC. This project has supported the previous study performed in late 1990's in New Zealand and has demonstrated that we have not learnt from the results of the previous study as we are still detecting microbiological contamination in bottled waters 10 years later. The microbiological monitoring procedures of bottled waters sold in New Zealand need to be reassessed. This study also supported the need for a review of the microbiological quality monitoring criteria in order to ensure safe and acceptable quality of bottled waters around the world.

5.2. Recommendations

During my literature review I noted that several authors raised the importance of Good Manufacturing Practice (Defives et al., 1999), indicated the need to improve Hazard Analysis Critical Control Points (HACCP) systems (Kokkinakis et al., 2007) and highlighted the need for an improved surveillance system for the bottled water industry (Zamberlan da Silva et a., 2008). Some studies, such as Ribeiro et al. (2006) recommended the implementation of a HACCP programme to improve quality control and the introduction of the Best Practice Guidelines.

While New Zealand Microbiological Reference Criteria for Food (1995) Guidelines would appear more stringent than the Australia New Zealand Food Standards Code Requirements for the Microbiological Compliance of Bottled

Water under the Food Standards Code, it is important to note that the Microbiological Reference Criteria are not part of New Zealand law. They are to be used where no standard exists in law to monitor the microbiological safety of a manufacturing process or the safety of a food. They may be used as supplements to existing standards where public health concerns dictate.

Microbiological Standards were part of the New Zealand Food Regulations 1984, which clearly established a microbiological content or level that it was unlawful to exceed. They were legislative and mandatory. The Food Regulations 1984 were revoked on 20 December 2002 and the Food Standards Code came into full effect with the transitional provisions. This was in order to allow stock in trade to be sold for a further 12 months and 24 months in the case of long shelf-life foods. Food Standards Code 2002 is the legal instrument that incorporates the Australia New Zealand Food Standards Code into New Zealand law. The Code separates the new microbiological standard into the standard for mineral water and standard for packaged water and ice.

As an outcome of my research I recommend the following:

1. I recommend that the current microbiological quality standards, which are a part of Food Standards Code, are revised to cover microbiological quality of mineral water and that of packaged water and ice combined.
2. I also recommend that more stringent microbiological testing criteria are applied. Currently mineral waters are subject to Coliform and *Pseudomonas aeruginosa* testing criteria, while packaged water and ice are also subject to TVC control.
3. More regular routine microbiological monitoring should be adopted by the water bottling operators.
4. Jeena et al. (2006) found that the majority of the Heterotrophic Plate Count (HPC) bacteria strains acquired resistance against antibiotics. Since bottled drinking water is a ready to drink commodity, the high levels of heterotrophic bacteria with multiple drug resistance poses a significant health hazard to the consumers, especially to immunocompromised individuals. Any future studies of bottled water

microbiological quality in New Zealand should include TVC in the microbiological testing criteria.

5. Yeasts and moulds should be included in the Microbiological Reference Criteria for Packaged Water and Ice and for Mineral Waters under the Australia and New Zealand Food Standards Code as a measure of product safety and process quality control.
6. The findings of the survey indicated a need to develop a Code of Practice for the water bottling operators in Australia and New Zealand. Any initiative on development of a joint Australia and New Zealand Code of Practice for Bottled Water Manufacturers is to be congratulated. A Code of Practice with a special legal status should be developed and implemented to regulate bottled water manufacturing and the microbiological quality of bottled water in New Zealand.

5.3. Possible Future Implications

The development of a joint Australia and New Zealand Code of Practice for water bottling operators may take some time.

Once more stringent microbiological criteria are applied and implemented some bottled water brands may fail to comply with the new standard. Some brands may be required to invest back into their bottling water plant or operation, whether regarding staff training, procedural matters, covering the cost of increased microbiological testing or upgrading the plant and the operation.

A development of a joint Australia and New Zealand Code of Practice for Bottled Water Manufacturers would not only provide best practice, advice and guidance to the manufacturers but would further demonstrate commitment to the "Food Standards Treaty", which Australia and New Zealand signed in 1995 with the vision to develop and implement a single set of food standards. The underlying aims of the joint system were to consider the needs of both New Zealand and Australia, to protect the public health of both countries and to reduce unnecessary barriers to trade. The outcome of the Food Standards

Treaty was the development of the joint Australia New Zealand Food Standards Code based on the review of the Australian Food Standards Code, undertaken by FSANZ. The Food Standards Code was adopted in New Zealand in February 2001 and took full effect on 20 December 2002. Thus a development of a joint Australia and New Zealand Code of Practice for Bottled Water Manufacturers would take us one step further towards protection of public health in both countries.

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<http://www.norlandintl.com/blog/2009/04/types-of-bottled-water.html>. Accessed on 24/09/2009.

http://www.premiumrefreshment.com/bottled_water/types_bottled_water.aspx.

Accessed on 24/09/2009.

<http://www.unwater.org/worldwaterday/about.html>. Accessed on 09/09/2009.

<http://www.wisegeek.com/what-is-epi-infotrade.htm> - 21k - [Cached](#). Accessed on 29/09/2008.

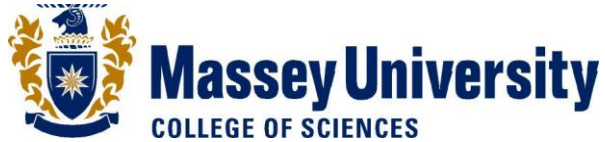
<http://www.which.co.uk/advice/switching-from-bottled-to-tap-water/tap-vs-bottled-water/index.jsp>. Accessed on 29/11/2008.

<http://www.which.co.uk/environment-and-saving-energy/environment-and-greener-living/guides/switching-from-bottled-to-tap-water-tap-vs-bottled-water>.

Accessed on 27/09/10.

APPENDICES

Appendix I. A Copy of the Survey Questionnaire.



I. Contact details

1. Trading name
-
2. Physical address:
-
- 3 .Phone:.....(Business)Fax:.....
- E-mail:
4. How many brands of water do you bottle and/or import?.....
5. What are the brands that you import or sell?.....
6. How long have you been operating the current business?.....
7. 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
- Other (specify).....
2. Location of the source:
3. Location of 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 (circle one)(specify other)

Containers Yes No

If yes, are the containers stainless steel/PVC/other (circle 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

Other.....

Other (please describe in detail or attach protocols if possible):.....

V. Bottles.

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

2. What volume: 250ml/300ml/500ml/600ml/750ml/800/1L/1.5L/2L/5L/10L/20L?(circle all that apply)?

Other.....

3. Do the bottles have a plastic screw-on cap/pump top/metal beer top/metal screw-on top? (Circle all that apply)

Other.....

3. Where are the bottles purchased from?.....

4. Are the bottles sterilised by the supplier?

	<input type="checkbox"/> Yes	<input type="checkbox"/>	No
If Yes, how: Autoclaving	<input type="checkbox"/> Yes	<input type="checkbox"/>	No
Gamma irradiation	<input type="checkbox"/> Yes	<input type="checkbox"/>	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 bottling? Yes No

3. Is the water chilled post 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? Yes No

2. Do you test microbiological quality of the water that you bottle?

Yes No

If yes, what tests are routinely undertaken?

Total coliforms Yes No

E. coli Yes No

Enterococci Yes No

Pseudomonas aeruginosa Yes No

Yeasts & moulds Yes No

Other.....

VIII. Staff.

1. How many staff do you have?..... (F/T)..... (P/T)

2. What are their qualifications?.....

3. What sort of training is provided for the staff?.....

.....

4. What are their responsibilities?.....

.....

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

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

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

X. Other.

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.

XI. Comments:

.....
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.....

.....

Thank you for participating in this survey. All information obtained will be used for the completion of my thesis, which is a part of Master of Philosophy Programme at Massey University in Wellington.

If you have any queries or require further information please contact Ruta Svagzdiene on:
021-1195084 or e-mail: R.Svagzdiene@massey.ac.nz

Appendix II. Campylobacteriosis Trends in New Zealand.

Campylobacteriosis is the leading cause of gastrointestinal disease in New Zealand and its incidence is ten times higher than salmonellosis. The number of campylobacteriosis cases reported to Public Health Units in New Zealand is growing each year. While this may partially be attributed to the development of more advanced *Campylobacter spp.* detection methods, it is evident that the number of reported campylobacteriosis cases are increasing each year.

In New Zealand campylobacteriosis occurs at a higher rate than in Australia and the USA. While reporting systems may account for some of these differences, the high rate of campylobacteriosis in New Zealand is a concern. (<http://www.esr.cr.nz/competencies/foodsafety/Pages/Campylobacter.aspx> on 29/09/2008 10:01:58).

In 2007 there were 12 776 cases of campylobacteriosis notified to the Public Health Units in New Zealand (302.2 cases per 100 000 population), which was significantly lower than the 2006 rate of 379.3 per 100 000 population (15 873 cases). Campylobacteriosis continued to be the most commonly notified disease, comprising 65.9% of all notifications (19 383) in 2007 (Table 1).

The illness is highly seasonal with a summer peak and winter trough. The pattern in 2007 is similar to 2006, where there was a second peak in early winter. The highest monthly campylobacteriosis total for 2007 was for the month of January when 2045 cases were notified. This can be associated with the holiday period, BBQs, camping and hiking.

Campylobacteriosis rates varied throughout the country as demonstrated in Figure 1. The highest rates were reported for South Canterbury and Taranaki District Health Boards (DHBs) (398.3 per 100 000 population, 220 cases; 382.1 per 100 000 population, 410 cases, respectively) and the lowest rates were reported for Tairāwhiti and Wairarapa DHBs (106.8 per 100 000 population, 49 cases; 172.0 per 100 000 population, 68 cases, respectively). However, it must be noted, that the geographical areas covered by Tairāwhiti and Wairarapa

DHBs are less densely populated than some other regions. The socioeconomic status of the population in the region has a direct correlation with the notification of communicable diseases, for example, the cases may not seek medical help. While medical practitioners are legally obliged to report the cases of communicable diseases (Health Act 1956), in reality only a rather low percentage of these diseases are reported to the Public Health Units. Taking this into account the true number of campylobacteriosis cases would be much higher than the number of reported cases.

In 2007 gender was recorded for the majority of cases (97.9%). The figures demonstrated that there were more male campylobacteriosis cases (327.9 per 100 000 population, 6790 cases) notified than female (265.1 per 100 000 population, 5719 cases).

Ethnicity was recorded for 76.0% (9714/12 776) of the cases. 87.7% of notified cases were of European ethnicity, followed by Maori (6.7%), other (4.2%) and Pacific people (1.4%).

Age was recorded for 99.0% (12 648/12 776) of cases. The highest age-specific rate occurred for children aged 1-4 years (449.3 per 100 000 population, 1036 cases), followed by the 20-29 year age group (389.6 per 100 000 population, 2178 cases) and 60-69 year age group (333.2 per 100 000 population, 1202 cases).

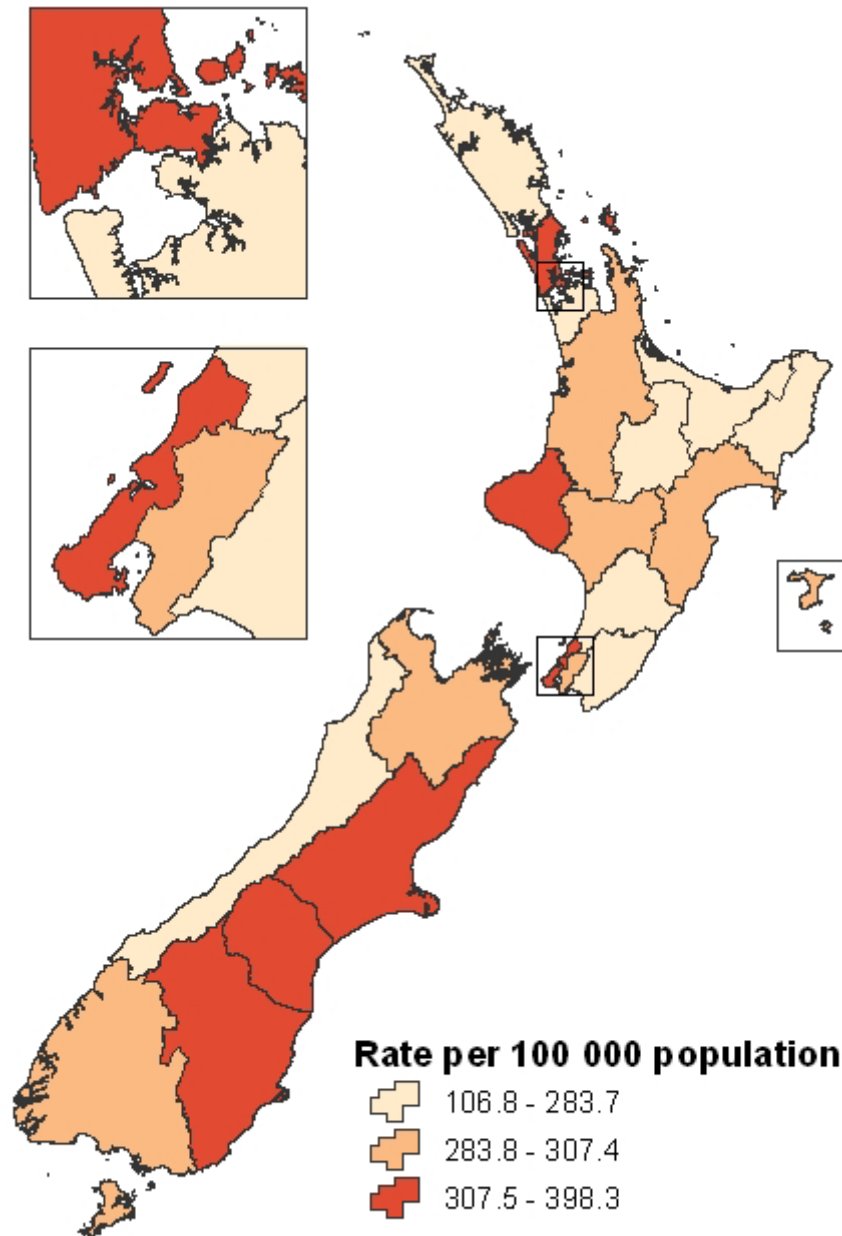
The hospitalisation status was recorded for 6916 cases and 8.4% (581 cases) of cases were hospitalised. During 2007 one death from campylobacteriosis was reported. The risk factors recorded for campylobacteriosis are those similar to previous years, the most common risk factor is consumption of food from retail premises. In 2007, 20 outbreaks of campylobacteriosis were reported involving 54 cases.

Table 1. Exposure to risk factors associated with campylobacteriosis, 2007 (Population and Environmental Health Group Institute of Environmental Science and Research Limited (May 2008). Notifiable and Other Diseases in New Zealand Annual Report 2007)

RISK FACTOR	YES	NO	UNKNOWN	% *
Consumed food from retail premises	1488	1632	9656	47.7
Contact with farm animals	1117	2414	9245	31.6
Consumed untreated water	558	2329	9889	19.3
Contact with faecal matter	384	2878	9514	11.8
Contact with other symptomatic people	367	2985	9424	10.9
Recreational water contact	337	2839	9600	10.6
Travelled overseas during the incubation period	283	3891	8602	6.8
Contact with sick animals	135	2942	9699	4.4

* % refers to the percentage of cases that answered “yes” out of the total number of cases for which this information was supplied. Cases may have more than one risk factor recorded.

Figure 1. Campylobacteriosis Notifications in New Zealand by District Health Board, 2007.



Appendix III. Campylobacter spp. detection methodology (ELS Ltd).

Appendix IV. List of Bottled Water Brands Tested.

1	Aqua Luna
2	Aquana
3	Charlie's Sports Water Feijoa Flavoured
4	CH'I Herbal Mineral Water Drink
5	Crystal Valley
6	Deep Spring Carbonated Natural Mineral Water
7	Deep Spring New Zealand Natural Water
8	Evian
9	Ferrarelle
10	H2GO (Kaiapoi)
11	Hedonic Cactus Sparkling Water
12	Hedonic Pure Drinking Pleasure
13	Infinity
14	Kiwi Blue
15	Kiwi Blue
16	Mizone Sports Water
17	Monsoon Energy Water Peach Flavoured
18	New Zealand Natural
19	New Zealand Natural Sparkling Mineral Water Kaiapoi
20	Organic Still Spring Water
21	Pam's Spring Water
22	Perrier Lime Flavoured
23	Phoenix Watermelon Flavoured
24	Powearge Grapefruit Flavoured
25	Pump (Putaruru)
26	Pure Dew
27	Sanitarium Peach Flavoured
28	Santa Vittoria
29	Santa Vittoria
30	Santa Vittoria (Azur)
31	Santa Vittoria (Azur)
32	Signature Range Spring Water
33	Snowy Mountain
34	St. Pelegrino
35	Team New Zealand Waiwera
36	Waimak
37	Waiwera New Zealand
38	Waters

*The order of brands tested in this list does not relate in any way to the coding of brands or listing of brands in other tables throughout the thesis.

Appendix V. Survey Questionnaire Covering Letter.

24/03 2004 18:31 FAX 004380285827024

RED BULL

001

15/03 2004 17:58 FAX 004380285827024

RED BULL

001



5th March 2004



Wellington Campus
Private Box 750
Wellington
New Zealand
Telephone: 64 4 487 3722
Facsimile: 64 4 487 3892

Quality Manager
Red Bull Australasia
838 Elizabeth Street
Waterloo
NSW 2017
AUSTRALIA

Dear Sir/Madam,

MASTER'S (MPhil) STUDIES AT MASSEY UNIVERSITY IN WELLINGTON

My name is Ruta Svagdiene, and currently I am enrolled in the postgraduate Master of Philosophy programme at the Institute of Food, Nutrition, and Human Health (IFNHH), Massey University in Wellington.

For my thesis I am researching the microbiological quality of bottled water.

I would like to collect data about bottled water that is sold in the retail outlets in New Zealand. Therefore, I would be very grateful if you could complete the enclosed questionnaire and post it back to us in the enclosed envelope.

The data that you provide us via this questionnaire will be confidential and will be used solely for part of my thesis.

Thank you very much for your kind consideration and assistance.

Regards,

Dr Rachel Page
Director
Sport & Life Sciences
IFNHH
Massey University
MPhil Supervisor

Dr Robert Lau
Lecturer
Life Sciences
IFNHH
Massey University
MPhil Supervisor
Tel.: 64-801 2794 ext.6661
Email: R.Lau@massey.ac.nz

Ruta Svagdiene
MPhil Student
Life Sciences
IFNHH
Massey University
Tel: 021-119 5084
E-mail: R.Svagdiene@massey.ac.nz

Tē Kōwhiri ki Pūrehurua

Incessant in te hītori Massey University's commitment to learning as a life-long journey

Appendix VI. Copy of Ethic's Committee Approval.



Massey University

**COPY FOR YOUR
INFORMATION**

OFFICE OF THE ASSISTANT
TO THE VICE-CHANCELLOR
(ETHICS & EQUITY)
Private Bag 11 222
Palmerston North
New Zealand
T 64 6 390 5573
F 64 6 390 5522
humanethics@massey.ac.nz
www.massey.ac.nz

6 September 2005

Ruta Svagzdiene
46 Wilford Street
Wallaceville
UPPER HUTT

Dear Ruta

Re: Microbiological Quality of Bottled Water

Thank you for the Low Risk Notification that was received on 6 September 2005.

Your project has been recorded on the Low Risk Database which is reported in the Massey University Human Ethics Committee Annual Report.

Please notify me if situations subsequently occur which cause you to reconsider your initial ethical analysis that it is safe to proceed without approval by a campus human ethics committee.

Please ensure that the following statement is used on Information Sheets:

"This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named above are responsible for the ethical conduct of this research."

If you have any concerns about the conduct of this research that you wish to raise with someone other than the researcher(s), please contact Professor Sylvia Rumball, Assistant to the Vice-Chancellor (Ethics & Equity), telephone 06 350 5249, email humanethics@massey.ac.nz."

Please note that if a sponsoring organisation, funding authority, or a journal in which you wish to publish requires evidence of committee approval (with an approval number), you will have to provide a full application to a Campus Human Ethics committee. You should also note that such an approval can only be provided prior to the commencement of the research.

Yours sincerely

Professor Sylvia V Rumball, Chair
Assistant to the Vice-Chancellor (Ethics & Equity)

cc Dr Rachel Page
Institute of Food, Nutrition and Human
Health
Wellington

Dr Robert Lau
Institute of Food, Nutrition and Human
Health
Wellington

Mr Dennis Thomas, Acting HoI
Institute of Food, Nutrition and Human
Health
Palmerston North

Massey University Human Ethics Committee
Accredited by the Health Research Council

Appendix VII. Researcher Qualifications.

Degree in Biology (Genetics), Department of Natural Sciences, Vilnius University, Lithuania, 1987-1992.

Bachelor of Applied Science (Environmental Health), Institute of Food, Nutrition and Human Health, College of Science, Massey University at Wellington, New Zealand, 2000- 2001.

I am a Full Voting Member of the United Kingdom Chartered Institute of Environmental Health (2007), a member of the International Water Association (2005) and a member of the Australian Institute of Environmental Health (2001).

At present I work as a Senior Environmental Health Officer at the Royal Borough of Kensington and Chelsea in London, UK. I have been working as an Environmental Health Officer in the UK now since 2005.

Previously I have worked as a Health Protection Officer in Public Health Units in New Zealand – at first in the Wanganui Public Health Centre (Mid Central Health) and then at the Regional Public Health (Hutt Valley District Health Board). During the course of my Health Protection Officer work I attended and completed Drinking Water Treatment and Assessment courses.

Appendix VIII . Research outputs.

1. Svagzdiene, R., Page, R. A. & Lau, R. (2004). Bugs in your bottled water? Massey University, Wellington. Conference Contribution – Poster.

2. Svagzdiene, R., Page, R. A. & Lau, R. (2009). Microbiological Quality of Bottled Water Brands Sold in New Zealand. *International Water Association 15th International Symposium of the Health-Related Water Microbiology Group*, Naxos, Greece. Conference Contribution – Poster.

3. Svagzdiene, R., Lau, R. & Page, R.A. (2010). Microbiological Quality of Bottled Water Brands Sold in Retail Outlets in New Zealand. *Water Science & Technology: Water Supply—WSTWS Vol 10 No 5 pp 689–699* © IWA Publishing 2010 doi:10.2166/ws.2010.384.

