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Effect of *Escherichia coli* and *Aeromonas hydrophila* on Some Abiotic Properties of Water Stored in Aerobic and Anaerobic Conditions

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Abstract

Few studies have been carried out to date on the influence of bacteria on variations in the abiotic properties of water. The aim of the present work was to evaluate the influence of *Escherichia coli* and *Aeromonas hydrophila* on some abiotic properties of groundwater stored under aerobic or anaerobic conditions. Experiments were performed in the presence of monospecific cells and bispecific cells. The incubation temperatures were 4˚C and 23˚C. The incubation (storage) times were 6 h, 12 h, 24 h, 48 h and 72 h. Bacteriological analyses were carried out using culture and physicochemical analyses using appropriate techniques. The results show that in the presence of monospecific cells and under aerobic conditions, *E. coli* abundances were relatively lower than under anaerobic conditions. The opposite was observed in the presence of bispecific cells. In most cases, minimum cell abundances were observed after 6 hours of storage. The same was true for *A. hydrophila cells*. The values of pH, electrical conductivity and ammonium ions in the water on the day of sampling varied over time. The pH values were relatively higher at 4˚C in the presence of *E. coli* cells alone, and at 23˚C in the presence of *A. hydrophila* cells. Ammonium ion levels were relatively higher at 4˚C under anaerobic conditions for both bacterial species. The highest electrical conductivity value was observed at

23˚C in the presence of *E. coli* cells, and at 4˚C in the presence of *A. hydrophila* cells, under anaerobic conditions. Electrical conductivity is higher in the presence of *A. hydrophila* with fluctuations from 169 to 285 µS/cm after 24 h incubation and 259 µS/cm after 6 hours incubation at 4˚C and 23˚C respectively. Under anaerobic conditions and in the presence of *E. coli*, the conductivity varied from 169 µS/cm at sampling to 274 µS/cm after 72 hours of incubation at 4˚C and from 169 to 289 after 24 hours. Under the same experimental conditions and in the presence of *A. hydrophila*, the electrical conductivity is higher at 4˚C with a value of 299 µS/cm after 24 hours. In the monospecific condition and under aerobic and anaerobic conditions and at an incubation temperature of 4˚C, the increase in the abundance of *E. coli* cells is significantly and positively correlated with the increase in pH values. In the mixed condition, pH is very significantly and positively correlated with the abundance of commensal *Escherichia coli* in anaerobic conditions ($P < 0.01$). Ammonium ions (NH_4^+) is significantly and negatively correlated with cellular concentrations of *A. hydrophila* in both aerobic and anaerobic conditions.

Keywords

Groundwater, *Escherichia coli*, *A. hydrophila*, Storage, Variation in Abiotic Factors

1. Introduction

Water is an essential resource for life and a very precious resource on Earth. It is used by humans both for food needs and for agricultural and industrial purposes [1]. On a planetary scale, it seems insufficient to meet the needs of human populations. In fact, of the 13,600 million km^3 of water covering our planet, only 0.014% is fresh water in the form of surface water [2]. This low availability puts a third of humanity today in a situation known as "water stress", with less than 1700 cubic metres of fresh water available per inhabitant per year. Fresh water is therefore a scarce resource. In addition to this scarcity, water faces many problems including pollution from the population explosion, making the issue of water supply more and more worrying every day [3]. Faced with the threat of water scarcity, international organisations have taken the lead in organising a growing number of conferences and meetings to address the problem on a planetary scale. To this end, at the first World Water Forum, held in Marrakech, Morocco, in 1997, the participating countries expressed their concern about the scarcity and quality of drinking water. Poor water quality is often due to the presence of protozoa, viruses or bacteria, and to deterioration caused by physico-chemical parameters. It is the cause of many water-related diseases [4]. The most commonly isolated bacteria from freshwater human consumption include species of the genera *Aeromonas*, *Salmonella*, *Shigella*, *Escherichia*, *Yersinia*, *Vibrio* and *Campylobacter* [4]. These bacteria can be opportunistic pathogens, strict pathogens or commensal organisms

[5]. Strict or opportunistic pathogens can cause major waterborne epidemics such as dysentery, cholera, typhoid and paratyphoid fever, acute gastroenteritis and diarrhoea [4]. In developing countries, rapid population growth and uncontrolled urbanisation are increasing the demand for drinking water. Unfortunately, supply is less than demand [6] and populations are turning to groundwater as a source of drinking water to meet their needs [7]. Many studies carried out in the city of Yaoundé have focused on determining the bacteriological quality of groundwater. It appears that these waters are acidic, and soft and host a diverse microflora, including faecal bacteria and opportunistic pathogenic bacteria, whose population dynamics are influenced by rainfall and certain physico-chemical factors [8]. However, few studies have been carried out on the importance of bacteria in the variation of physicochemical properties of water. Little information is available on the relationship between the composition of the bacterial microflora and the variation in the physicochemical properties of water. Based on this observation, the study of the temporal variation of some physico-chemical properties of groundwater associated with the dynamics of some bacteria was carried out. The present work aims to evaluate the influence of the presence of *Escherichia coli* and *Aeromonas hydrophila* on the temporal variation of some abiotic properties (pH, electrical conductivity and ammoniacal nitrogen concentration) of groundwater stored under aerobic and anaerobic conditions, depending on the incubation temperature.

2. Materials and Methods

2.1. Choice of Bacteria and Inoculation

The study focused on two bacterial species: commensal *Escherichia coli* and *Aeromonas hydrophila*. These two species were chosen because of their sanitary and hygienic importance and their use as indicators of the microbiological quality of water intended for consumption [9]. These bacteria generally indicate that the bacteriological quality of the water has deteriorated. For each of the two bacterial species, a pre-culture was prepared by inoculation on specific culture media Endo and ADA (Ampicillin Dextrin Agar) for *E. coli* and *A. hydrophila*, respectively. After 24 hours of incubation at 44˚C for *E. coli* and 37˚C for *A. hydrophila*, the colonies were picked with a platinum loop, and were then subcultured on a basic culture medium of base, PCA (plate count agar), sloped into the test tubes and incubated at 37˚C for 24 hours to obtain young colonies. These pure colonies were then added to 10 ml of sterile physiological water (0.85% NaCl). The concentration of bacteria in each stock solution was adjusted to 2.10^8 CFU/ml by reading the optical density between 0.8 and 400 nm using a spectrophotometer.

2.2. Collection of Water Samples

Water samples were collected monthly from September to December 2020 from a number of wells in the city of Yaounde. A volume of 5 litres of water was collected in a container previously rinsed with water from the well. In the laboratory, this water was filtered using a vacuum pump through a filter membrane with a porosity of 0.45 µm and then through a membrane with a porosity of 0.20 µm, with the aim of eliminating all bacteria present in this water. The physico-chemical analysis is carried out on the part of this filtered water sample.

2.3. Experimental Design

For each set, 60 Erlenmeyer flasks of 200 ml were then sterilised in the autoclave, including one for the control. These flasks were divided into 2 series, AER and ANAER, of 30 flasks each. For each series, the flasks were divided into 2 batches, T_4 and T_{23}, of 15 flasks each. Then, for each batch, they were arranged in triplets. For each triplet, the conical flasks were coded EC, AH and EC + AH. The flasks from batch T_4 were then labelled $T_{4\text{-}6h}$, $T_{4\text{-}12h}$, $T_{4\text{-}24h}$, $T_{4\text{-}48h}$, and $T_{4\text{-}72h}$. Those from batch T_{23} were labelled $T_{23\text{-}6h}$, $T_{23\text{-}12h}$, $T_{23\text{-}24h}$, $T_{23\text{-}48h}$, and $T_{23\text{-}72h}$. Then, 100 ml of the water sample filtered as described above is added into each flask.

For the bacterial suspensions, 0.2 ml of the solution of this suspension was added to each flask. The suspensions of *E. coli* cells are placed in the tubes marked EC. Suspensions of *A. hydrophila* cells are placed in tubes marked AH. A suspension of each of the 2 bacterial cells was added to the tubes coded EC+AH. A cell suspension was repeatedly introduced into a primed control vial. The bacteriological analysis was carried out on the solution of the control vial and allowed to know the concentration of cells in the vials at the beginning. With the exception of the control flasks, all other flasks were incubated. The AER series was incubated under aerobic conditions and the ANAER series under anaerobic conditions.

Batch T_4 flasks were incubated at 4˚C and T_{23} flasks were incubated at laboratory temperature. The incubation times were 6 h, 12 h, 24 h, 48 h and 72 h. The experiment was first carried out in the presence of a single bacterial species (pure culture) and then in the presence of two bacterial species at the same time (mixed culture). During the study, changes in some chemical properties were observed. The parameters studied were pH, electrical conductivity and ammoniacal nitrogen. These parameters were measured before the introduction of the different bacterial strains and then at each hour of incubation [9] [10].

The bacteriological analysis is based on a quantitative study aimed at evaluating the temporal variation in the abundance of commensal *E. coli* and *A. hydrophila*. The results were expressed in colony forming units (CFU) per 100 ml of analysed water [11].

2.4. Statistical Analysis

The temporal variations of the abiotic variables considered (pH, conductivity, ammoniacal nitrogen) were illustrated by graphs. The temporal evolution of the abundances of *E. coli* and *A. hydrophila* was presented by means of histograms. The correlations between the variables considered in this study were evaluated by the Spearman correlation test using SPSS 25.0 software.

3. Results and Discussion

3.1. Pre-Storage Groundwater Physico-Chemical and Bacteriological Properties

During the study, the physico-chemical parameters of the well water sampled varied over time. The pH of a water represents its acidity or alkalinity. The pH of natural waters is linked to the nature of the land they pass through. On the day of sampling, the pH of the waters studied varied relatively little. Values ranged from 6.54 to 7.32. Several analyses of groundwater in Yaounde showed that the pH ranged between 4 and 6. The acidic nature of the soils of Yaounde could explain the acidic pH values of the water analysed on the days of sampling [12]. The acidic nature of groundwater is due to the presence of organic acids in the soil and atmospheric water originating from infiltration into the water table [13].

The measurement of electrical conductivity allows a rapid but very approximate assessment of the global mineralisation of water and follows its evolution [9]. The temporal variations in the electrical conductivity and, consequently, in the degree of mineralisation of the groundwater analysed would be due to the variations in the nature and concentration of the salts dissolved in the water. All groundwater contains various salts in solution resulting from the location and movement of water in the past [14]. The type and concentration of salts depends on the environment, movement and origin of the groundwater. The electrical conductivity of the well water studied varied between 112 and 237 μS/cm during the study. It is slightly mineralised [9]. The temporal variations of the values of electrical conductivity on the days of water abstraction would be related to the temporal variations of the solubility of the minerals of the soil and to the importance of mineral contributions of superficial origin, resulting from anthropogenic activities in the soil. Above the watershed of the water points [15].

In fact, the study well is located close to latrines and the Olézoa stream. This faecal pollution could also explain the relatively high levels of ammoniacal nitrogen observed in the water from the study wells, which are the result of human activities above the water catchment area of the water points [15]. Ammoniacal nitrogen concentrations ranged from 1.4 to 6.66 mg/l (3.77 ± 2.21 mg/l). In general, groundwater does not naturally contain nitrogen compounds, which are derived from the decomposition of living matter by soil microorganisms and may be converted to molecular nitrogen or remain in the soil in very small amounts [16]. This phenomenon artificially increases the amount of combined nitrogen available in the soil, creating an imbalance between supply and consumption and an excess of nitrogen that eventually leaches into the groundwater.

In monospecific culture and under aerobic conditions, the abundance of *E. coli*, expressed in Ln units (CFU/100 ml), varied from 13.62 to 18.15 and from 12.77 to 18.36 at 4˚C and 23˚C, respectively (**Figure 4**). Under anaerobic conditions and at the same temperature, these densities varied between 12.14 and 17.76 units (Ln (CFU/100 ml)) and between 13.71 and 18.31 units (Ln (CFU/100 ml)), respectively. The minimum values were observed in most cases after 12 hours of incubation (**Figure 1**). In mixed culture, *E. coli* concentrations varied from 13.59 to 17.93 units (Ln (CFU/100ml)) and from 14.48 to 18.07 units (Ln (CFU/100ml)) at 4˚C and 23˚C respectively under aerobic conditions. Under anaerobic conditions, these concentrations varied from 13.08 to 18.10 and from 14.29 to 17.81 units (Ln (CFU/100ml)), respectively. In the majority of cases, minimum cell counts were recorded after 12 hours of incubation (**Figure 1**).

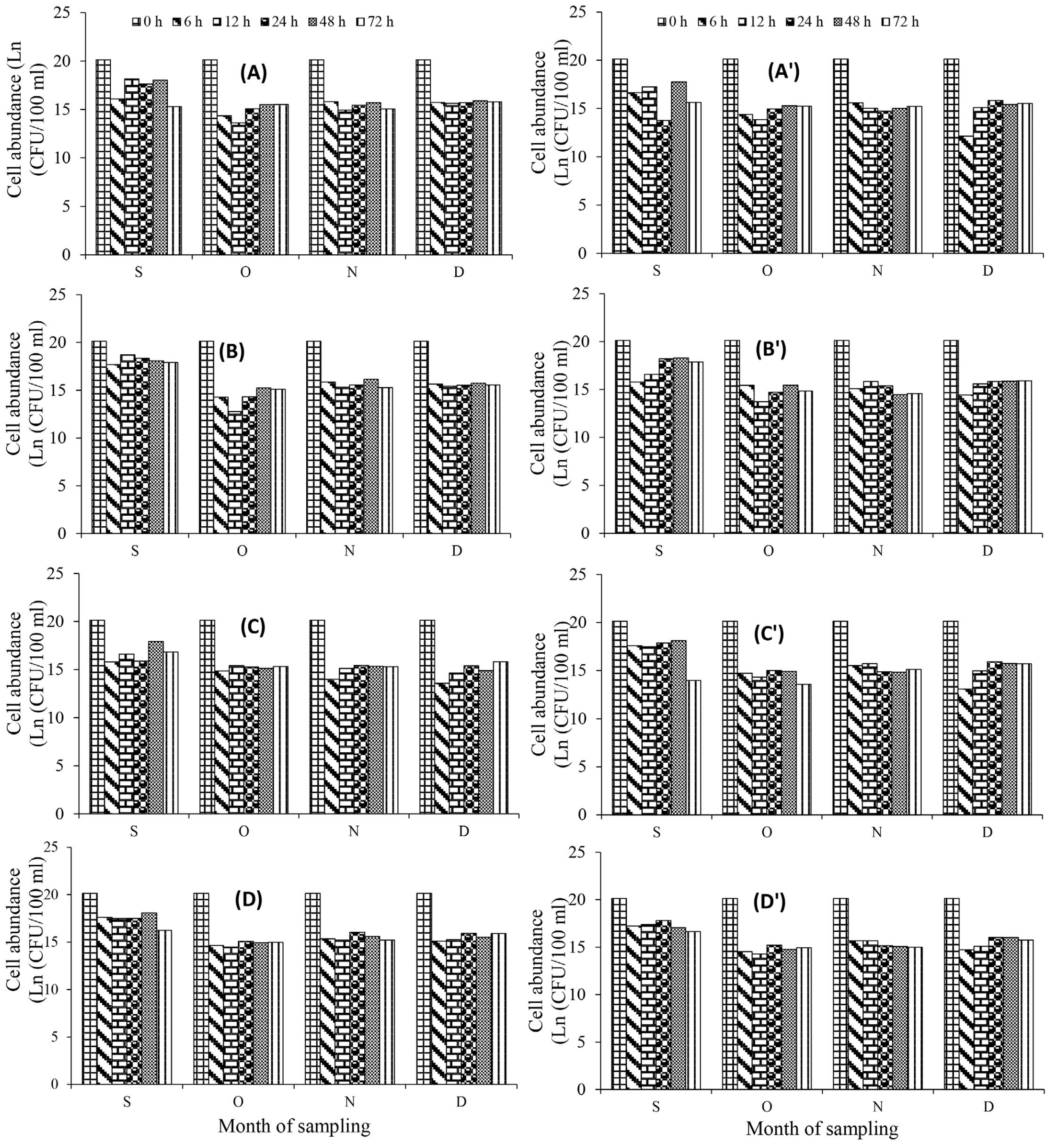

Figure 1. Temporal variation of *Escherichia coli* in monospecific culture incubated under aerobic conditions at 4˚C (A) & 23˚C (B), under anaerobic conditions at 4˚C (A’) & 23˚C (B’), then in mixed culture under aerobic conditions at 4˚C (C) & 23˚C (D), under anaerobic conditions at 4˚C (C’) et 23˚C (D’).

In monospecific culture, *A. hydrophila* cell densities varied from 14.06 to 17.97 units (Ln (CFU/100ml)) and from 14.09 to 17.81 units (Ln (CFU/100ml)), respectively, at incubation temperatures of 4˚C and 23˚C under aerobic conditions. Under anaerobic conditions, they varied from 13.29 to 17.40 units (Ln (CFU/100ml)) and from 14.19 to 17.10 units (Ln (CFU/100ml)), respectively, at the same temperatures. Under mixed culture conditions and under aerobic conditions, the abundance of *A. hydrophila* cells varied from 14.09 to 17.31 units (Ln (CFU/100ml)) and from 13.08 to 17.52 units (Ln (CFU/100 ml)) at 4˚C and 23˚C, respectively. Under anaerobic conditions, the cellular concentrations of *Aeromonas hydrophila* varied from 13.51 to 17.44 units (Ln (CFU/100ml)) and from 13.22 to 17.53 units (Ln (CFU/100ml)) at 4˚C and 23˚C, respectively (**Figure 2**).

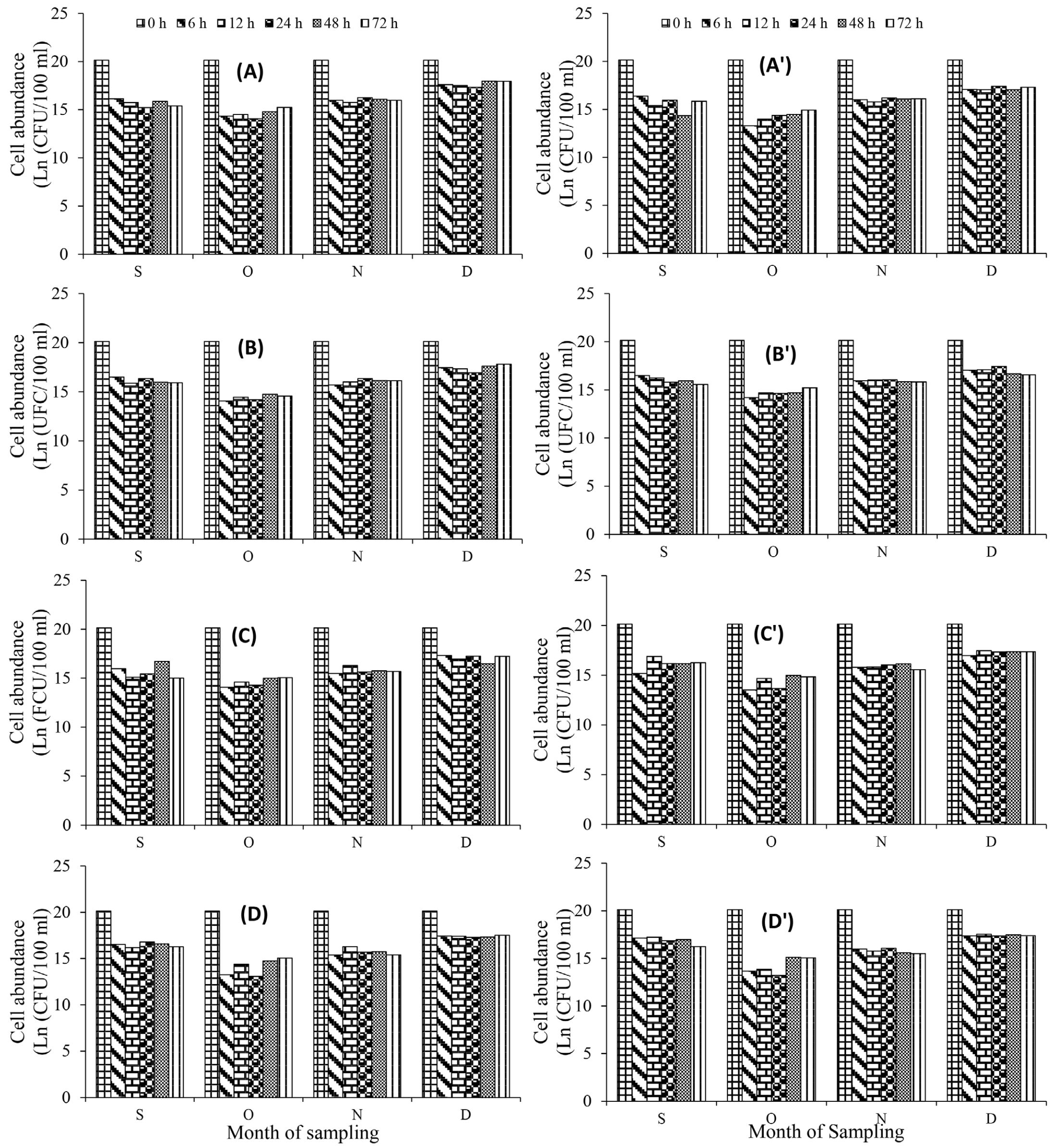

Figure 2. Temporal variations during each study campaign, of the abundances *of Aeromonas hydrophila* cells in monospecific culture incubated in aerobic conditions at 4˚C (A) and 23˚C (B), in anaerobic conditions at 4˚C (A') and 23˚C (B'), then in mixed culture aerobically at 4˚C (C) and 23˚C (D), and anaerobic condition at 4˚C (C') and 23˚C (D').

3.2. Physicochemical Changes in Groundwater Observed When Stored

During the study, a temporal change in the chemical properties (pH, electrical conductivity and ammonium ions) of water was observed in the presence of a single bacterial species. Variations in these parameters were observed according to experimental conditions (aerobic/anaerobic, incubation time and temperature). Figure 3 shows temporal changes in pH, conductivity and ammonia nitrogen in water stored with *E. coli* and *A. hydrophila*.

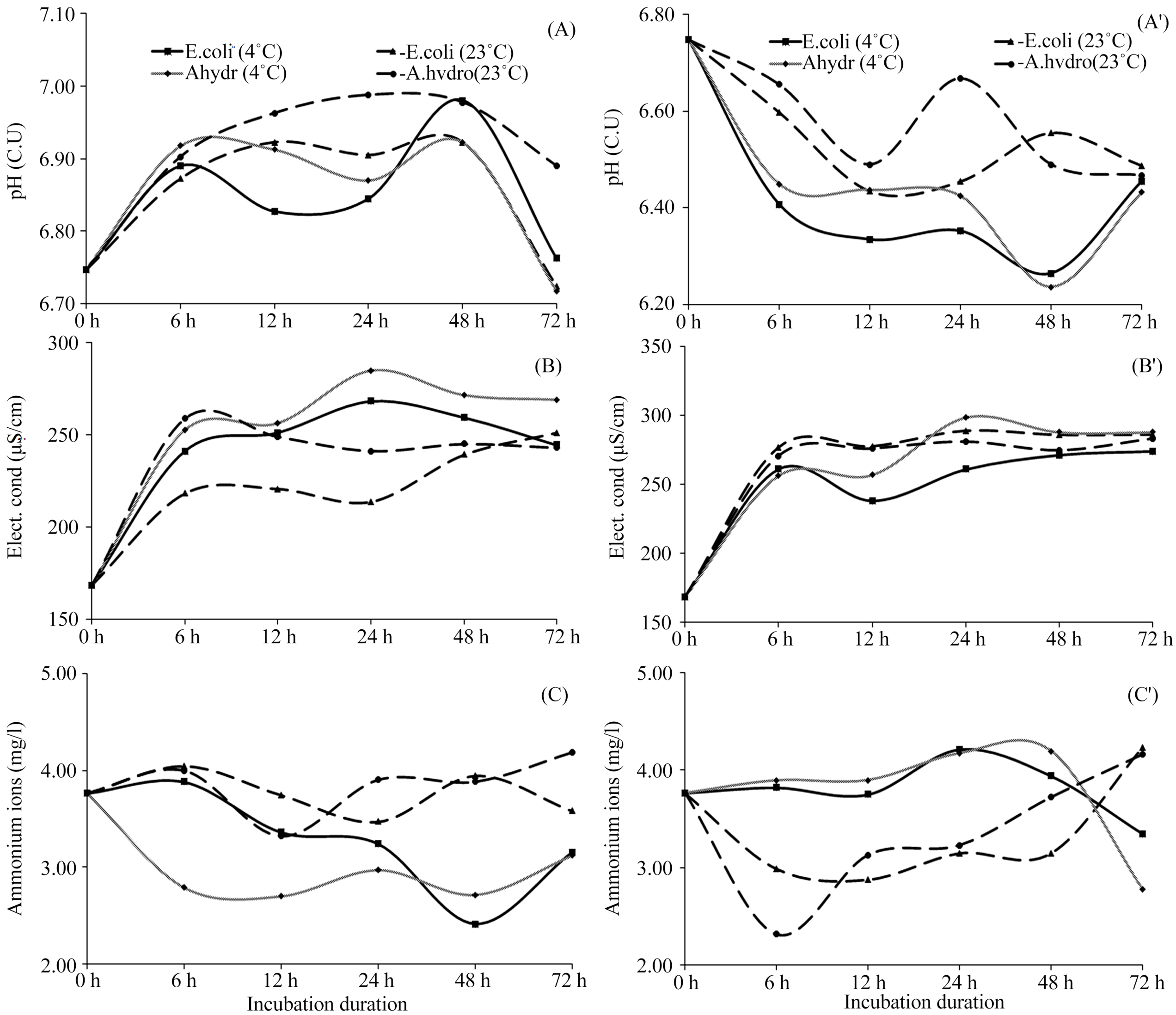

Figure 3. Temporal variation of the values of pH, electrical conductivity and ammonium ions in water stored in the presence of *E. coli* and *A. hydrophila*, at storage temperatures of 4˚C and 23˚C, under aerobic conditions (A, B & C) and then under anaerobic conditions (A', B' & C').

Under aerobic conditions and in the presence of *E. coli*, pH values were relatively higher at 4˚C than at 23˚C. On average, the pH increased from 6.75 to 6.98 after 24 h storage at 4˚C. The same trend is observed for pH variation from 6.75

to 6.92 at 2˚C storage (**Figure 3(A)**). On the other hand, under anaerobic conditions, the pH decreases slightly with storage time, with minimum values of 6.27 after 48 h incubation and 6.44 after 12 h incubation at 4˚C and 23˚C respectively. Overall, the pH is slightly higher at 23˚C (**Figure 3(A')**). For water incubated in the presence of *A. hydrophila* and under aerobic conditions, the pH increased from 6.75 to 6.92 after 6 h and to 6.99 after 24 h of incubation for temperatures of 4˚C and 23˚C respectively. Under anaerobic conditions, the pH decreases with the incubation time regardless of the incubation temperature. At a temperature of 4˚C, the pH drops from 6.75 to 6.43 after 24 hours, then to 6.24 after 48 hours of incubation. At 23˚C, the pH drops from 6.75 to 6.67 after 24 hours and then to 6.47 after 72 hours of incubation. Overall, however, the pH values at 4˚C are slightly lower than those obtained at 23˚C (**Figure 3(A')**).

For electrical conductivity, there is a strong increase with incubation time, with much higher values in the presence of *E. coli* at 4˚C. The electrical conductivity varies from 169 to 268 µS/cm after 24 hours of incubation at 4˚C and 251 µS/cm after 72 hours of incubation at 23˚C. This electrical conductivity is higher in the presence of *A. hydrophila* with fluctuations from 169 to 285 µS/cm after 24 h incubation and 259 µS/cm after 6 hours incubation at 4˚C and 23˚C respectively (**Figure 3(B)**). Under anaerobic conditions and in the presence of *E. coli*, the conductivity varied from 169 µS/cm at sampling to 274 µS/cm after 72 hours of incubation at 4˚C and from 169 to 289 after 24 hours. Under the same experimental conditions and in the presence of *A. hydrophila*, the electrical conductivity is higher at 4˚C with a value of 299 µS/cm after 24 hours.

Ammonium ions (NH_4^+) in the presence of *E. coli* and under aerobic conditions, increased slightly from 3.77 to 3.88 mg/l after 6 hours of incubation and then decreased to 2.41 mg/l after 48 hours of incubation for a storage temperature of 4˚C. At 23˚C, the same trend is observed with an increase from 3.77 to 4.05 mg/l after 6 hours and a decrease to 3.47 after 24 hours of incubation. However, in water stored at 4˚C in the presence of *A. hydrophila*, a decrease in these values is observed, from 3.77 to 2.72 mg/l after 48 hours of incubation. Under anaerobic conditions, the ammonium ions content is generally much higher. In the presence of *E. coli* and at an incubation temperature of 4˚C, there is a fluctuation from 3.77 to 4.21 mg/l after 24 hours of storage and at 23˚C, the value of 4.23 mg/l is reached after 72 hours. Under the same experimental conditions and in the presence of *A. hydrophila*, there is an increase from 3.77 to 4.20 mg/l after 48 hours and from 3.77 to 4.16 mg/l after 72 hours of storage at 4˚C and 23˚C respectively (**Figure 3(C')**).

The values of pH, electrical conductivity and ammoniacal nitrogen also change over time when both bacterial species are present in the medium (**Figure 4**). The pH values are generally higher under aerobic conditions with maximum values of 6.95 after 12 h of incubation at 4˚C and 7.05 after 48 h of incubation at 23˚C. These values decrease from 6.75 to 6.27 and to 6.44 at incubation temperatures of 4˚C and 23˚C respectively (**Figure 4(A)**).

As for the electrical conductivity, the values are generally higher under anaerobic conditions. Under aerobic conditions it varies from 169 to 311 µS/cm at 4˚C and from 169 to 331 µS/cm at 23˚C. These maximum values are obtained after 72 hours of incubation. Under anaerobic conditions, it varies from 169 to 341 µS/cm at 4˚C and from 169 to 348 µS/cm at 23˚C. These maximum values are obtained after 72 and 24 hours of incubation respectively (**Figure 4(B)**).

Ammonium ions increased significantly under all experimental conditions. Under aerobic conditions, they vary from 3.77 to 5.43 mg/l at an incubation temperature of 4˚C and from 3.77 to 6.82 mg/l at 23˚C. These maximum values are obtained after 24 hours of incubation. Under anaerobic conditions, they range from 3.77 to 5.56 mg/l at 4˚C and from 3.77 to 6.04 mg/l at 23˚C. These maximum values are obtained after 24 hours of incubation (**Figure 4(C)**).

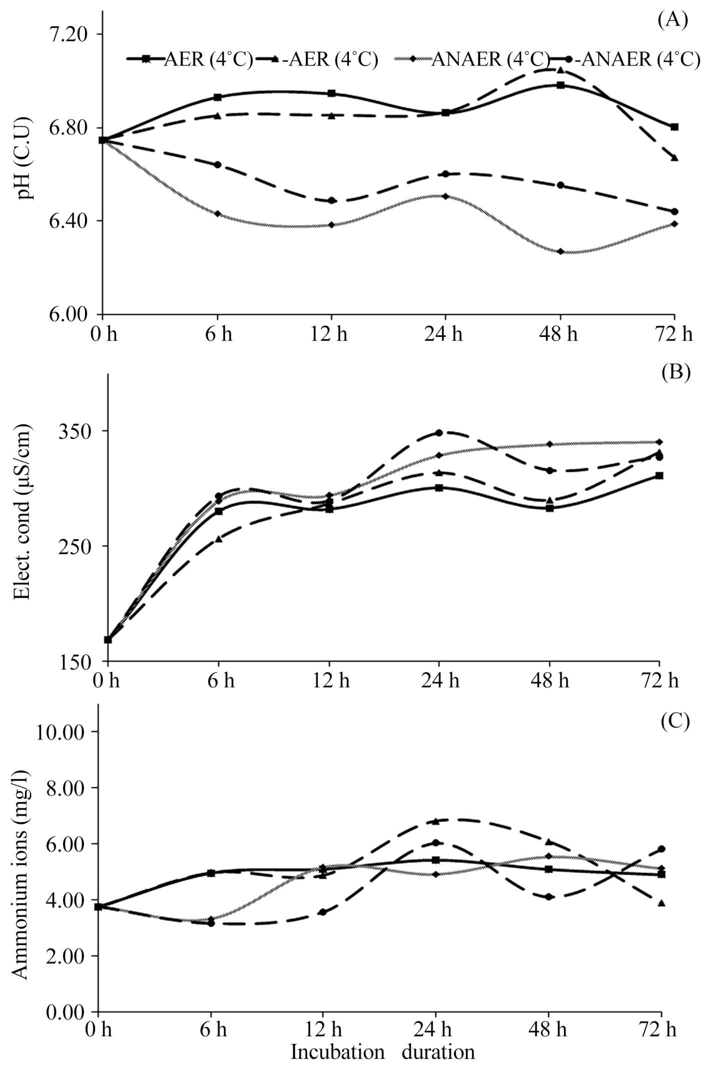

Figure 4. Temporal variation in the values of pH (A), electrical conductivity (B) and ammoniacal ions (C) in water stored in the simultaneous presence (mixed condition) of *E. coli* and *A. hydrophila*, at storage temperatures of 4˚C and 23˚C, under aerobic and anaerobic conditions.

3.3. Relationships between the Different Parameters Considered in This Study

The Spearman correlation coefficient r between the different variables analysed was calculated in the monospecific condition.

Under aerobic and anaerobic conditions and at an incubation temperature of 4˚C, the increase in the abundance of *E. coli* cells is significantly and positively correlated with the increase in pH values ($P < 0.05$) (Table 1). The increased abundance of *A. hydrophila* under anaerobic conditions is accompanied by a significant decrease in the pH of the medium ($P < 0.05$) (Table 1). At 4˚C, the temporal variations of the electrical conductivity and of the NH_4^+ content are not significantly related to the variations of the cell abundances of each of the two bacterial species in both experimental conditions ($P > 0.05$). At an incubation temperature of 23˚C and under anaerobic conditions, negative and significant correlations were observed between the ammonium ions (NH_4^+) content and the abundance of commensal *E. coli* ($P < 0.01$) and *A. hydrophila* ($P < 0.05$) (Table 1). No significant correlation was observed with either electrical conductivity or pH ($P > 0.05$).

Table 1. Spearman correlation coefficients between cell bacteria and abiotic factors at each experimental condition in monospecific culture.

Bacterial species and experimental conditions		Incubation storage temperature and abiotic factors					
		4˚C			23˚C		
		pH	Elec.cond	NH_4^+	pH	Elec.cond	NH_4^+
E. coli	Aerobic conditions	0.631**	−0.009	−0.541	0.191	−0.065	−0.142
	Anaerobic conditions	0.445*	0.022	−0.228	0.094	−0.137	−0.567**
A. hydrophila	Aerobic conditions	0.062	0.150	−0.107	0.018	0.340	−0.057
	Anaerobic conditions	−0.486*	0.408	−0.355	−0.265	0.044	−0.548*

Number of observation = 20; *: $P < 0.05$; **: $P < 0.01$.

In the mixed condition, when *E. coli* and *A. hydrophila* bacteria are present simultaneously, pH is very significantly and positively correlated with the abundance of commensal *Escherichia coli* in anaerobic conditions ($P < 0.01$). Ammonium ions (NH_4^+) is significantly and negatively correlated with cellular concentrations of *A. hydrophila* in both aerobic and anaerobic conditions (Table 2).

Table 2. Spearman correlations coefficients between cell bacteria and abiotic factors at each experimental condition in mixed culture.

Bacteria species and experimental conditions		Incubation storage temperature and abiotic factors					
		4˚C			23˚C		
		pH	Elec.cond	NH_4^+	pH	Elec.cond	NH_4^+
E. coli	Aerobic conditions	0.437	0.175	−0.092	0.352	0.235	−0.067
	Anaerobic conditions	0.585**	−0.041	−0.341	0.360	0.081	−0.367
A. hydrophila	Aerobic conditions	−0.093	0.148	−0.600**	−0.049	0.183	−0.478*
	Anaerobic conditions	−0.093	0.248	−0.461	−0.184	0.062	−0.602**

Number of observation = 20; *: $P < 0.05$; **: $P<0.01$.

After the introduction of the bacterial strains into the medium, these physicochemical properties appear to change during incubation. Thus, under aerobic conditions and in the presence of *Escherichia coli*, the pH values vary little, whereas under anaerobic conditions, the pH drops to 5.48 at 4˚C. Although the variation is not statistically significant, the relative decrease in pH of the water during incubation would be due to the increase in dissolved CO_2 [9]. The same observation is made when *A. hydrophila* is present in the medium. As our study bottles are sealed, CO_2 (acidifying gas) cannot easily escape from the stored water.

Bacterial assimilation of different organic or mineral substrates is influenced by the levels of H^+ and OH^- ions. The alteration of this assimilation leads to the penetration of toxic compounds present in the medium into the bacterial cell. The effect of pH on productivity varies from one microorganism to another. In groundwater, increases in pH sometimes favour the development of *Aeromonas hydrophila* and increases in the abundance of faecal coliforms and faecal streptococci [17] [18]. A number of studies have shown that, for example, the enzymatic activity can also be strongly influenced by the pH value of the medium [19]. The electrical conductivity values increase as a function of the incubation time in aerobic or anaerobic conditions. This increase would be due to the presence of biodegradable organic compounds in the water tested, which would then be broken down by the bacteria present in the environment. The increase in water mineralization during storage would therefore result from a strong mineralizing activity of water microorganisms [20]. Bacterial metabolism, during which neutral macromolecules are for example converted into electrically charged molecules, contributes to significant fluctuations in the electrical conductivity of water [21]. It is therefore noted that the initial mineralogical characteristics of these waters are modified. These waters change from weakly mineralised to moderately mineralised [9]. This change in water mineralisation is observed both aerobically and anaerobically. Temperature plays a role in the solubility of salts, their dissociation and therefore in electrical conductivity. It is an important factor in the aquatic environment because it controls almost all physical, chemical and biological reactions [13].

In both aerobic and anaerobic conditions, the variation in ammoniacal nitrogen content is significantly and negatively correlated with the abundance of *E. coli* and *A. hydrophila* in the monospecific culture condition ($p \le 0.05$). Ammonium ions are the form of nitrogen that is preferred by bacteria for their metabolism. Its influence on the growth of bacteria has already been pointed out by a number of authors [22]. The latter point out that bacterial production in an aquatic environment can be maximal when the uncoloured dissolved organic carbon and ammonium ions of the environment are in high concentration. This increase in ammonium ions would be due to the fact that the groundwater collected contains nitrogenous organic compounds (proteins, amino acids, urea, etc.), the molecules of which are first converted into ammonium ions (NH_4^+), which are then oxidised to nitrites and then to nitrates under the action of bacterial nitrification [23]. In the absence of oxygen, the opposite reaction to that described above occurs: anaerobic bacteria convert nitrates and to produce ammonia (NH_3) or ammonium ions (NH_4^+). *E. coli* and *A. hydrophila* are both facultative aero-anaerobic bacteria. In the presence of both species simultaneously in water, high levels of ammoniacal nitrogen were recorded, reaching 10.08 mg/l at 4˚C and 11.20 mg/l at 23˚C under aerobic conditions, and values of 11.20 mg/l and 9.28 mg/l at 4˚C and 23˚C respectively. We can therefore assume that the presence of these two species in the environment ensures that the maximum amount of nitrogenous organic matter is converted into ammoniacal nitrogen.

4. Conclusion

The well water sampled for the study has an acidic pH and a low degree of mineralisation. The ammonium ions content is relatively low. The values of these physico-chemical parameters show relative temporal variations depending on the storage conditions and the bacterial strain present in the medium. The degree of relationship between the dynamics of bacterial abundance and the chemical characteristics of the stored water also varies with the microorganisms present and the chemical characteristics considered. The deterioration of the water quality of the wells would be due to the change in these physico-chemical parameters. Groundwater storage favours the development of certain bacteria to the detriment of others. The study recommends that groundwater should be properly disinfected before storage. This would reduce changes in the chemical properties of the water as a result of bacterial metabolism.

Conflicts of Interest

The authors have declared that no competing interests exist.

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