Survey of the Microbiological Quality of Bottled Waters

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Executive Summary

A total of 1082 bottles of still natural mineral and other bottled water, comprising 17 different brands, were purchased from a variety of retailers around the Midlands and North West.

Samples were analysed for total colony counts at 37°C and 22°C, total coliforms, *E. coli, Pseudomonas aeruginosa*, aeromonads, faecal streptococci and sulphite-reducing clostridia according to the standard methods outlined in Report 71 (HMSO, 1982 and 1994) and were examined for compliance with the Natural Mineral Water Regulations 1985 and the Drinking Water in Containers Regulations 1994.

No samples were found to contain *E. coli*, faecal streptococci or aeromonads, one sample contained sulphite reducing clostridia, four samples contained total coliforms and 13 samples contained *Ps. aeruginosa*. There was little difference in the percentage of failures between natural mineral water and other bottled water.

Significantly lower (37°C) colony counts were measured in natural mineral water samples when compared with other bottled water (commonly called 'spring' water) samples.

Overall, container type (i.e. clear plastic versus coloured plastic versus glass.), was found to affect the microbiological quality of the water, with clear materials yielding lower colony counts than coloured materials. Overall, glass was found to produce lower colony counts than plastic, although there was an individual exception to this, with significantly higher colony counts recorded from one brand of water stored in clear glass than in clear plastic.

Large variations in colony counts, at both 22°C and 37°C, were seen at different sell-by dates, and no consistent pattern emerged.

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1 Introduction

The UK Department of the Environment is promoting several research projects to investigate whether some, as yet unspecified, proportion of gastrointestinal illness in the community might be associated with the consumption of drinking water. Work is underway to facilitate objective risk assessment from a knowledge of the microbiological quality of drinking water. A significant proportion of the UK population is known to consume bottled water, however, little data are available on the microbiological quality of bottled water at the point of sale. This study, which has been supervised by the Drinking Water Inspectorate, seeks to provide such data, through the purchase and analysis of a sample of bottled waters from UK supermarkets (Anon, 1995).

2 Background

For the purposes of this report bottled waters fall into one of two categories:

- natural mineral water
- other bottled water.

2.1 Legislation

Natural mineral water derives from officially recognised sources and is governed by The Natural Mineral Water Regulations 1985. In order to gain recognition as a natural mineral water the source must be protected against pollution and must be subjected to a hydrogeological survey. The source water must be physico-chemically described and it must meet a number of microbiological and chemical standards. Disinfection is not allowed, with purity depending on the protected nature of the source. Filtration or decanting and addition or removal of carbon dioxide are the only treatments authorised for natural mineral water. The names of waters which have been recognised as natural mineral waters are published in the Official Journal of the European Communities.

After bottling the microbiological standards outlined in Table 1 apply.

Table 1 **Microbiological Standards for Natural Mineral Waters Required after Bottling**
(Natural Mineral Water Regulations 1985)

Parameter	Units of Measurement	Maximum Concentration
E. coli	number/250 ml	0
Coliforms	number/250 ml	0
Faecal streptococci	number/250 ml	0
Sporulated sulphite-reducing anaerobes	number/50 ml	0
Psuedomonas aeruginosa	number/250 ml	0
Total viable colony count at 20-22°C (72 hours)*	number/1 ml	100
Total viable colony count at 37°C (24 hours)*	number/1 ml	20

* Measured within 12 hours after bottling, the water being maintained at 4°C +/- 1°C during this period

In addition, the Regulations state that the water should be free from parasites and pathogenic micro-organisms.

With the exception of the total colony counts, all standards remain in force up to and including the point of sale. Colony counts are allowed to increase but according to the Regulations *"the total viable colony counts shall be no more than that which results from the normal increase in the bacterial content which the water had at source."*

Table 2 **Microbiological Standards for Other Bottled Waters Required after Bottling**
(Drinking Water in Containers Regulations 1994)

Parameter	Units of Measurement	Maximum Concentration
Total coliforms	number/100 ml	0
Faecal coliforms	number/100 ml	0
Faecal streptococci	number/100 ml	0
Sporulated sulphite-reducing clostridia	number/20 ml	≤ 1†
Colony counts*	number/1 ml at 22°C	100
	number/1 ml at 37°C	20

† Analysis by multiple tube method
* The total viable colony count should be measured within 12 hours of bottling with the sample water being kept at a constant temperature during that 12-hour period. Any increase in the total viable colony count of the water between 12 hours after bottling and the time of sale shall not be greater than that normally expected.

'Other bottled water', for the purposes of this report, is taken to mean all bottled water which is not recognised as a natural mineral water. Legislation is provided by The Drinking Water in

Containers Regulations 1994. In contrast to natural mineral waters, other bottled waters can be subject to chemical treatment, physical treatment and disinfection.

2.2 Brief Literature Review

Bottled water is now the biggest selling soft drink in Europe (van Musschenbroek, 1995), with sales topping 700 million litres in 1994 in the UK alone (Natural Mineral Water Association, pers. com.).

Studies examining the microbiological quality of bottled waters from retail outlets have often found high total colony counts. In Canada, Warburton *et al.* (1986) reported that 16% (n= 136) of mineral water samples examined exhibited counts of between 1,000 and 10,000 cfu/ml at 35°C. None of the mineral waters they examined contained *E. coli* or faecal coliforms but two samples were positive for total coliforms.

In the UK, Hunter and Burge (1987) examined a total of 58 bottles of mineral water, 29 carbonated and 29 still. The still water samples were found to yield significantly higher colony counts than carbonated water at both 22°C ($p<0.001$) and 37°C ($p<0.01$). Over 50% of the still water samples (15/29) yielded colony counts (22°C) of greater than 1000 cfu/ml, compared with only 1 sample (3%) of carbonated water. The authors attributed this difference to the antibacterial action of carbon dioxide. This work was extended by examining a further 44 bottles of still water (Hunter *et al.*, 1990). In this study, 7 samples were found to yield counts greater than 100,000 cfu/ml at 22°C and 2 samples were found to exceed 100,000 cfu/ml at 37°C.

A similar small scale study is reported by Richards *et al.* (1992). Of sixty nine still water samples, 50 were found to have colony counts greater than 1000/ml A number of samples (4/104) were positive for *Pseudomonas aeruginosa.*

Mavridou (1992) examined water from the Campsie spring in Scotland at different stages of the bottling procedure. Water prior to bottling had very low colony counts ranging from <1 - 25 cfu/ml. Samples analysed shortly after bottling were also found to yield low colony counts. Additional analyses were conducted up to 46 days after bottling on the same samples. During storage, Mavridou reported that greater numbers of bacteria grew in glass (dark green) than PVC bottles.

Opinions vary on the effect of the bottle material on water quality. A number of authors report higher colony counts in waters from plastic bottles compared to glass (Del Vecchio and

Fischetti, 1972; De Felip *et al.*, 1976; Yurdusev and Ducluzeau, 1985 - all cited by Hunter, 1993). Morais and da Costa (1990) found no difference between the two materials. Bischofberger *et al.* (1990) initially found significantly lower colony counts from glass containers than plastic (100,000 cfu/ml in plastic versus 10,000 cfu/ml in glass). This difference, however, was thought to be due to residual detergent in the glass bottles and after thorough cleaning similar counts were found from each type of container. Although Gonzalez *et al.* (1987) found higher overall levels of colony counts (22°C) from glass, these were not significantly different from plastic. In a study of bacterial growth she found that populations increased between days 1 to 6 in plastic containers and between days 6 to 12 in glass containers. After the initial increase the bacterial population then remained practically constant until the end of the experiment (60 days). Moreira *et al.* (1994) found that, while the type of container did not affect the survival of the enterobacteria *E. coli*, *Enterobacter cloacae* or *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* exhibited a lower mortality rate in water bottled in PVC than in glass. The authors speculate that this may reflect the capacity *of Ps. aeruginosa* to colonise this type of material.

3 Methodology

A total of 17 different brands of still water (both natural mineral and other bottled water) in a variety of different containers (plastic and glass, clear and coloured) were purchased from a variety of retailers around the Midlands and North West during January 1996. Table 3 lists the brands of water and types of containers.

Table 3 Water Types and Brands

Water Type	Brand	Material
Natural Mineral	Aqua Pura	Plastic
	Ashbrook Spring	Plastic
	Buxton Water	Glass
	Buxton Water	Plastic
	Caledonian Spring	Plastic
	Evian	Glass
	Evian	Plastic
	Glenburn Spring	Plastic
	Highland Spring	Glass
	Highland Spring	Plastic
	Malvern	Plastic
	Vittel	Plastic
	Volvic	Plastic
Other bottled	Farm Stores	Plastic
	National Trust	Plastic
	Peak	Plastic
	Pennine	Plastic
	St. Michael	Plastic
	Strathmore	Glass
	Ty Nant	Glass

A code is used to distinguish between the different brands and container types, it is used throughout the statistical analyses.

3.1 Microbiology

Samples were transported in cool (2 - 8°C), dark conditions to Acer Environmental in Daresbury, a NAMAS accredited laboratory, for analysis within six hours of purchase.

Samples were analysed for total colony counts at 37°C and 22°C, total coliforms, *E. coli, Pseudomonas aeruginosa*, aeromonads, faecal streptococci and sulphite-reducing clostridia according to the standard methods outlined in Report 71 (HMSO, 1982 and 1994).

3.2 Statistical Analysis

Microbiological data were received as 'Excel 5.0' files, these were imported into the statistical package 'Minitab'. Statistical tests involving analysis of variance (ANOVA) were applied to the data to test for significant differences in water quality exhibited by different brands of water and different container materials (i.e. glass or plastic; clear or coloured).

Where colony count data were reported by the laboratory as >3,000 cfu/ml (i.e. the early sample runs) it was difficult adequately to characterise the upper part of the data distribution for the purposes of parametric analysis. However, this problem was addressed by calculating the theoretical probability density function from all available data (including those from later sample runs with enumerations of > 3,000 cfu/ml) assuming a $\log_{10}$ normal distribution. This was not done where there were no full enumerations and the number of >3,000 cfu/ml results exceeded 50% of the sample measurements.

4 Results

A total of 1,082 bottles were purchased, of which 708 (65.4%) were natural mineral water and 374 (34.6%) were other bottled water. No samples were found to contain *E. coli*, faecal streptococci or aeromonads. A total of four samples contained total coliforms, one sample contained sulphite-reducing clostridia and 13 samples (1.2%) were found to contain *Pseudomonas aeruginosa* up to 13/250ml. These 18 samples represented nine different brands of water, 11 samples were from plastic coloured bottles, 6 were from plastic clear bottles with only 1 sample being from a glass container. Of these samples 13 were natural mineral water (13/708 - 1.8%) and 5 were other bottled water (5/374 - 1.3%).

In order to examine the possibility of microbiological variability within bottles, a number of samples were analysed for colony counts in triplicate. There were no significant differences between these data, suggesting that there is little intra-bottle variability.

Colony count data at 22°C and 37°C were found to be $\log_{10}$ normally distributed, all statistical analyses were, therefore, conducted on the $\log_{10}$ transformed data. An exception to this was '14'; colony counts were either very low (generally < 20 cfu/ml) or greater than 3,000 cfu/ml. These data were omitted from the statistical analyses.

For each brand of water geometric mean three day colony counts (22°C) were significantly higher than geometric mean one day colony counts (37°C), as shown in Table 4.

Table 4 Summary of Differences in Geometric Mean Colony Counts at 22°C and 37°C.

Brand	Container	Material	n	CC22 GM Log_{10}	CC37 GM Log_{10}	p
Natural Mineral Water						
1	Clear	Plastic	50	2.559	1.373	0.000
2	Coloured	Plastic	49	3.234	1.709	0.000
3	Clear	Glass	50	3.255	1.259	0.000
4	Clear	Plastic	53	1.627	0.460	0.000
5	Coloured	Plastic	50	2.707	1.722	0.000
6	Clear	Glass	50	2.089	0.591	0.000
7	Clear	Plastic	55	2.650	1.633	0.000
9	Coloured	Plastic	50	2.888	1.919	0.000
10	Coloured	Glass	50	2.520	1.424	0.000
11	Coloured	Plastic	75	4.733	2.760	0.000
12	Clear	Plastic	51	2.980	1.050	0.000
19	Coloured	Plastic	55	3.137	1.140	0.000
20	Coloured	Plastic	70	4.514	3.290	0.000
Other Bottled Water						
8	Clear	Plastic	54	2.976	2.484	0.008
13	Coloured	Plastic	51	2.460	0.670	0.000
15	Clear	Plastic	50	2.612	1.932	0.000
16	Clear	Plastic	70	4.448	3.100	0.000
17	Coloured	Glass	49	2.157	0.818	0.000
18	Coloured	Glass	50	3.224	1.810	0.000

GM - geometric mean
p - t test p value

4.1 Container Differences

Brands were separated according to water type (i.e. natural mineral water or other bottled water) and container type (plastic clear, plastic coloured, glass clear and glass coloured) and examined for statistical differences using analysis of variance (ANOVA). Figure 1 shows the results of the ANOVA test for $\log_{10}$ transformed colony counts at 22°C from natural mineral water bottled in clear plastic containers.

Figure 1 ANOVA for Transformed Colony Count Data (22°C) from Natural Mineral Water in Clear Plastic Containers

```
Source     DF        SS        MS        F        p
TYPE        3     53.12     17.71    13.64    0.000
Error     205    266.07      1.30
Total     208    319.19
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Brand       N      Mean     StDev  ---------+---------+---------+-------
    1      50     2.559     0.980                 (-----*----)
    4      53     1.627     1.503   (----*----)
    7      55     2.650     0.955                    (----*----)
   12      51     2.982     1.024                         (-----*----)
                                   ---------+---------+---------+-------
Pooled StDev =    1.139                  1.80      2.40      3.00
```

It is clear from this that there are significant differences between different brands regardless of the common container type. This is even more clearly shown when examining the ANOVA results from natural mineral waters bottled in coloured plastic containers, as shown in Figures 2 and 3.

Figure 2 ANOVA for Transformed Colony Count Data (22°C) from Natural Mineral Water in Coloured Plastic Containers

```
Source     DF        SS        MS        F        p
TYPE        5   236.399    47.280   126.03    0.000
Error     343   128.671     0.375
Total     348   365.070
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Brand       N      Mean     StDev  ----+---------+---------+---------+--
    2      49    3.2336    0.5433             (-*--)
    5      50    2.7078    0.6813  (--*-)
    9      50    2.8882    0.5225     (-*--)
   11      75    4.7331    0.6299                                (-*-)
   19      55    3.1366    0.5703         (--*-)
   20      70    4.5135    0.6751                            (-*--)
                                   ----+---------+---------+---------+--
Pooled StDev =   0.6125               2.80      3.50      4.20      4.90
```

A further test, Tukey's pairwise comparisons, shows which pairs are significantly different. In this instance water 2 is significantly different to waters 5, 9, 11 and 20; type 5 is significantly different to types 11, 19 and 20; type 9 is significantly different to types 11 and 20; type 11 is significantly different to type 19 and type 19 is significantly different to type 20.

Figure 3 ANOVA for Transformed Colony Count Data (37°C) from Natural Mineral Water in Coloured Plastic Containers

```
Source     DF        SS        MS         F        p
TYPE        5    196.30     39.26     26.26    0.000
Error     343    512.77      1.49
Total     348    709.07
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Brand       N      Mean     StDev ---------+---------+---------+------
    2      49     1.709     1.103        (---*----)
    5      50     1.722     1.064        (----*---)
    9      50     1.919     0.755           (---*---)
   11      75     2.764     1.595                     (---*--)
   19      55     1.140     0.904 (---*---)
   20      70     3.292     1.418                           (--*---)
                                  ---------+---------+---------+------
Pooled StDev =    1.223                 1.60      2.40      3.20
```

Tukey's test shows the following statistically significant differences: 11 vs 2; 11 vs 5; 11 vs 9; 19 vs 9; 19 vs 11; 20 vs 2; 20 vs 5; 20 vs 9; 20 vs 11 and 20 vs 19.

ANOVA produces an apparent difference between the microbiological quality of water bottled in clear plastic and that in coloured plastic (Figure 4) with the coloured plastic producing apparently higher colony counts at 37°C. A parallel analysis of 22°C colony counts produces similar results.

Figure 4 ANOVA for Transformed Colony Count Data (37°C) from Natural Mineral Water in Clear versus Coloured Plastic Containers

```
Source     DF        SS        MS         F        p
Bottle      1    148.29    148.29     81.36    0.000
Error     556   1013.34      1.82
Total     557   1161.63
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
            N      Mean     StDev -------+---------+---------+---------
    1     209     1.130     1.209  (---*----)
    2     349     2.196     1.427                           (---*--)
                                  -------+---------+---------+---------
Pooled StDev =    1.350               1.20      1.60      2.00
```

1 - clear plastic bottles; 2 - coloured plastic bottles.

However, this result might be influenced by a 'brand' effect. Figure 5 presents an ANOVA for each 'brand' in plastic containers. The 'coloured' containers are indicated with the *. It can seen from this figure that there is considerable overlap between 'clear' and 'coloured' container types when the data are split by 'brand'. The same is true if the comparison is made between 'coloured' and 'clear' glass containers split by 'brand' (see Figure 6).

Figure 5 **ANOVA for Transformed Colony Count Data (37°C) from Natural Mineral Water in Clear versus Coloured Plastic Containers**

```
Source     DF        SS        MS        F        p
TYPE        9    385.56     42.84    30.25    0.000
Error     548    776.07      1.42
Total     557   1161.63
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Brand       N      Mean     StDev  ---------+---------+---------+-------
    1      50     1.373     1.354            (---*--)
   *2      49     1.709     1.103                (--*--)
    4      53     0.460     0.700  (---*--)
   *5      50     1.722     1.064                (--*---)
    7      55     1.633     1.316               (--*--)
   *9      50     1.919     0.755                  (--*--)
  *11      75     2.764     1.595                           (--*-)
   12      51     1.048     1.045         (--*---)
  *19      55     1.140     0.904          (--*---)
  *20      70     3.292     1.418                                (--*--)
                                   ---------+---------+---------+-------
Pooled StDev =    1.190                   1.0       2.0       3.0

* indicates a 'coloured'  container
```

Figure 6 **ANOVA for Transformed Colony Count Data (37°C) from Natural Mineral Water in Clear versus Coloured Glass Containers**

```
Source     DF        SS        MS        F        p
TYPE        2    19.452     9.726    11.32    0.000
Error     147   126.352     0.860
Total     149   145.804
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Brand       N      Mean     StDev  --+---------+---------+---------+----
    3      50    1.2587    0.9976                     (-----*------)
    6      50    0.5909    0.7404  (------*-----)
  *10      50    1.4239    1.0174                         (------*-----)
                                   --+---------+---------+---------+----
Pooled StDev =   0.9271            0.40      0.80      1.20      1.60

* indicates a 'coloured'  container
```

All brands are sold in either clear or coloured containers (see Table 3). Hence, the unique effect of container colour cannot be separated from the brand effect with this data set.

Accepting this limitation, Figure 7 shows the ANOVA results examining plastic and glass bottles containing natural mineral water. It demonstrates that water from glass containers sampled in this survey yielded lower colony counts at 37°C than water bottled in plastic (the same is also true for colony counts at 22°C).

Figure 7 ANOVA for Transformed Colony Count Data (37°C) from Natural Mineral Water in Plastic versus Glass Containers

```
Source     DF        SS        MS        F        p
Material    1     58.84     58.84    31.77    0.000
Error     706   1307.43      1.85
Total     707   1366.27
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
           N      Mean     StDev  -+---------+---------+---------+-----
    1    558     1.797     1.444                              (---*---)
    2    150     1.091     0.989  (-------*-------)
                                  -+---------+---------+---------+-----
Pooled StDev =   1.361           0.90      1.20      1.50      1.80
```

1 - plastic bottles; 2 - glass bottles.

However, when looked at in more detail the apparent difference between glass and plastic containers is due to the high colony counts observed in water from the coloured plastic containers (see Figure 8).

Figure 8 ANOVA for Transformed Colony Count Data (37°C) from Natural Mineral Water in Plastic (clear and coloured) versus Glass (clear and coloured) Containers

```
Source     DF        SS        MS        F        p
Bottle      3    215.43     71.81    43.93    0.000
Error     704   1150.84      1.63
Total     707   1366.27
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
           N      Mean     StDev  -------+---------+---------+---------
    1    209     1.130     1.209        (---*--)
    2    349     2.196     1.427                               (--*--)
    3    100     0.925     0.936  (----*-----)
    4     50     1.424     1.017          (------*-------)
                                  -------+---------+---------+---------
Pooled StDev =   1.279                 1.00      1.50      2.00
```

1 - clear plastic bottles; 2 - coloured plastic bottles; 3 - clear glass bottles; 4 - coloured glass bottles.

A number of brands were available in both glass and plastic containers. This enabled the effect of container type to be examined within brand. In one brand, significantly higher one day colony counts (p=0.000) were measured in clear glass containers compared to clear plastic bottles. This pattern was reversed in the other two brands (Table 5).

Table 5 Summary of Differences in Geometric Mean Colony Counts Between Glass and Plastic Containers

Brand	Container	Material	GM 22°C Log_{10}	p	GM 37°C Log_{10}	p
A	Clear	Glass	3.255	0.000	1.258	0.000
	Clear	Plastic	1.627		0.460	
B	Clear	Glass	2.089	0.011	0.591	0.000
	Clear	Plastic	2.650		1.633	
C	Coloured	Glass	2.519	0.000	1.424	0.000
	Coloured	Plastic	4.733		2.764	

GM - Geometric Mean
p - ANOVA p value

A different pattern was seen with other bottled water with coloured plastic containers yielding the lowest colony counts, followed by coloured glass, with clear plastic producing the highest counts (see Table 6).

Table 6 Geometric Mean Colony Counts in Other Bottled Water Bottled in Glass and Plastic Containers

Container	Material	n	GM 22°C Log_{10}	GM 37°C Log_{10}
Clear	Plastic	96	3.999	2.574
Coloured	Plastic	51	2.458	0.669
Coloured	Glass	99	2.695	1.319

GM - Geometric Mean

Examining container type, irrespective of whether the water was natural mineral water or other bottled water, colony counts were significantly lower from glass than plastic containers (Table 7).

Table 7 Geometric Mean Colony Counts in All Water Bottled in Glass and Plastic Containers

Material	n	GM 22°C Log_{10}	p	GM 37°C Log_{10}	p
Glass	249	2.651	0.000	1.182	0.000
Plastic	783	3.267		1.896	

GM - geometric mean
p - ANOVA p value

4.2 Water Type

Colony count results from each brand of natural mineral water were pooled, irrespective of container, and compared with pooled other bottled water results. Natural mineral water yielded significantly lower colony counts (37°C) than other bottled water (geometric mean 1.647 vs 1.891 p=0.008).

4.3 Sell-by Date

Sell-by dates were converted into months of life remaining, for example a sell-by date of December 1996 is equivalent to 11 months remaining. The following Table summarises the range of sell-by dates for each brand and whether there were any statistically significant differences in colony counts between different sell-by dates.

The longest remaining shelf life was 35 months ('18'), the shortest was 1 month ('12'). There is no clear relationship between shelf life and microbiological quality of the bottled waters examined. ANOVA analyses were completed for each brand and are presented in Appendix A.

Table 8 Summary of Shelf Life and ANOVA Test Results

Brand	Container	Material	Months Remaining	CC22 p	CC37 p
Natural Mineral Water					
1	Clear	Plastic	10,12,13, 14,15,16	0.078	0.004
2	Coloured	Plastic	3,4,5,6,7, 8,9	0.047	0.537
3	Clear	Glass	12,14,15, 16,17,20	0.000	0.031
4	Clear	Plastic	7,11,12,13,14, 15,16,17,18,23	0.069	0.237
5	Coloured	Plastic	7,8,9,10 11,12	0.015	0.000
6	Clear	Glass	8,9,20	0.000	0.003
7	Clear	Plastic	10,16,17,18, 19,20,22,23	0.000	0.002
9	Coloured	Plastic	7,8	0.564	0.033
10	Coloured	Glass	7,8,9,14,16, 18,19,20,21	0.055	0.019
11	Coloured	Plastic	4,6,7,8,9, 10,18,20	0.003	0.002
12	Clear	Plastic	1,5,7,8,9	0.069	0.338
19	Coloured	Plastic	23,24,25, 26,29,33	0.057	0.268
20	Coloured	Plastic	23		
Other bottled Water					
8	Clear	Plastic	3,4,5,6,7		0.000
13	Coloured	Plastic	12,13,16,17	0.004	0.188
15	Clear	Plastic	9,10		0.847
16	Clear	Plastic	10,11,12,13	0.086	0.007
17	Coloured	Glass	7,19	0.155	0.032
18	Coloured	Glass	32, 33, 35	0.000	0.000

CC22 p - ANOVA test p value for colony counts (22°C)
CC37 p - ANOVA test p value for colony counts (37°C)

5 Discussion

Almost 2% of the bottled water samples examined failed to meet the required microbiological standards laid down in the Natural Mineral Water Regulations and the Drinking Water in Containers Regulations. The percentage of samples failing to comply with the standards was similar for both natural mineral water (1.8%) and other bottled water (1.5%). *E. coli* and faecal streptococci were not isolated, however, a number of samples (1.2%) were found to contain *Ps. aeruginosa.* Other studies have also isolated *Ps. aeruginosa* from bottled water, including Richards *et al.* (1992), who found 4 positive samples from 104, and Rosenberg (1990; cited by Hunter, 1993) who found *Ps. aeruginosa* in 1.2 - 10.2% of samples. Based on the results of this study and those reported by Richards *et al.* (1992) and Rosenberg (1990) *Ps. aeruginosa* would seem to be the most common cause of failure. No aeromonads were isolated from any of the bottled water samples examined. Previous studies (Hunter and Burge, 1987 and Havelaar *et al.*, 1990) have also failed to isolate *Aeromonas* spp., in waters of low nutrient content Havelaar suggests that the normal bottled water flora is inhibitory to *Aeromonas* spp.

In this study, almost 60% of samples incubated at 22°C and over 20% incubated at 37°C yielded colony counts greater than 1,000 cfu/ml. Other authors in the UK have reported similar figures. Hunter and Burge (1987) reported 52% of still waters examined after incubation at 22°C and 10% incubated at 37°C had colony counts in excess of 1,000 cfu/ml. Hunter *et al.* (1990) found that 70% of samples had counts over 1,000 at 22°C and 20% exceeded 1,000 at 37°C.

No attempt was made to characterise the autochthonous bacteria making up the total viable colony counts. Mavridou (1992), identified a number of genera from pre-bottling water samples, taken from the Campsie spring, including *Micrococcus, Moraxella, Pseudomonas, Acinetobacter, Flavobacterium* and *Xanthomonas.* Following bottling and during storage *Pseudomonas* species (excluding *Ps. aeruginosa*) were found to predominate. In addition to the genera identified in pre-bottling samples *Alteromonas, Alcaligenes, Cytophaga, Staphylococcus* and Coryneforms were also identified from storage samples. Other studies (Bischofberger *et al.*, 1990) have also shown differences between the pre- and post-bottling bacterial population, suggesting that colonisation from pipework biofilms may have taken place.

Overall the container material appears to affect the microbiological quality of natural mineral waters. This study found that significantly lower colony counts were obtained from waters stored in glass than in plastic bottles. This is in agreement with a number of studies including

Del Vecchio and Fischetti (1972); De Felip *et al.* (1976) and Yurdusev and Ducluzeau (1985). Other studies, however, have produced conflicting results (Morais and da Costa, 1990; Mavridou, 1992). These apparent inconsistencies may be related to the type or brand of bottled water examined. In this study, although overall glass bottles yielded lower colony counts than plastic bottles, an exception, was '3'. The reason for this difference is not known, but may be related to differing bottling lines or to the growth characteristics and preferences of the natural flora of the source water.

In addition to the type of material, the colour of the container also appears to affect total colony counts, with generally higher colony counts (37°C) obtained from both coloured glass and coloured plastic when compared to their clear counterparts (at 22°C, there was a significant difference between only coloured versus clear plastic). This difference may be due to the coloured materials providing a protective effect from ultra violet light. Whether there is any difference in the materials' efficacy in supporting bacterial growth is impossible to determine within the constraints of this study. Precise definition of the contribution of 'container type' to microbiological concentration within the water examined is difficult because the 'brand' exerts a degree of unquantified influence which causes interference with the relationship between 'container type' and 'water quality'. The unique contribution of container materials to the microbiological quality of bottled waters would best be quantified with a controlled 'experimental' protocol rather than the 'empirical' design adopted in this study.

It has been found in previous studies that the bacterial population of bottled water increases after bottling, reaching a peak between the first and second weeks (Gonzalez *et al.*, 1987; Bischofberger *et al.*, 1990), it then remains reasonably constant for at least 6 months. In this study it was found that there was a large variation in the colony counts at different sell-by dates, both within and between brands, this made generalisations about length of shelf life and microbiological quality difficult. The growth characteristics are likely to depend on the typical natural flora of the source water and, thus, are likely to vary widely between different sources. A more appropriate way to look at the effect of remaining shelf life on microbiological quality may, therefore, be to purchase a large number of bottles of a single water from the same batch at the start of the experiment, store it and progressively analyse samples through time.

The concept of natural mineral water is based on non-intervention (the only treatment allowed is filtration to remove solid particles) and a naturally protected source. The fact that this study found that overall natural mineral water yielded a significantly lower colony count at 37°C than other bottled water (there was no difference at 22°C) would suggest that there is some validity to this approach. Contamination during extraction and bottling, however, may be a significant problem since an approximately equal percentage of natural mineral water and other bottled water samples failed to meet the required microbiological standards.

Regardless of differences between water types, containers and shelf life, many of the waters examined contained very high levels of bacteria including one measurement of over 300,000 cfu/ml (22°C). It is unknown whether this autochthonous flora has the potential to cause illness, although Payment *et al.* (1991a,b) found an association between colony count at 37°C and gastrointestinal illness in consumers drinking water in a study employing point-of-use domestic reverse-osmosis filtration units. These filtration units were found to produce water yielding colony counts of 1,000 - 100,000 cfu/ml. Interestingly, the bacteria isolated were similar to those isolated from bottled waters namely, *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Chromobacterium*, *Alcaligenes* and *Moraxella.*

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Appendix A

Individual Brand Results of ANOVA Tests for Transformed Colony Count Data (37°C) by Remaining Shelf Life

Months - Months Remaining

1

```
Source      DF       SS       MS       F        p
months       5    28.51     5.70    4.10    0.004
Error       44    61.27     1.39
Total       49    89.79
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Months       N     Mean    StDev  --+---------+---------+---------+----
    10       1    2.643    0.000      (---------------*---------------)
    12       4    1.849    0.978        (--------*--------)
    13      15    2.169    1.478              (---*----)
    14       3    2.289    1.587          (---------*--------)
    15      12    0.396    0.673  (----*---)
    16      15    0.964    1.128      (---*----)
                                  --+---------+---------+---------+----
Pooled StDev =    1.180             0.0       1.5       3.0       4.5
```

2

```
Source      DF       SS       MS       F        p
months       6     6.34     1.06    0.85    0.537
Error       42    52.02     1.24
Total       48    58.35
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Months       N     Mean    StDev  --+---------+---------+---------+----
     3       2    0.906    0.293         (----------*----------)
     4       2    0.766    0.657         (----------*----------)
     5       2    1.653    0.000              (----------*----------)
     6      13    2.072    1.036                        (---*---)
     7      14    1.680    1.423                     (---*---)
     8      15    1.745    0.938                      (---*--)
     9       1    0.477    0.000  (---------------*---------------)
                                  --+---------+---------+---------+----
Pooled StDev =    1.113            -1.5       0.0       1.5       3.0
```

3

```
Source      DF       SS       MS       F        p
months       5   11.532    2.306    2.73    0.031
Error       44   37.231    0.846
Total       49   48.763
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Months       N     Mean    StDev  -------+---------+---------+---------
    12       1   2.4914   0.0000            (------------*------------)
    14       2   1.6751   1.9433          (---------*---------)
    15       8   1.9653   1.1725                 (---*---)
    16       4   0.2940   0.3514     (-----*-----)
    17       2   0.3010   0.4257  (---------*---------)
    20      33   1.1998   0.8529              (-*-)
                                  -------+---------+---------+---------
Pooled StDev =   0.9199                 0.0       1.5       3.0
```

4

```
Source      DF        SS        MS        F        p
months       9     5.639     0.627     1.36    0.237
Error       43    19.835     0.461
Total       52    25.474
                                     Individual 95% CIs For Mean
                                     Based on Pooled StDev
Months       N      Mean     StDev   ----+---------+---------+---------+--
     7       3    0.3597    0.3177             (-------*-------)
    11       5    0.3204    0.5635              (-----*-----)
    12       3    1.3554    0.7119                       (-------*------)
    13       7    0.2653    0.2632              (-----*----)
    14      11    0.4349    0.6978                 (---*---)
    15       6    0.9222    1.3447                     (----*-----)
    16       3    0.0000    0.0000          (-------*-------)
    17       9    0.2310    0.5079               (---*----)
    18       5    0.6760    0.4897                  (-----*-----)
  . 23       1    0.0000    0.0000   (-------------*-------------)
                                     ----+---------+---------+---------+--
Pooled StDev =    0.6792               -1.0       0.0       1.0       2.0
```

5

```
Source      DF        SS        MS        F        p
months       5    24.855     4.971     7.13    0.000
Error       44    30.666     0.697
Total       49    55.521
                                     Individual 95% CIs For Mean
                                     Based on Pooled StDev
Months       N      Mean     StDev   -----+---------+---------+---------+-
     7      10    1.1925    0.4440     (----*----)
     8      10    2.6610    0.9160                   (-----*----)
     9      12    1.4595    1.0238        (----*---)
    10      11    1.0196    0.9550   (----*----)
    11       4    2.9053    0.4116                   (-------*-------)
    12       3    2.4001    0.3018            (---------*---------)
                                     -----+---------+---------+---------+-
Pooled StDev =    0.8348                 1.0       2.0       3.0       4.0
```

6

```
Source      DF        SS        MS        F        p
months       2     6.006     3.003     6.77    0.003
Error       47    20.858     0.444
Total       49    26.864
                                     Individual 95% CIs For Mean
                                     Based on Pooled StDev
Months       N      Mean     StDev   ------+---------+---------+---------+
     8      37    0.5372    0.6119              (--*--)
     9       6    1.4524    1.1877                      (-------*-------)
    20       7    0.1363    0.2328    (------*------)
                                     ------+---------+---------+---------+
Pooled StDev =    0.6662                 0.00      0.70      1.40      2.10
```

7

```
Source     DF        SS        MS         F        p
months      7     33.83      4.83      3.81    0.002
Error      47     59.65      1.27
Total      54     93.48
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  ----------+---------+---------+------
    10      1     1.919     0.000          (-----------*----------)
    16      1     0.301     0.000  (-----------*----------)
    17      5     0.843     0.833          (----*----)
    18     22     1.136     0.862              (--*-)
    19      9     1.442     1.375              (---*---)
    20      4     2.675     0.555                   (----*-----)
    22      6     2.007     2.095                (----*----)
    23      7     3.233     0.740                      (---*---)
                                   ----------+---------+---------+------
Pooled StDev =    1.127                     0.0       2.0       4.0
```

8

```
Source     DF        SS        MS         F        p
months      4    43.196    10.799     16.18    0.000
Error      49    32.700     0.667
Total      53    75.896
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  -+---------+---------+---------+-----
     3      2    1.1722    0.0824   (---------*--------)
     4      3    0.9186    0.8017   (-------*-------)
     5      8    0.8329    0.4509     (----*----)
     6      4    3.2463    0.3702                      (------*------)
     7     37    2.9567    0.9064                        (--*-)
                                   -+---------+---------+---------+-----
Pooled StDev =   0.8169            0.0       1.2       2.4       3.6
```

9

```
Source     DF        SS        MS         F        p
months      1     2.536     2.536      4.79    0.033
Error      48    25.391     0.529
Total      49    27.928
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  ------+---------+---------+---------+
     7     35    2.0663    0.6717                    (------*------)
     8     15    1.5748    0.8474  (----------*----------)
                                   ------+---------+---------+---------+
Pooled StDev =   0.7273                 1.40      1.75      2.10      2.45
```

10

```
Source     DF        SS        MS        F        p
months      8    17.287     2.161     2.65    0.019
Error      41    33.437     0.816
Total      49    50.724
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  ----------+---------+---------+------
     7      6    1.6342    0.3018                 (---*---)
     8     11    1.9484    1.2422                    (--*-)
     9     11    0.6270    0.9136              (--*--)
    14      1    0.0000    0.0000    (---------*---------)
    16      1    1.1761    0.0000          (---------*---------)
    18      1    2.3304    0.0000                (---------*---------)
    19      9    1.8255    0.6382                   (--*--)
    20      6    0.9021    0.8098               (---*--)
    21      4    1.9276    0.9429                  (----*---)
                                   ----------+---------+---------+------
Pooled StDev =   0.9031                     0.0       2.0       4.0
```

11

```
Source     DF        SS        MS        F        p
months      7     53.37      7.62     3.79    0.002
Error      67    134.81      2.01
Total      74    188.18
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  ----------+---------+---------+------
     4      1     0.000     0.000    (---------*---------)
     6      3     1.466     1.622            (-----*----)
     7     36     3.359     1.263                       (*-)
     8     11     3.276     1.144                     (--*--)
     9      9     1.959     2.124                (---*--)
    10     13     1.652     1.430                (--*-)
    18      1     2.380     0.000           (---------*---------)
    20      1     4.500     0.000                   (---------*---------)
                                   ----------+---------+---------+------
Pooled StDev =    1.418                     0.0       3.0       6.0
```

12

```
Source     DF        SS        MS        F        p
months      4      5.02      1.26     1.17    0.338
Error      46     49.53      1.08
Total      50     54.56
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  ---+---------+---------+---------+---
     1      9     1.115     1.242                  (-----*-----)
     5      3     1.199     0.439               (---------*---------)
     7      2     0.000     0.000    (-----------*-----------)
     8     19     0.814     1.109                  (---*---)
     9     18     1.353     0.930                      (---*---)
                                   ---+---------+---------+---------+---
Pooled StDev =    1.038            -1.2       0.0       1.2       2.4
```

13

```
Source      DF        SS        MS        F        p
months       3     2.027     0.676     1.66    0.188
Error       47    19.124     0.407
Total       50    21.151
                                       Individual 95% CIs For Mean
                                       Based on Pooled StDev
Months       N      Mean     StDev     ---+---------+---------+---------+---
    12       3    0.6344    0.5515       (--------------*--------------)
    13       3    0.9584    0.4493             (--------------*--------------)
    16      33    0.5328    0.5794               (----*---)
    17      12    0.9799    0.8185                     (-------*------)
                                       ---+---------+---------+---------+---
Pooled StDev =    0.6379                 0.00      0.50      1.00      1.50
```

15

```
Source      DF        SS        MS        F        p
months       1     0.031     0.031     0.04    0.847
Error       48    39.335     0.819
Total       49    39.366
                                       Individual 95% CIs For Mean
                                       Based on Pooled StDev
Months       N      Mean     StDev     ---------+---------+---------+-------
     9      18    1.9652    0.7041     (-----------------*-----------------)
    10      32    1.9135    0.9985        (------------*------------)
                                       ---------+---------+---------+-------
Pooled StDev =    0.9052                     1.75      2.00      2.25
```

16

```
Source      DF        SS        MS        F        p
months       3     14.88      4.96     4.38    0.007
Error       66     74.71      1.13
Total       69     89.60
                                       Individual 95% CIs For Mean
                                       Based on Pooled StDev
Months       N      Mean     StDev     ---+---------+---------+---------+---
    10      12     3.420     0.371                       (-------*------)
    11      16     2.928     1.280                 (------*-----)
    12      32     3.391     1.103                         (---*----)
    13      10     2.076     1.101      (-------*-------)
                                       ---+---------+---------+---------+---
Pooled StDev =     1.064                 1.60      2.40      3.20      4.00
```

17

```
Source     DF        SS        MS        F        p
months      1     2.199     2.199     4.87    0.032
Error      47    21.241     0.452
Total      48    23.440
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  -----+---------+---------+---------+-
     7     12    1.1904    0.7153              (------------*------------)
    19     37    0.6978    0.6585   (-------*-------)
                                   -----+---------+---------+---------+-
Pooled StDev =   0.6723               0.60      0.90      1.20      1.50
```

18

```
Source     DF        SS        MS        F        p
months      2    27.508    13.754    25.21    0.000
Error      47    25.644     0.546
Total      49    53.152
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  ----------+---------+---------+------
    32     12    1.2478    0.6911   (-----*-----)
    33     16    1.0830    0.8378  (----*-----)
    35     22    2.6428    0.6853                        (----*---)
                                   ----------+---------+---------+------
Pooled StDev =   0.7387                    1.40      2.10      2.80
```

19

```
Source     DF        SS        MS        F        p
months      5     5.268     1.054     1.33    0.268
Error      49    38.872     0.793
Total      54    44.139
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Months      N      Mean     StDev  --------+---------+---------+--------
    23      6    1.0909    1.1998              (---------*--------)
    24      6    1.5695    1.2616                    (---------*--------)
    25     10    1.1348    1.0045                 (------*------)
    26     16    1.4145    0.7402                      (-----*----)
    29     14    0.8189    0.6866              (-----*-----)
    33      3    0.4337    0.3787   (-----------*------------)
                                   --------+---------+---------+--------
Pooled StDev =   0.8907                  0.00      0.80      1.60
```