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*Full Length Research Paper*

# Assessment of the temporal change in groundwater quality when stored at different temperatures in household conditions, in the equatorial region of Central Africa

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**The study carried out aimed at assessing the impact of groundwater storage temperature at household conditions on the temporal evolution of electrical conductivity and the future of heterotrophic aerobe bacteria (HAB). The storage duration was 7 days and the considered temperatures were 3, 10, 18 and 25°C. The electrical conductivity during storage reached 829 µS/cm at 3 - 18°C and 850 µS/cm at 25°C. The maximum HAB abundance was 9 x $10^3$ cfu/ml at 3°C, 41 x $10^3$ cfu/ml at 10°C, 44 x $10^3$ cfu/ml at 18°C and 93 x $10^3$ cfu/ml at 25°C. At the 3rd and 7th days storage at 3°C, changes in bacterial abundances values were significantly in the same direction as those of electrical conductivity ($P < 0.05$). The highest cell apparent growth rate at the 3rd day storage was 0.249 $d^{-1}$ at 3°C, 0.559 $d^{-1}$ at 10°C, 0.924 $d^{-1}$ at 18°C and 1.233 $d^{-1}$ at 25°C. However, at the 7th day storage, it was 0.362 $d^{-1}$ at 3°C, 0.497 $d^{-1}$ at 10°C, 0.690 $d^{-1}$ at 18°C and 0.672 $d^{-1}$ at 25°C. At the 3rd day storage, a decrease in cell abundance was noted in 90% of samples at 3°C and the cell apparent inhibitory rate varied from 0.012 to 0.989 $d^{-1}$. The storage of groundwater in households' conditions for a long period would alter its bacteriological quality.**

**Key words:** Bacterial abundance's evolution, electrical conductivity, groundwater, storage temperature, duration.

## INTRODUCTION

In most regions of the world, groundwater is one of the main drinking water supplies. Their dating using isotopic tracers and taking into account their flows and the soil depth at which it appeared showed that they are sometimes many decades old (Portniaguine and Solomon, 1998; Cook and Herczeg, 2000). Their origins are sometimes explained by several theories such as the infiltration theory which shows the impact of the soils porosity and permeability, that of water vapour condensation implying the water vapour contained in the air which penetrates in the ground and the youthful theory showing the implication of gas emanations from magma in the depths of the ground (Banton and Bangoy, 1997).

The origin of bacteria in underground waters is often discussed. According to some authors, although the origin of other micro-organisms is uncertain, most of the

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**Abbreviation: HAB,** Heterotrophic aerobe bacteria; **CAGR,** cells apparent growth rate; **CAIR,** cell apparent inhibition rate.

underground bacteria are derived from infiltrated bacteria through propagation, due to pollution of underground water by runoffs (Mayer et al., 1997; Dzeda et al., 1998). For other authors, the ancestors of natives in the underground ecosystem originated from anoxigenic microorganisms which likely were mutated (Fenchel, 2001). In natural environments, bacterial survival is supported by various parameters. The systematic changes in microbial community composition are related to the salinity (Jiang et al., 2007). According to Lozupone and Knight (2007), the major environmental determinant of microbial community composition is salinity rather than extremes pH or other physical and chemical factors. Furthermore and depending on the mineralogy, compatible solutes in water sample can be used as sources of nutrients for bacterial populations and therefore be responsible for the marked enhancement of bacterial growth (Krammer et al., 2008). Water storage conditions sometimes affect some of the bacterial properties such as culturability and cell activity, but would not affect others such as structural and genomic integrity (Caro et al., 1999).

Water from springs and wells are often stored in various conditions for many days drinking in households. This is often due to the long distances between groundwater points and households related to drought in the region. Many studies have been carried out on groundwater quality in the equatorial area of Central Africa. They show that bacterial distribution undergoes fluctuations in space and time and is influenced by physicochemical and weather factors (Nola et al., 2001, 2002). However, little is known about the bacteriological quality of this water at the moment of their consumption. Little data is available on the change of this microbial quality during household storage conditions. This study aims was to determine the impact of the storage temperature of groundwater on the temporal evolution of its bacterial microflora and electrical conductivity.

## MATERIALS AND METHODS

### Description of study sites

The Yaounde region (Cameroon) is located at latitude 3°52'N and longitude 11°32'E, with average altitude of 760 m. The climate is of typical equatorial type, with 4 seasons (Succhel, 1988); a mild rainy season from April to June, a mild dry season from July to August, a peak rainy season from September to November and a peak dry season from December to March of the next year. Its soil is ferro-lateritic and acidic, the pH values in general is lower than 6 (Bachelier, 1959). Two well water points coded $W_1$ and $W_2$ were chosen, based on their highest importance as a drinking water supply for the population, the higher density of these neighboured populations and the permanent presence of water in these wells during all seasons of the year.

### Water samples collection

Samples were collected once every 15 days during peak dry season from mid-November 2008 to mid- April 2009. This period was chosen because it corresponds to the peak dry season during which ground waters are least affected by precipitation. Ten study campaigns were carried out. At each site, water samples were first collected in a 100 ml sterile glass bottle coded $A_{d0}$ and in a 100 ml clean polyethylene bottle coded $B_{d0}$. Second samples were collected in 4 series of 7 sterile glass bottles of 100 ml each coded $A^1_{d1}$, $A^1_{d2}$, $A^1_{d3}$,…, $A^1_{d7}$; $A^2_{d1}$, $A^2_{d2}$, $A^2_{d3}$…, $A^2_{d7}$; $A^3_{d1}$, $A^3_{d2}$, $A^3_{d3}$, …, $A^3_{d7}$; $A^4_{d1}$, $A^4_{d2}$, $A^4_{d3}$, …, $A^4_{d7}$, respectively and in 4 other series of 7 clean polyethylene bottles of 100 ml coded $B^1_{d1}$, $B^1_{d2}$, $B^1_{d3}$,…, $B^1_{d7}$; $B^2_{d1}$, $B^2_{d2}$, $B^2_{d3}$…, $B^2_{d7}$; $B^3_{d1}$, $B^3_{d2}$, $B^3_{d3}$, …, $B^3_{d7}$ and $B^4_{d1}$, $B^4_{d2}$, $B^4_{d3}$, …, $B^4_{d7}$ respectively. Each sample series was done in triplicate. All samples were then transported to the laboratory in cool conditions (6 ± 1°C) after 45 min following their collection. Samples in glass bottles were used for bacteriological analysis and those in polyethylene bottles were used for chemical analysis.

### Storage and sample analyses

In the laboratory, couple of samples $A_{d0}$ and $B_{d0}$ were immediately analyzed. Couples of samples $A^1$ and $B^1$, $A^2$ and $B^2$ and $A^3$ and $B^3$ were stored respectively in the refrigerators named R1, R2 and R3, in which temperatures were respectively adjusted to 3, 10 and 18°C. Samples $A^4$ and $B^4$ were stored at room temperature (25 ± 1°C). The duration of sample storage varied according to their codes. Samples $A^1_{d1}$, $A^2_{d1}$, $A^3_{d1}$, $A^4_{d1}$ , $B^1_{d1}$, $B^2_{d1}$, $B^3_{d1}$ and $B^4_{d1}$ were analyzed after 24 h (1 day storage). Samples $A^1_{d2}$, $A^2_{d2}$, $A^3_{d2}$, $A^4_{d2}$, $B^1_{d2}$, $B^2_{d2}$, $B^3_{d2}$ and $B^4_{d2}$ were analyzed after 48 h (2 days storage), … and samples $A^1_{d7}$, $A^2_{d7}$, $A^3_{d7}$, $A^4_{d7}$, $B^1_{d7}$, $B^2_{d7}$, $B^3_{d7}$, $B^4_{d7}$ were analyzed after 168 h (7 days storage).

The bacteriological parameter considered was the heterotrophic aerobe bacteria (HAB). Analyses were performed on standard agar medium (Bio-Rad), using plate count method and incubations were done at room temperature (25 ± 1°C) during the 7 days. Physico-chemical parameters considered were the pH, electrical conductivity and biochemical oxygen demand in 5 days (BOD5). The pH and BOD5 values were measured only on the sampling days (d0). All analyses were performed according to standard techniques (Rodier, 1996; APHA, 1998).

### Data analysis

The daily mean values of each parameter were calculated. The relationship between temporal evolution of the HAB abundance and that of electrical conductivity at each storage temperature was assessed. The straight log (number of CFUs) lines against storage duration were plotted. The slope a of each regression line was considered as the apparent evolution rate of the HAB abundance at the 3rd and 7th day of storage in each condition. This slope was then assimilated as the cell apparent growth rate (CAGR) when it was positive, or to the cell apparent inhibition rate (CAIR) when it was negative.

## RESULTS

### Chemical and bacteriological characteristics of water samples of the days ($d_0$)

The abundances of HAB isolated from well $W_1$ collected water samples varied from $46 \times 10^1$ to $67 \times 10^2$ cfu/ml (Figure 1). The highest abundance was observed at campaign $C_4$ and the lowest at campaign C1 (Figure 1). In wells $W_2$, HAB abundances varied from $39 \times 10^1$ to $61 \times 10^2$ cfu/ml. The highest value was recorded during

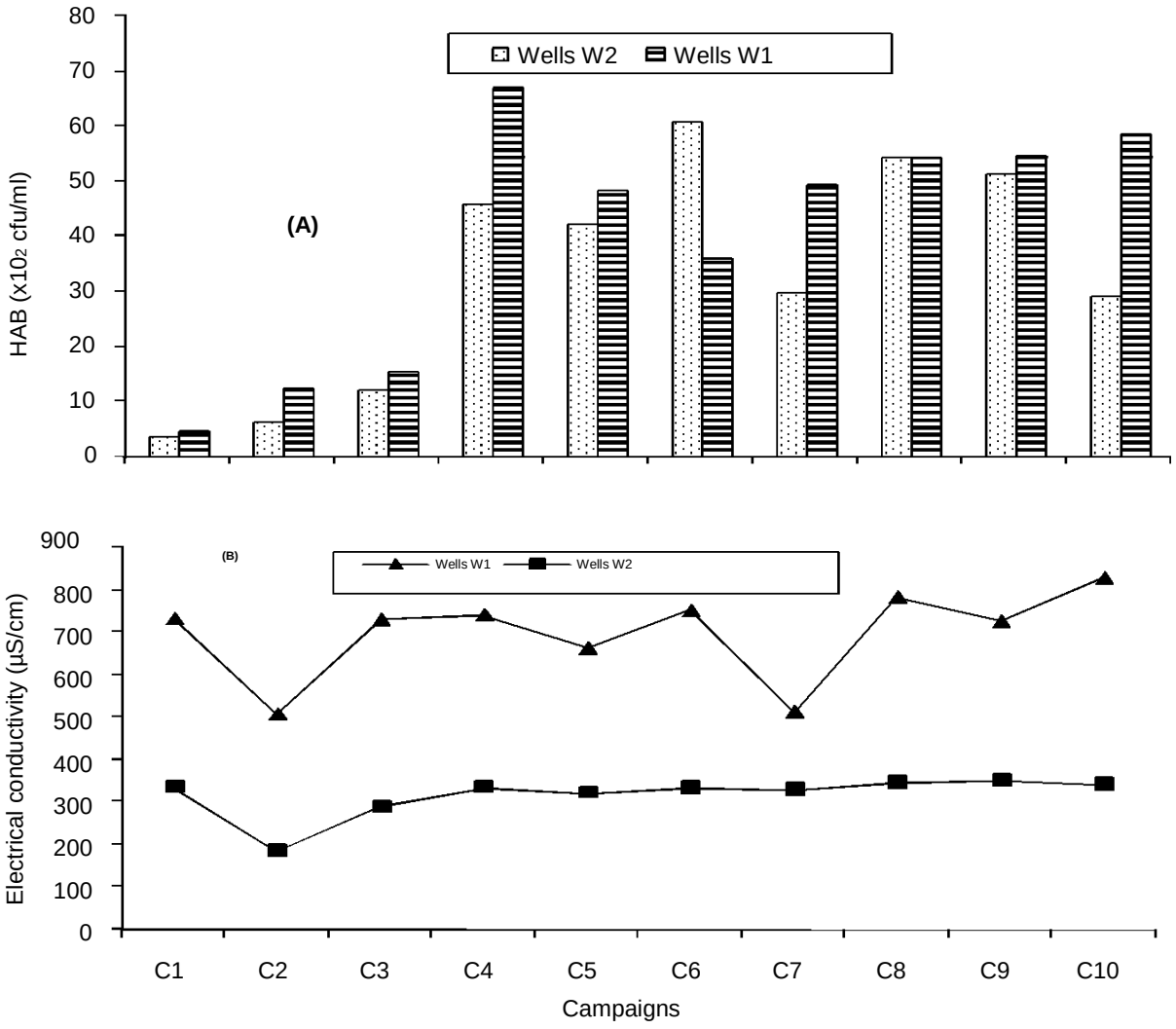


**Figure 1.** Abundances of HAB (A) and electrical conductivity values (B) in water samples during each campaign at each wells point, at the sampling day ($d_0$).

campaign C6 and the lowest during campaign C1 (Figure1). Abundances of HAB on the sampling days on the whole underwent spatial and temporal fluctuations (Figure 1).

Electrical conductivity values varied from 507 to 829 µS/cm in well $W_1$ and from 185 to 346 µS/cm in well $W_2$ (Figure 1). The values of this parameter also underwent spatio- temporal fluctuations. pH values in both wells varied between 4.9 and 5.4 (Figure 1). $BOD_5$ values remained 0.00 mg/l in samples over the campaigns in wells $W_1$ and $W_2$.

## Chemical characteristic of water samples during storage

In samples from well $W_1$, mean electrical conductivity values for all campaigns varied from 279 to 829 µS/cm in samples stored at 3°C, from 301 to 829 µS/cm in those stored at 10°C, from 433 to 829 µS/cm at 18°C and from 507 to 850 µS/cm in samples stored at 25°C. In samples from well $W_2$, mean values of this parameter in water stored at 3, 10, 18 and 25°C varied from 159 to 352 µS/cm, from 148 to 378 µS/cm, from 149 to 352 µS/cm and from 185 to 376 µS/cm, respectively. The variation rate seemed to vary from one sampling campaign to another. During water sample storage at all incubation temperatures, the electrical conductivity values underwent temporal fluctuations. The variations of the mean values are presented in Figures 2 and 3. Because most of the curves were superposed, the standard deviations were not mentioned on the graphs. Their scale values are presented in Table 1. It was noted, in most cases and almost at all incubation temperatures, that there was a decrease in electrical conductivity values after 24 h of storage (Figures 2 and 3). After this period, the evolution

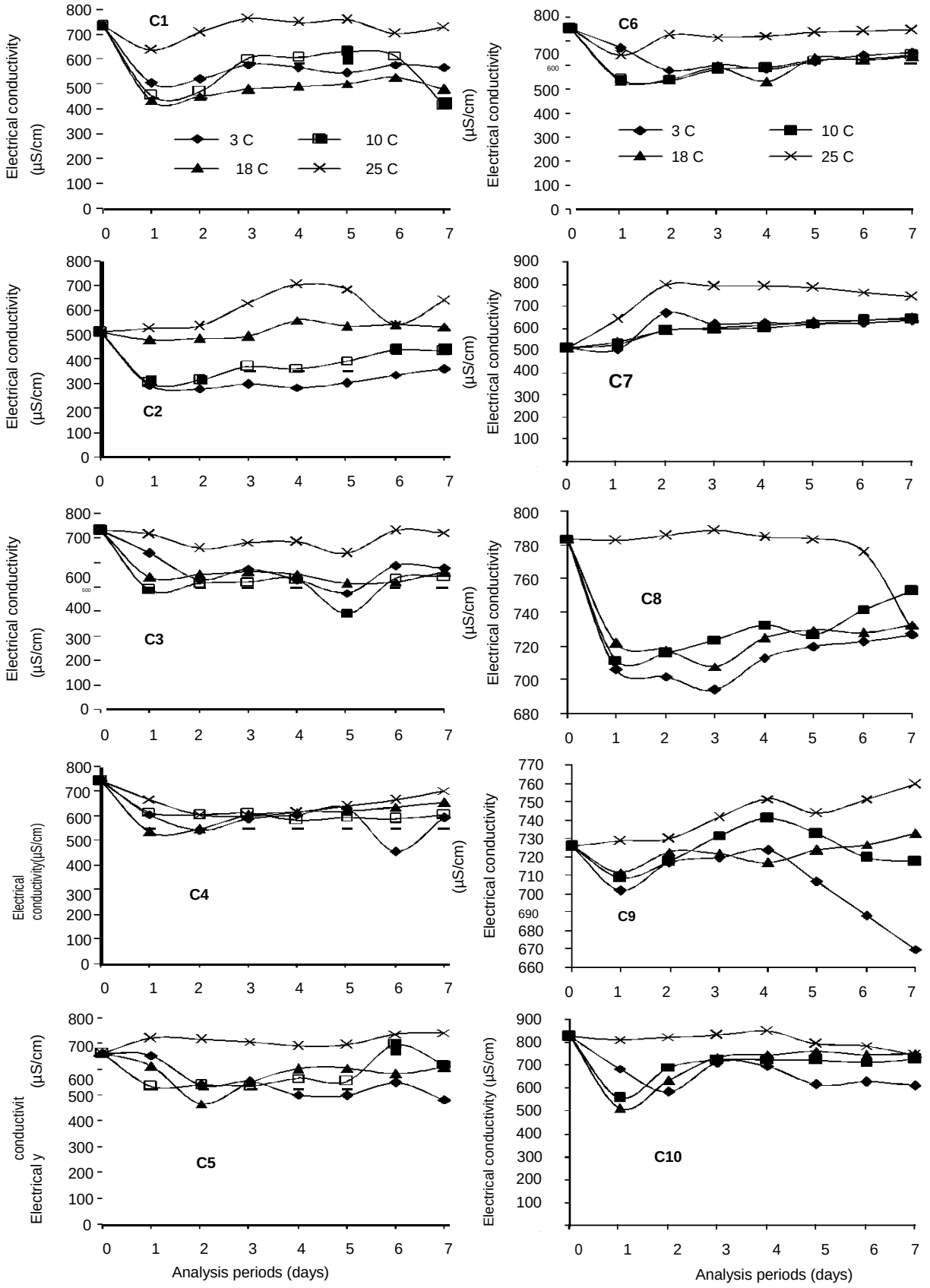


**Figure 2.** Temporal variation of the mean electrical conductivity values in samples stored at 3, 10, 18 and 25°C, collected from each of campaign (C1 to C10) from well $W_1$.

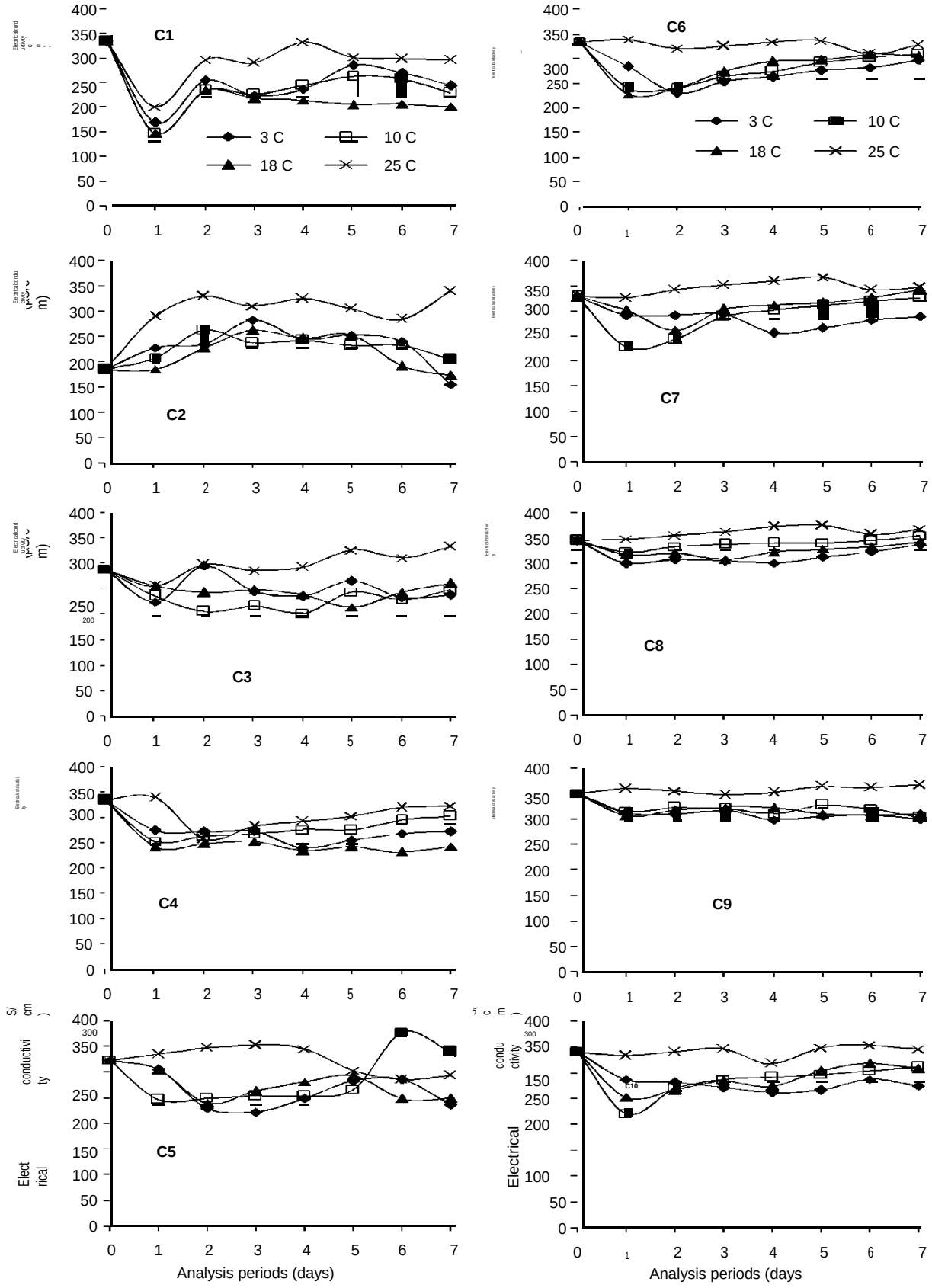


**Figure 3.** Temporal variation of the mean electrical conductivity values in samples stored at 3, 10, 18 and 25°C, collected from each of campaign (C1 to C10) from well $W_2$.

**Table 1.** Variation of standard deviation values of electrical conductivity and HAB abundances in water samples from each wells, stored at each temperature.

| Storage temperature | Electrical conductivity (µS/cm) | | HAB abundance ( x $10^2$ cfu/ml) | |
|---|---|---|---|---|
| (°C) | $W_1$ | $W_2$ | $W_1$ | $W_2$ |
| 3 | 5.6 - 12.06 | 0.9 - 3.89 | 1 - 3 | 1 – 3 |
| 10 | 4.55 - 11.7 | 0.71 - 4.75 | 1 - 3 | 1 – 4 |
| 18 | 1.15 - 7.75 | 1.2 - 4.77 | 1 - 4 | 1 – 5 |
| 25 | 1.01 - 7.5 | 0.86 - 3.45 | 1 - 6 | 1 – 6 |

of this factor varied with the sample (Figures 2 and 3). During some rare cases as it was observed in samples from campaign C7 in well $W_1$ and in samples from campaign C2 in well $W_2$, electrical conductivity values during the first 5 days storage at all temperatures were higher than the recorded values on the sampling day ($d_0$) (Figures 1 to 3).

**Bacteriological characteristics of water samples during storage**

In water samples collected from wells $W_1$ for the whole investigation campaigns, mean values of HAB abundance during storage varied from 50 to 9 x $10^3$ cfu/ml at 3°C, from 1.2 x $10^2$ to 27 x $10^3$ cfu/ml at 10°C, from 4.1 x $10^2$ to 41 x $10^3$ cfu/ml at 18°C and from 3.4 x $10^2$ to 93 x $10^3$ cfu/ml at 25°C. In samples from wells $W_2$, HAB abundance during storage varied from 30 to 26 x $10^3$ cfu/ml at 3°C, from 1.3 x $10^2$ to 41 x $10^3$ cfu/ml at 10°C, from 2.1 x $10^2$ to 44 x $10^3$ cfu/ml at 18°C and from 13 x $10^2$ to 69 x $10^3$ cfu/ml at 25°C. During water sample storage at all incubation temperatures, the HAB abundance values underwent temporal fluctuations. The variations of the mean values are presented in Figures 4 and 5. The standard deviations were not mentioned on the graphs because most of the curves were superposed. Their scale values are presented in Table 1. In some cases for both wells, a decrease of HAB abundance was observed after one or two day's storage, mainly when storage temperatures were lower than 25°C. This was followed by the increase in HAB amount. In other cases, a relative decrease in HAB abundance was observed for more than 4 days storage (Figures 4 and 5) . For the whole in both wells, abundances of HAB in water samples stored at 25°C were relatively higher than those in samples stored under other temperatures, at least during the first 5 days incubation. HAB abundance in water samples stored at 3°C seemed relatively lower (Figures 4 and 5).

The Spearman correlation test was performed between the average values of bacterial abundances and those of electrical conductivities recorded after 3 and 7 days and at each storage temperature for the 10 campaigns. It appeared that after 3 days of storage of water samples from wells $W_1$, changes in bacterial abundances values were significantly in the same direction as those of electrical conductivity ($P < 0.05$) at 3°C (Table 2). In samples from well $W_2$, this relationship was highly significant ($P < 0.005$) at 3 and 10°C (Table 2). After 7 days of storage, modifications of the 2 parameters were significant in the same way ($P < 0.05$) in water samples from wells $W_1$ stored at 3°C and very significant ($P < 0.01$) in those from $W_2$ stored at 10°C. No significant relationship was found between variations in the two parameters in water samples stored at 18 and 25°C (Table 2).

The HAB abundance's apparent evolution rates were estimated for each storage temperature, per investigation campaign and for each well point. The straight log (number of HAB counted) lines against storage duration was plotted. The slope *a* of each regression line was considered as the apparent evolution rate of the HAB abundance. This slope was assimilated to the cells apparent growth rate (CAGR) if it was positive or to the cells apparent inhibition rate (CAIR) if it was negative. The values of these evolution rates are given in Table 3. It was noted that in water samples from wells $W_1$ stored at 3°C, the HAB abundance's apparent evolution rates were negative at the 3rd day of storage, resulting in cell apparent inhibition. The CAIRs varied from 0.012 $d^{-1}$ (campaign C8) to 0.738 $d^{-1}$ (campaign C10) (Table 3). In water samples from wells $W_2$ and stored at the same temperature, most of the cell abundance's apparent evolution rates were also negative and the CAIRs varied from 0.042 $d^{-1}$ (campaign C4) to 0.989 $d^{-1}$ (campaign C10) (Table 3). CAGR was observed in samples from wells $W_2$ during the campaigns C2 (0.249 $d^{-1}$) and C8 (0.087 $d^{-1}$). At the 7th day of storage of samples from both wells at this temperature, either a decrease of CAIR or an increase of CAGR was noted. The CAGRs at the 7th day of storage ranged from 0.012 $d^{-1}$ (campaign C8) to 0.362 $d^{-1}$ (campaign C1) in samples from wells $W_1$ and from 0.041 $d^{-1}$ (campaign C9) to 0.311 $d^{-1}$ (campaign C10) in those from $W_2$ (Table 3).

In the water samples stored at 10°C, some CAIRs were observed at the 3rd day storage and their values ranged from 0.110 $d^{-1}$ (campaign C5) to 0.994 $d^{-1}$ (campaign C9) in samples from well $W_1$ and from 0.011 $d^{-1}$ (campaign C4) to 0.708 $d^{-1}$ (campaign C9) in those from the well $W_2$. The CAGRs ranged from 0.003 $d^{-1}$ (campaign C7) to 0.559 $d^{-1}$ (campaign C1) in samples from wells $W_1$ and

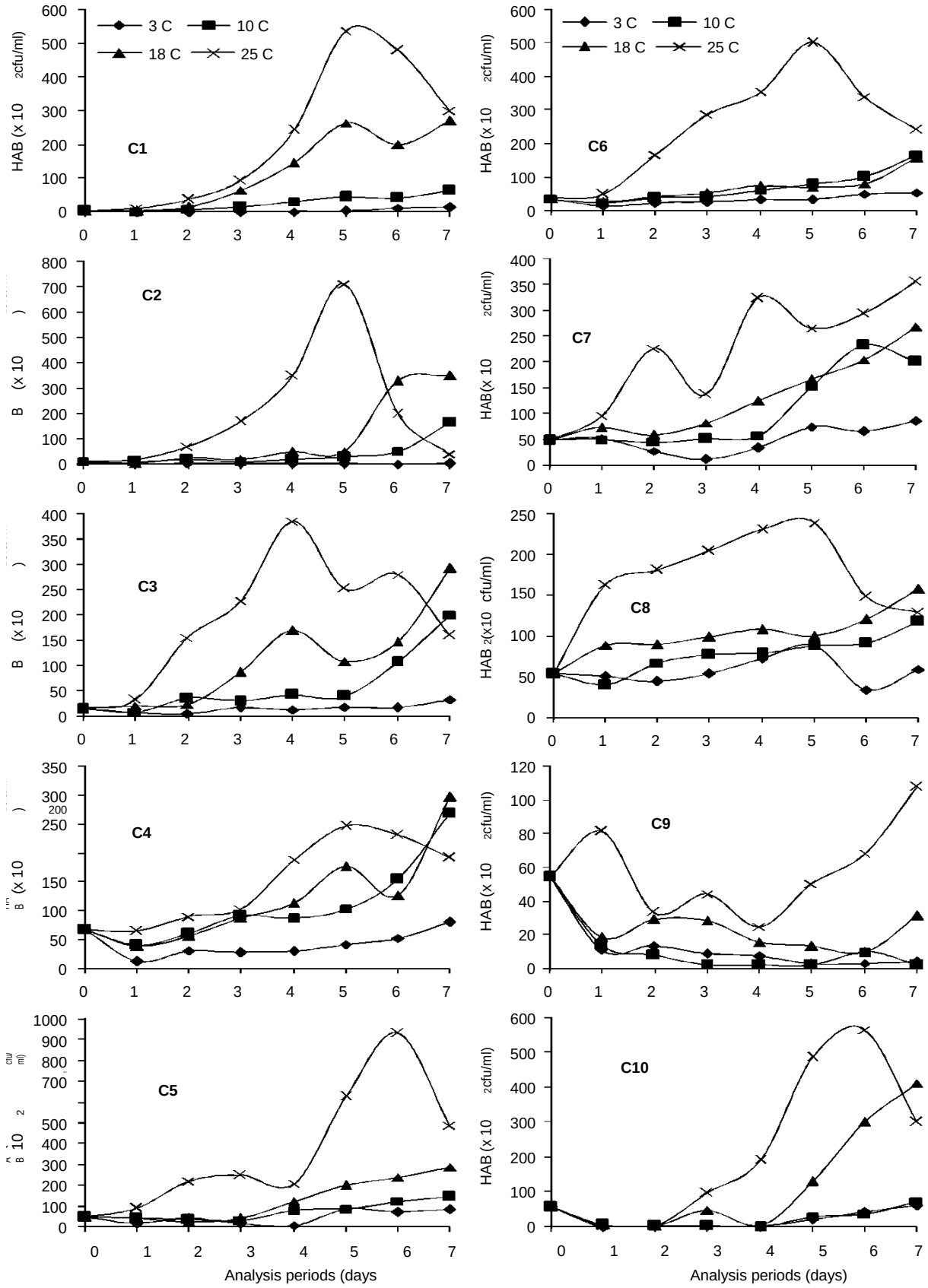


**Figure 4.** Temporal variation of the mean abundances of HAB in samples stored at 3, 10, 18 and 25°C, collected from each of campaign (C1 to C10) from well $W_1$.

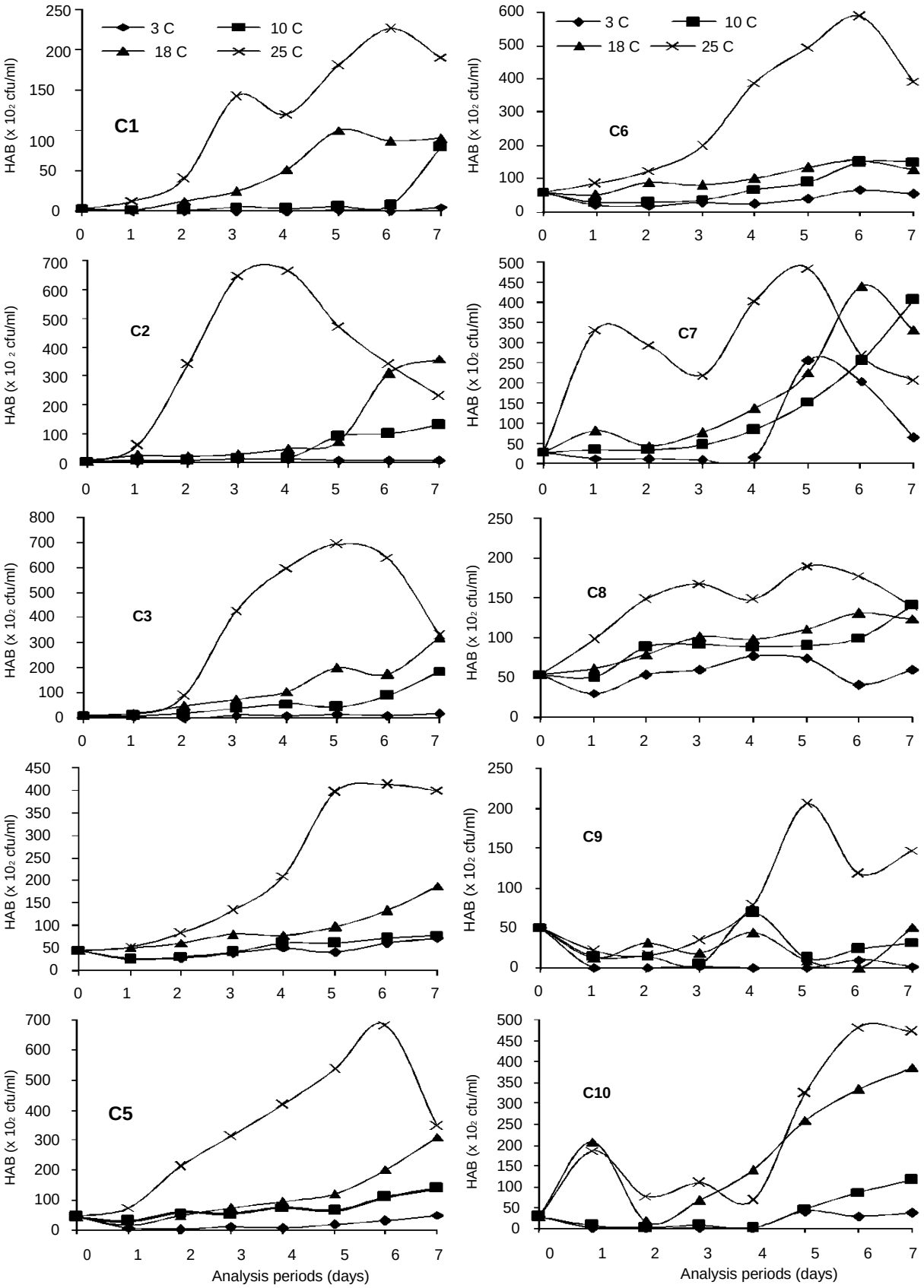


**Figure 5.** Temporal variation of the mean abundances of HAB in samples stored at 3, 10, 18 and 25°C, collected from each of campaign (C1 to C10) from well $W_2$.

**Table 2.** Spearman correlation coefficients between evolution rate values of cell abundance and mean values of electrical conductivity in water samples at the 3rd and 7th day of water sample storage, at each storage temperature.

| Well | Correlation coefficient | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3°C | | 10°C | | 18°C | | 25°C | |
| | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day |
| $W_1$ | 0.413* | 0.271* | 0.309 | 0.047 | 0.313 | -0.011 | 0.162 | 0.158 |
| $W_2$ | 0.520*** | 0.201 | 0.468*** | 0.312** | 0.089 | 0.008 | 0.126 | 0.004 |

3rd day of storage, n= 40 samples; 7th day of storage, n= 80 samples; *, P < 0.05; **, P < 0.01 ***; P < 0.005.

from 0.041 $d^{-1}$ (campaign C1) to 0.409 $d^{-1}$ (campaign C3) in those from well $W_2$ (Table 3). With the exception of the samples from well $W_1$ during the campaign C9; all HAB abundance's apparent evolution rates were positive at the 7th day storage at this temperature. The CAGRs ranged from 0.122 $d^{-1}$ (campaign C8) to 0.0497 $d^{-1}$ (cam-paign C1) in samples from well $W_1$ and from 0.017 $d^{-1}$ (campaign C9) to 0.481 $d^{-1}$ (campaign C2) in those from well $W_2$ (Table 3).

In water samples stored at 18°C, all HAB abundance's apparent evolution rates were positive with the exception of samples from well $W_1$ collected during the campaigns C5, C9 and C10. At the 3rd day storage, the CAGRs ranged from 0.117 $d^{-1}$ (campaign C4) to 0.924 $d^{-1}$ (campaign C1) in samples from well $W_1$ and from 0.005 $d^{-1}$ (campaign C10) to 0.740 $d^{-1}$ (campaign C1) in those from $W_2$. At the 7th day storage, it ranged from 0.112 $d^{-1}$ (campaign C8) to 0.690 $d^{-1}$ (campaign C1) in those from well $W_1$ and from 0.125 $d^{-1}$ (campaign C8) to 0.565 $d^{-1}$ (campaign C1) in those from well $W_2$ (Table 3).

In the samples stored at 25°C, negative HAB abundance's apparent evolution rates were observed at the 3rd day of storage in water samples collected from the well $W_2$ during the campaigns C2, C6 and C9 and from $W_1$ during the campaign C9 (Table 3). The CAGR values at the 3rd day storage varied from 0.123 $d^{-1}$ (campaign C10) to 0.1012 $d^{-1}$ (campaign C1) in samples from $W^1$ and from 0.312 $d^{-1}$ (campaign C10) to 1.233 $d^{-1}$ (campaign C3) in those from $W_2$. At the 7th day storage, the CAGRs ranged from 0.054 $d^{-1}$ (campaign C9) to 0.672 $d^{-1}$ (campaign C1) in samples from $W_1$ and from 0.121 $d^{-1}$ (campaign C8) to 0.547 $d^{-1}$ (campaign C1) in those from $W_2$ (Table 3).

It was noted that in the water samples collected from $W_1$ during the campaign C5 and from well $W_2$ during the campaign C9, the CAIR decreased gradually with the increase in storage temperature after 3 days of storage. However, in samples collected from $W_1$ during the campaigns C9 and C10, the CAIR values registered at 3rd storage at 10°C were greater than those recorded at 3°C (Table 3). In majority of samples from both wells which were stored at 3 - 18°C, the CAGR values increased from the 3rd to the 7th day of storage. However, in those stored at 25°C, the CAGR values decreased from the 3rd to 7th day of storage in most cases. On the whole, the negative bacterial abundance's evolution rates registered in some cases at 3rd day storage were followed by the positive values at the 7th day. No CAIR changed to any CAGR from the 3rd to 7th day of samples storage (Table 3). It was also noted that bacterial abundance's evolution rates in the stored water samples varied from one campaign to another, from one storage temperature to another and underwent temporal fluctuations at the same storage temperature (Table 3).

## DISCUSSION

The investigations showed that water sample storage can result in an increase in HAB abundances (Figures 4 - 5, Table 3). This suggests the presence of biodegradable energetic substances. These waters might have contained biodegradable organic matter in microgram/l or nanogram/l level (the minimum concentration of the analysis method used could record 0.01 mg/l), or inorganic compounds that may have been used as an energy source through chemolithotrophic metabolism (Gounot, 1994; Holt et al., 2000; Fenchel, 2001). These processes may have been responsible for temporal variations in electrical conductivity values in water samples during storage.

A decrease of CAGR and the increase of CAIR at the 3rd day of storage were noted in most cases at 3°C. The lower temperature may have resulted in large number of cells in stress. This state would have induced some cells in a viable non-cultivable state, or would have destroyed others and the surviving cells then made use of the substances released. This may have led to an increase of CAGR or the decrease of CAIR noted on the 7th day of storage. These processes could have included among others the degradation of some macromolecules and the conversion of electrically neutral molecules to electrically charged molecules, which led to the variability of electrical conductivity of the medium (Nola et al., 1998). This explains the significant correlations shown in Table 2. These processes can also affect other cell properties. Caro et al. (1999) investigating the genomic integrity of the stressed *Salmonella typhimurium* population by salinity (0.9 and 3.8%) at 23°C, noted that the evolution of culturability and cellular activities followed a pattern different from that of the structural and genomic integrities. A slight decrease in cell structural and

**Table 3.** Apparent evolution rate values of HAB abundance (and regression coefficient) at the 3rd and 7th day of water sample storage at each temperature, from each campaign.

| Cp | Apparent evolution rate (day$^{-1}$) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Well $W_1$ | | | | | | | | Well $W_2$ | | | | | | | |
| | 3°C | | 10°C | | 18°C | | 25°C | | 3°C | | 10°C | | 18°C | | 25°C | |
| | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day | 3rd day | 7th day |
| C1 | -0.457 | 0.362 | 0.559 | 0.497 | 0.924 | 0.690 | 1.012 | 0.672 | -0.891 | 0.111 | 0.041 | 0.375 | 0.740 | 0.565 | 1.192 | 0.547 |
| | (0.319) | (0.442) | (0.432) | (0.800) | (0.836) | (0.880) | (0.999) | (0.864) | (0.788) | (0.052) | (0.007) | (0.567) | (0.719) | (0.855) | (0.999) | (0.807) |
| C2 | -0.349 | -0.068 | 0.069 | 0.343 | 0.346 | 0.570 | 0.897 | 0.324 | 0.249 | 0.069 | 0.225 | 0.481 | 0.467 | 0.535 | -1.567 | 0.416 |
| | (0.524) | (0.130) | (0.081) | (0.778) | (0.285) | (0.839) | (0.982) | (0.324) | (0.853) | (0.345) | (0.813) | (0.890) | (0.681) | (0.919) | (0.946) | (0.412) |
| C3 | -0.023 | 0.157 | 0.341 | 0.370 | 0.528 | 0.423 | 0.965 | 0.348 | -0.077 | 0.115 | 0.409 | 0.383 | 0.625 | 0.451 | 1.233 | 0.568 |
| | (0.002) | (0.423) | (0.461) | (0.835) | (0.795) | (0.880) | (0.949) | (0.558) | (0.020) | (0.239) | (0.832) | (0.926) | (0.989) | (0.956) | (0.938) | (0.703) |
| C4 | -0.171 | 0.109 | 0.133 | 0.212 | 0.117 | 0.238 | 0.164 | 0.206 | -0.042 | 0.102 | -0.011 | 0.136 | 0.192 | 0.190 | 0.365 | 0.361 |
| | (0.114) | (0.220) | (0.273) | (0.812) | (0.199) | (0.800) | (0.851) | (0.834) | (0.046) | (0.544) | (0.003) | (0.684) | (0.971) | (0.955) | (0.964) | (0.947) |
| C5 | -0.207 | 0.143 | -0.110 | 0.193 | -0.094 | 0.338 | 0.585 | 0.366 | -0.588 | 0.176 | 0.137 | 0.186 | 0.269 | 0.352 | 0.705 | 0.343 |
| | (0.292) | (0.192) | (0.992) | (0.743) | (0.174) | (0.774) | (0.933) | (0.829) | (0.320) | (0.139) | (0.339) | (0.832) | (0.305) | (0.854) | (0.971) | (0.744) |
| C6 | -0.060 | 0.123 | 0.083 | 0.243 | 0.158 | 0.214 | 0.747 | 0.316 | -0.255 | 0.083 | -0.144 | 0.216 | 0.137 | 0.144 | -0.389 | 0.326 |
| | (0.042) | (0.503) | (0.217) | (0.889) | (0.462) | (0.863) | (0.948) | (0.641) | (0.387) | (0.167) | (0.311) | (0.659) | (0.491) | (0.827) | (0.995) | (0.872) |
| C7 | -0.486 | 0.106 | 0.003 | 0.252 | 0.130 | 0.244 | 0.390 | 0.246 | -0.358 | 0.134 | 0.131 | 0.398 | 0.226 | 0.367 | 0.589 | 0.173 |
| | (0.840) | (0.164) | (0.005) | (0.775) | (0.536) | (0.943) | (0.619) | (0.754) | (0.864) | (0.408) | (0.824) | (0.934) | (0.360) | (0.876) | (0.451) | (0.237) |
| C8 | -0.012 | 0.012 | 0.155 | 0.122 | 0.183 | 0.112 | 0.408 | 0.078 | 0.087 | 0.041 | 0.209 | 0.119 | 0.213 | 0.125 | 0.381 | 0.121 |
| | (0.029) | (0.011) | (0.519) | (0.831) | (0.750) | (0.813) | (0.742) | (0.161) | (0.134) | (0.110) | (0.781) | (0.805) | (0.981) | (0.912) | (0.929) | (0.517) |
| C9 | -0.513 | -0.331 | -0.994 | -0.331 | -0.152 | -0.118 | -0.150 | 0.054 | -0.907 | -0.124 | -0.708 | 0.017 | -0.211 | -0.238 | -0.153 | 0.291 |
| | (0.665) | (0.747) | (0.977) | (0.457) | (0.194) | (0.276) | (0.270) | (0.079) | (0.363) | (0.038) | (0.891) | (0.002) | (0.202) | (0.131) | (0.137) | (0.585) |
| C10 | -0.738 | 0.262 | -0.948 | 0.181 | -0.088 | 0.483 | 0.123 | 0.607 | -0.989 | 0.311 | -0.504 | 0.356 | 0.005 | 0.349 | 0.312 | 0.333 |
| | (0.383) | (0.203) | (0.814) | (0.084) | (0.007) | (0.433) | (0.008 | (0.557) | (0.667) | (0.207) | (0.430) | (0.318) | (0.001) | (0.540) | (0.265) | (0.652) |

Cp = Campaign.

genomic integrity was observed after 24 h incubation, but this increased quickly at the 4th day whereas, the culturability and the cellular activities decreased after 7 days of starvation; the percentages of structural and genomic integrity were still very high. In other respects, cell growth noted in samples stored at 3 and 10°C (Figures 4 - 5, Table 3), reflected the presence of psychrophilictolerantmicroorganisms.

Psychrophilic bacteria possess great concentration of unsaturated fats in their cytoplasm. Some of them contain polyunsaturated fats, the degree of unsaturation being related to the thermic transition point T (temperature at which fat melts or solidifies). Unsaturated fats remain liquid at low temperature, but are denatured at moderate temperature. Saturated fats are solid at ambient temperature meanwhile unsaturated ones remain liquid even at temperatures lower than 15°C (Todar, 2007).

Furthermore, it has been indicated that some bacteria strains such as *Bacillus subtillis* have multiple transportation systems leading to cell osmoprotection and others such as *Corynebacterium* genera are equipped with several systems of chemical molecules disintegration systems in the environment

(Wood et al., 2001).

At relatively high storage temperatures, no significant relationship was noted between cell abundance evolution rates and the electrical conductivity values (Table 2). The highest temperatures considered in this investigation relatively sped up the bacterial enzyme kinetics. Cell activities then occurred through various pathways and various chemicals were released into the medium and some of them interacted. In the aquatic environment, multiple microorganisms coexist as communities, competing for resources and are often associated as biofilms. Bacterial growth rate and production of quorum-sensing inhibitors constituted an attempt to identify attributes and allowed bacteria to effectively interact and coexist in a drinking- water environment. Simoes et al. (2007) when studying interactions amongst most species of *Methylobacterium, Sphingomonas, Burkholderia, Staphylococcus* and *Acinetobacter* genera noted synergy /cooperation between some species and antagonism and neutral interaction between others. According to Krammer et al. (2008), compatible solutes can be a source of nutrients for bacterial populations and therefore be responsible for the observed marked enhancement of bacterial growth. The exhaustion of biodegradable organic or inorganic compounds initially present would lead to a modification of the chemical characteristics of the medium; this latter becomes favorable for the survival of some bacteria and unfavourable for the others. At relatively low temperatures, the moderate celerity of enzymatic activities leads to a low impoverishment in celerity of the medium with energetic compounds (Todar, 2007).

In the aquatic medium, chemical elements highly influence micro-organism survival. In return, micro-organisms influence water physico- chemical properties through release of diverse metabolic wastes, rather than in normal conditions, or after physiological changes resulting in response to variation of environmental conditions. Some of these metabolic wastes can be harmful to other living micro-organisms in the ecosystem. These processes often contribute to significant fluctuations of the electrical conductivity in the milieu (Grouhel et al., 1995). This would have been one of the origins of the relative temporal increases of electrical conductivity values in stored samples at various temperatures (Figures 2 - 3, Table 1). It then resulted in the production of diverse chemicals, some alkaline others acidic, in the medium. In addition, some authors have observed that systematic changes in microbial community composition are mainly linked to the salinity gradient (Jiang et al., 2007).

In this study, the pH was not daily recorded in storage samples. It has been indicated that groundwater harbors various bacterial groups and despite the pH of the environment, the vast majority of the populations are tolerant vis-à-vis this factor (Tiago et al., 2004).

Nevertheless, it is known that the indirect action of pH creates an unsuitable environment for the survival of micro-organisms and is not directly associated with $H^+$ or $OH^-$ concentrations, but acts through modification of the assimilation coefficient of different compounds by bacteria, which depends on the degree of bacterial tolerance vis-à-vis of acidity and of alkalinity of the environment (Nola et al., 2002). This modification can lead certain chemical substances towards the intracellular medium and are responsible for bacteria stress (Todar, 2007). This can partly be one of the causes of the decrease in HAB abundance in water samples during storage.

## Conclusion

Physiological adaptations to chemistry may be an integral part of the evolution, ecology and diversification of water organisms. Their versatile character allows them to adapt to numerous apparently hostile conditions as lower temperatures. Their activity even though lower in speed, can permit them to colonize and modify biotope properties. Storage of drinking waters originating from underground sources for a long period would increase the health risk to the consumers in the short term if it contains potentially pathogenic bacteria, due to their potential growth and activity.


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