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Research Article

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Assay of Potability with Bottled Mineral Water from Trademarks

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Abstract

The purpose of this test was to evaluate the physicochemical (ammoniacal nitrate, nitrogen nitrate, nitrogen nitrite, pH, orthophosphate and turbidity) and microbiological parameters (Escherichia coli and total coliforms) of mineral water samples bottled trademarks and compare the results obtained with Brasilian Ministery of Healty Ordinance 2.914/11. For this purpose, it was used trademarks mineral water bottled sold in pet 300 and 500 ml bottles. For microbiological analysis it was used Colipaper (Alfakit®), and all of them the analysis of physicochemical parameters was used Ecokit II (Alfakit®). From the five mineral water bottled samples, all were within the physicochemical parameters determined by Ordinance 2.914/11. In the microbiological findings, only one sample presented Escherichia coli. Therefore, all the analyzed brands meet the required physicochemical parameters of water quality for human consumption, except brand A, which did not meet the microbiological parameters. The results demonstrates the need for confirmatory tests performed by laboratories qualified to attest the result obtained, as well as the need to implement more effective monitoring by the responsible agency for monitoring the water quality for human consumption.

Keywords: *Escherichia Coli*, Microbiology, Contamination

Introduction

The humanity quality of life is directly linked to water for the proper functioning of the body, as well as for the preparation of food and for personal hygiene and the environment (home, clothes, utensils), among others. For the aforementioned functions, water for consumption must have satisfactory sanitary characteristics, such as being free of pathogenic organisms and toxic substances, thus avoiding damage to human health and well-being [1].

Usually the increase of parasites, bacteria and viruses in water is due to environmental changes, agriculture and livestock, uncontrolled urbanization or the industrialization of the region, which causes a low processing of fertilizers and other effluents, thus affecting the physical-chemical quality and microbiological analysis of water [2].

Pathogenic organisms are responsible for transmitting diseases through ingestion or contact with contaminated water. Bacteria, viruses, parasites, protozoa and helminths are capable of causing diseases such as dysentery, typhoid, cholera, hepatitis, leptospirosis, poliomyelitis, amebiasis, giardiasis, schistosomiasis, ascariasis, among others. The World Health Organization (WHO) estimates that 13.700 people die each day from waterborne diseases, with more than half of these people being children under 5 years of age [1].

In order to guarantee a quality water for packaging in Brazil, the agency responsible for its standardization is the National Health Surveillance Agency of the Ministry of Health (ANVISA-MS), which defines the physical, chemical and bacteriological parameters in accordance with Administrative Rule 1.469 from 2000, which establishes the procedures and responsibilities related to the water control for human consumption [3]. The treatment of water must follow the current regulations, such as Ordinance 2.914 of December 12, 2011 of the Ministry of Health (Ordinance 2.914/11), which establishes the surveillance and water quality directed to human consumption and its drinking water standard [4]. There are other legal provisions and standards that also establish standards and parameters for water intended for human consumption, in its various forms [5, 6].

Regarding the physical-chemical parameters, some can be considered pollution contamination indicators. Ammoniacal nitrate, as well as nitrogen nitrate and nitrogen nitrite, indicates the presence of animal or human fertilizers and manure. The pH indicates whether a solution is alkaline or acidic and, according to the Ministry of Health, it is desirable that water pH stays between 6.0 and 9.0. Orthophosphate may indicate the presence of detergents, fertilizers, pesticides and

domestic sewage. Finally, turbidity, which is the measure of the degree of interference from the passage of light to the liquid, may be increased due to the presence of suspended particles or by domestic and industrial sewage [4, 7].

Most of the bacteria found in the water are of enteric origin, coming from the gastrointestinal tract of humans and animals. Fecal coliforms are the most used parameters as bioindicators of water pollution, since they normally live in the human organism and of warm-blooded animals, and they are presented in large quantities in feces. Fecal coliforms are usually not pathogenic, but their presence in the water indicates that fecal coliforms have fecal material and may contain pathogenic microorganisms. Among the bacteria of the coliform group, the most used as bioindicator of fecal pollution is *Escherichia coli* [1, 2].

Escherichia coli is one of the most prolific microorganisms in the human intestinal tract, and there may be pathogenic and non-pathogenic strains. Pathogenic strains can cause severe infections in the urinary tract, bloody diarrhea, bacteremia and even meningitis. Bacteria of this lineage have specialized fimbriae that are capable of binding to intestinal epithelium cells and can be divided into three groups: the enterotoxigenic, noninvasive lineage produces an enterotoxin that causes an aqueous diarrhea; the enteroinvasive lineage, which invades the intestinal wall, resulting in fever, dysentery and inflammation; and enterohemorrhagic lineage, which adheres to the intestinal mucosa and produces toxins, which can lead to death. In all its forms, *E. coli* feeds on other bacteria and it is eliminated by feces [1, 8, 9].

Therefore, the objectives of the present study were to determine the presence of *Escherichia coli* and total coliforms, as well as to evaluate the physical-chemical parameters ammoniacal nitrate, nitrogen nitrate, nitrogen nitrite, pH, orthophosphate and turbidity and microbiological samples of mineral water trademarks and compare the results with the limits established by Ordinance 2.914/11 [4].

Materials and Methods

In this essay five commercial brands of mineral water bottled were analyzed in pet bottles, identified in the present study as samples A, B, C, D and E in order to preserve their identity. Microbiological analyzes were carried out using the Alfakit® Colipaper and the analysis of physico-chemical parameters such as ammoniacal nitrate, nitrogen nitrate, nitrogen nitrite pH, orthophosphate and turbidity was performed with Alfakit® II.

Samples

Five pet mineral water bottles of different brands were purchased, with a volume of 300ml to 500ml each, according to the manufacturer's information. The brands chosen for analysis belong to major brands marketed in Brazil, available in hypermarket chains, conveniences, wholesalers and restaurants throughout the country.

Physical and Chemical Tests

For the analyzes, the Ecokit II was used to determine the physicochemical parameters according to the manufacturer's instructions, as described below:

- **Ammoniacal nitrate:** Transfer the sample into the cuvet up to the mark (5 ml); add 3 drops of reagent 1, close and shake; add 3 drops of reagent 2, close and shake; add 3 drops of reagent 3, close and shake; wait 10 minutes; open the cuvet, position it on the chart and make the color comparison.
- **Nitrogen nitrate:** Transfer the sample to the cuvet to the mark (5 ml); add 1 measure of reagent 1 with blossom # 1 and shake vigorously until dissolved for 2 minutes; add 1 g of reagent 2 with blossom # 1 and stir until dissolved; add 2 drops of reagent 3 and shake well; wait 15 minutes; open the cuvet, position it on the chart and make the color comparison.
- **Nitrogen nitrite:** Transfer the sample to the cuvet up to the mark (5 ml); add 2 drops of reagent 1, close and shake; add 2 drops of reagent 2, close and stir until dissolved; wait 10 minutes; open the cuvet, position it on the chart and make the color comparison.
- **pH:** Transfer the sample to the cuvet up to the mark (5 ml); add 1 drop of PH reagent, close and shake; open the cuvet, place it on the carton and make the color comparison.
- **Orthophosphate:** Transfer the sample to the cuvet up to the mark (5 ml); add 5 drops of reagent 1, close and shake; add 1 measure of reagent 2, with pinion # 1, close and shake; wait 10 minutes; open the cuvet, place it on the carton and make the color comparison.

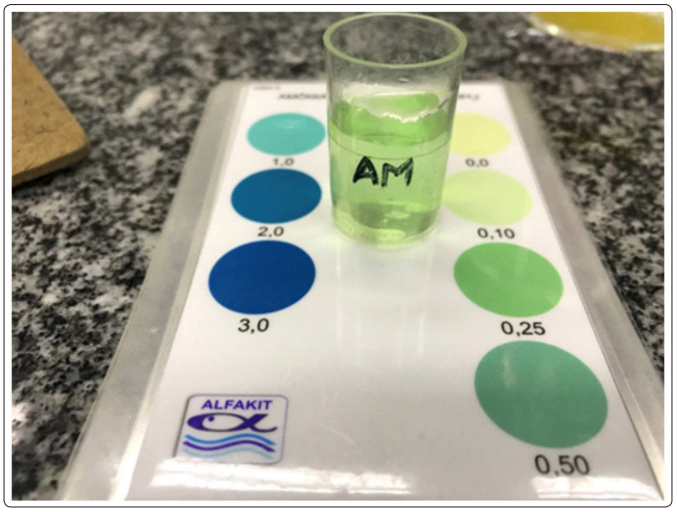

Figure 1: Determination of the Ammoniacal Nitrate Parameter

Microbiological assay

The Colipaper kit consists of microbiological cartons which are a culture medium in the form of a dehydrated gel. The product is intended for the detection and quantification of *Escherichia coli* and total coliforms. For the microbiological analysis, the step-by-step recommended by the manufacturer was followed and described in the explanatory leaflet: the microbiological carton was removed from the plastic package, touching only the top part of the pick; the carton was immersed in the sample, and waited until it was completely moist; the excess water was removed and the carton was replaced in the plastic and positioned within the squares drawn in the plastic package; being careful not to touch the carton; the sample was incubated for 15 hours at 37 °C.

The beaker used to store the sample and cartoon immersion was previously sanitized with 70 °C alcohol and latex gloves were used

for manipulation and procedure, thus minimizing contamination.

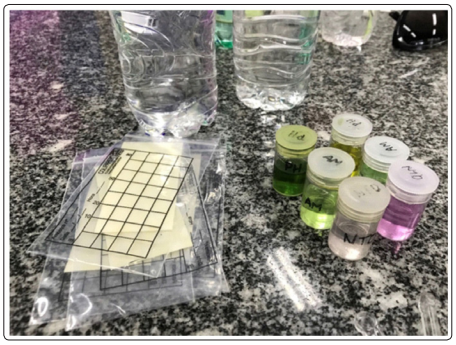

Figure 2: Colipaper Plates, Chemical Reagents and Samples

Count of colonies

After 15 hours of incubation, with the cartouche properly positioned in the squares drawn on the packaging, the colonies were counted in the carton. The counts of the colonies in dark blue and violet indicate the presence of *Escherichia coli*, and the pink to red colonies added to the blue and violet correspond to the total coliforms. The sums must be multiplied by 80.

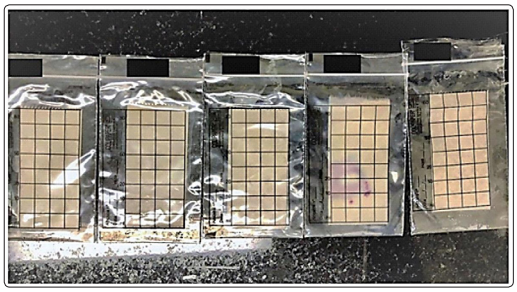

Figure 3: Colipaper Reaction Cards with Colonies for Counting

Results

The physical-chemical and microbiological parameters found in the five water samples are shown in Table I and figure 4.

Table I: Physical-chemical and microbiological parameters obtained in the samples in comparison to the established by the Ordinance 2.914/11 [4].

Physical-Chemical and Microbiological Parameters						
SAMPLES	**A**	**B**	**C**	**D**	**E**	**Ordinance 2.914/11**
Ammoniacal nitrate (mg/l)	0,25	0	0,25	0,10-0,25	0,10-0,25	1,5
Nitrogen nitrate (mg/l)	0,2	0	1,5	0,1	1,5	10
Nitrogen Nitrite nitrite (mg/l)	0	0,025	0,05	0	0	1
pH (mg/l^{-1} N)	7,5	7,5	6,5	7,5	8	6 a 9
Ortophosphate (mg/l)	1,5	1	1	1,5	1	Not applicable
Turbidity (NTU)	<25	<25	<25	<25	<25	5
E. coli (UFC/100ml)	960	absent	absent	absent	absent	absent
Coliforms Totals (UFC/100ml)	960	absent	absent	absent	absent	absent

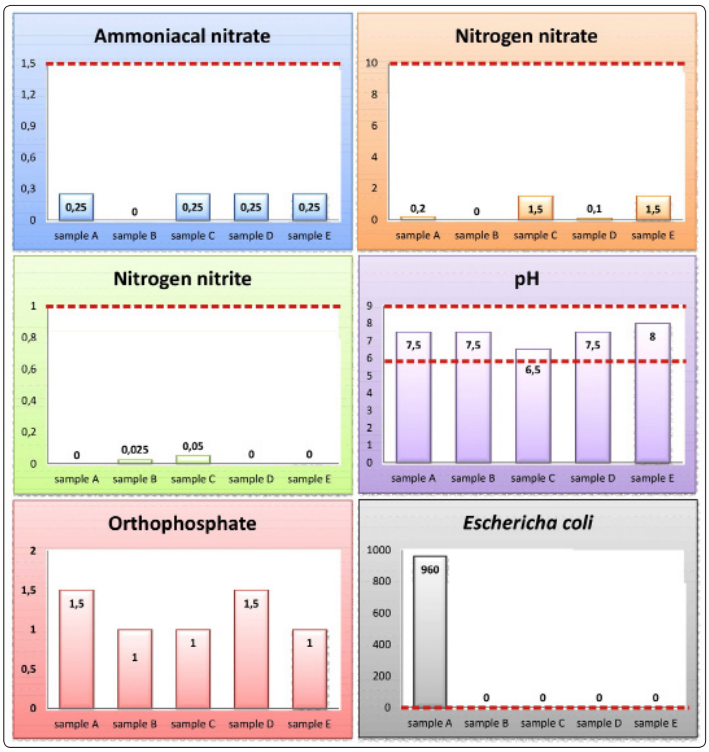

Figure 4: Physicochemical and microbiological parameters obtained in the samples in comparison to that established by Ordinance 2.914/11

Discussion

Ammoniacal nitrate is considered a pollution marker, as well as nitrogen nitrate and nitrogen nitrite, which indicate the presence of animal or human fertilizers and manures [7]. In relation to the nitrogen compounds, all the samples met the limits established by Ordinance 2.914/11.

pH indicates whether a solution is alkaline or acidic [7]. All samples were in the range of 6.5 to 8 mg / L-1 N. According to the Ministry of Health, it is desirable that the water pH stays between 6.0 to 9.0; thus, all samples are also within the limits accepted by Ordinance 2.914/11.

Orthophosphate may indicate the presence of detergents, fertilizers, pesticides and domestic sewage discharge [7]. In marks A and D the values were 1.5 mg / l-1 PO_4 and in marks B, C and E the values were 1 mg / l^{-1} PO_4. Ministry of Health Ordinance 2.914 / 11 [4] does not express a reference limit for this parameter, nor the complementary legal provisions (Resolution of the Collegiate Board of Directors - RDC No. 1736 and Consolidation Ordinance N º 5 of the Ministry of Health) [10], rendering the interpretation of the results obtained with regard to the current legislation.

Turbidity is the measure of the degree of interference from the passage of light to the liquid caused by suspended particles or by domestic and industrial sewage. All samples obtained results below <25 NTU, and therefore, although the applied scale is above the established limits, all the samples apparently fit the parameters established by Ordinance 2.914/11, which is of 5.0 NTU [4,7].

Fecal coliforms and *Escherichia coli* should be absent from the samples according to Ordinance 2.914/11 and there was nonconformity in sample A, where there was microbiological growth. Santos et al. (2017) also analyzed water for human supply in relation to the presence of fecal coliforms and *Escherichia coli*, and the results obtained were positive in some samples. The present study corroborates with the results of Santos et al. (2017), since it was also found coliforms quantity in disagreement with the current legislation. The conclusions of the study suggested a more effective inspection, as well as the implementation of water quality surveillance by the responsible organisms [5].

Silva et al. (2004) also carried out a study on the main microorganisms transmitted by water and concluded that the monitoring all pathogens in water for human consumption is unrealistic, since there is a great diversity of microorganisms that adapt to the environment, since there are several methods used to analyze them. The authors suggested that in each water basin where there is water collection, one should take into account the conditions and particularities of anthropogenic activities, relating them to the epidemiological context of the region, thus using sensitive and precise bioindicators for the region [2].

Conclusion

Through the analyzes carried out in the production of this test, it was noticed that all the analyzed brands met the required physico-chemical parameters of water quality, except brand A, which did not meet the microbiological parameters. However, confirmatory tests must be carried out to certify the result obtained, since the materials and equipment directed to this specific type of analysis were not used. In addition, the parameters analyzed may be insufficient to fully assess the good samples quality.

Although most of the results were satisfactory, the deficiency of the authorities responsible for the inspection and regulation of bottled water laws is notorious, so that companies that sell water are required to comply with the demand standards, and there is a need for unified parameters to be developed and followed. It is necessary to review these aspects in order to avoid major problems in the future, as well as to maintain the good quality of life of society as a whole, due to the ease of access we have to these brands in our daily lives.

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