LUMEN ET VIRTUS
ISSN: 2177-2789

MICROBIOLOGICAL EVALUATION OF THE QUALITY OF BOTTLED WATER – ADDED WITH SALTS – IN PET AND PP BOTTLES WITH A CAPACITY OF 20 L IN THE STATE OF CEARÁ (A CASE STUDY)

https://doi.org/10.56238/levv15n41-086

Submitted on: 09/23/2024 **Publication date:** 10/23/2024

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ABSTRACT

In this work, microbiological analyses were carried out in order to investigate the following microorganisms described in ANVISA's Normative Instruction No. 161, Total Coliforms, E.coli, Pseudonomonas aeruginosas and Enterococcus spp in water added to salts bottled in PET and PP bottles with a capacity of 20 L. Under the same analysis conditions, the results for PP bottles were unsatisfactory with 100% presence for Total Coliforms and Pseudonomonas aeruginosa, while in the 20 Liter PET packages all the results were satisfactory, that is, with the absence for all microorganisms of ANVISA's I.N. No. 161.

Keywords: Polyethylene terephthalate PET. Polypropylene. Water Added Salts. Microbiology. 20 Liter Carboys.

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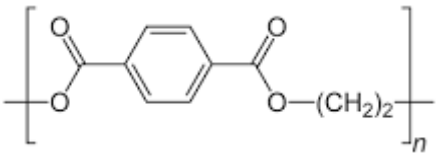
INTRODUCTION

The present study aims to evaluate the microbiological contamination in the Added Salts Water that were bottled in PET and PP bottles (20 L packages) and that underwent analysis in accordance with the current legislation, Normative Instruction No. 161 National Health Surveillance Agency (ANVISA) of July 1, 2022. These plastic packages are made up of polymers: Poly (Ethylene Terephthalate) PET and Polypropylene (PP).

In the State of Ceará there are many companies that produce, bottle and distribute Water Added with Salts and with that the consumption by the 20-liter capacity bottles (packages) also follow the same proportion, so there is a regulation that each bottle has a shelf life of 3 years for both PET and PP, so the great demand for these packages cross the borders of the municipality and the State for this material and some bottling companies, in the state itself, manufacture their PP bottles both for their own consumption and to meet the demand of other companies.

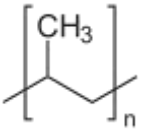
Poly (Ethylene Terephthalate) PET was introduced in the Brazilian market in 1988 and is one of the most widely used polymers today, mainly in the manufacture of packaging (J. THERM. SPRAY TECHNOL., 2, 2, p.145, 1993). Of the total PET consumed in Brazil, only about 21% is effectively recycled, despite the fact that PET is one of the most present components in plastic waste in Brazil. (PLÁSTICO MODERNO, 266, 8-18, 1996).

Figure 1. Chemical structure of PET.



Polypropylene PP was developed in 1954 and is used in wide applications such as the manufacture of sterilizable bottles, packaging films, luggage bags, car bumpers, toys, disposable injection syringes, household appliance housings, containers, ballpoint pen loading tubes, among other applications (MANRICH, 2005; MANO, 2001; CALLISTER, 2008). It has limited performance at low temperatures due to its glass transition temperature and has a molecular structure as shown in Figure 2.

Figure 2. Chemical structure of PP.



Currently, all types of bottled water, that is, Natural Mineral Water, Added Salts, Natural Water and Drinking Water from Tables and beverages in general use plastic packaging for bottling.

MATERIAL AND METHODS

Water samples were analyzed in 20 L bottles from 07 (seven) companies bottling water with added salts, where 5 (five) are located in the city of Fortaleza/Ceará, 1 (one) in the interior of the state and 1 (one) in the Metropolitan Region of Fortaleza, which will be called A, B, C, D, E, F and G.

In each company, 5 bottles were collected, in a total of 35, and in the tax expert analysis, the methodology was used with the objective of obtaining the necessary information and data on microbiological contamination only qualitative, that is, the presence or absence of the microorganisms contemplated in ANVISA's I.N. No. 161/2022. These analyses were performed by the Public Health Laboratory of the State of Ceará - LACEN with the enzymatic substrate method for *Total Colifortmes, E.coli (*Collilert*),* *Pseudonomonas aeruginosas* (Pseudalert), *Enterococcus spp* (Enterolert*)*. All substrates from the manufacturer Idexx, with detection in 24 hours in PP and PET carboys.

The method used for the analysis of total coliforms and fecal coliform (*Escherichia coli)* uses an enzymatic substrate and consists of an enzyme-substrate reaction, resulting in the release of a chromogenic and fluorogenic substance with the presence of yellow color (total coliforms) and fluorescence (*Escherichia coli*). The result is presented in a qualitative way (presence or absence). The sample should be analyzed at room temperature. The vial containing the specimen must be sterile, transparent, and identified with the specimen number. 250 mL of sample is measured and poured into the vial. The contents of a Colilert flask are added and the flask is homogenized with circular movements until the reagent is completely dissolved. It is incubated in an oven at 35 ± 0.5ºC for 24-28 hours.

The method applied for the analysis of *Pseudomonas aeruginosa* uses an enzymatic substrate and consists of an enzyme-substrate reaction. *Growing Pseudomonas aeruginosa* strains have an enzyme that cleaves the substrate in the reagent producing blue flowering under ultraviolet light. The result is presented in a qualitative way (presence or absence). The sample should be analyzed at room temperature. After identifying the sterile and transparent vial with the sample number, 250 mL of sample is measured and poured into the vial, the contents of a Pseudalert vial are added and the vial is homogenized with circular movements until the reagent is completely dissolved. It is

incubated in an oven at 35 ± 0.5ºC for 24-28 hours. Any fluorescence in blue indicates that the sample is positive for *Pseudomonas aeruginosa*.

The method applied for the analysis of *Enterococcus spp* uses an enzymatic substrate and consists of an enzyme-substrate reaction. When the substrate is metabolized by *Enterococcus spp,* it turns from blue to green, indicating positive detection. The result is presented in a qualitative way (presence or absence). The sample should be analyzed at room temperature, and after identifying a sterile and transparent vial with the sample number, 250 mL of sample is measured and poured into the vial, the contents of an Enterolert flask are added and the vial is homogenized with circular movements until the reagent is completely dissolved. It is incubated in an oven at 41 ± 1.0ºC for 24-28 hours.

Photo1. Substrates used by the Central Laboratory of Public Health of Ceará (LACEN) to identify microorganisms of ANVISA's I.N No. 161.



The analyses were carried out from December 2023 to September 2024 and companies A, B, C and D were bottled in PP carboys and companies E, F and G were bottled in PET carboys, making a total of 35 samples and all of Added Salts Water.

RESULTS AND DISCUSSIONS

The results in this study were obtained, in accordance with the current legislation, ANVISA's Normative Instruction (I.N.) No. 161 of 2022, which deals with the microbiological criteria for bottled waters.

Table 1 - Microbiological analyses carried out from December 2023 to September 2024 in 7 companies bottling water with added salts in the State of Ceará.

COMPANIES	PP carboys (quantity)	Results of qualitative analyses			
		Total coliforms	*E. coli*	*Pseudomonas aeruginous*	*Enterococcus*
A	5	presence	absence	presence	absence
B	5	presence	absence	presence	absence
C	5	presence	absence	presence	absence
D	5	presence	absence	presence	absence
COMPANIES	PET carboys (quantity)				
E	5	absence	absence	absence	absence
F	5	absence	absence	absence	absence
G	5	absence	absence	absence	absence

Source: The Author.

Therefore, the microbiological analyses of water that were bottled in the PP bottles, in this study, pointed to 100% non-conformity, that is, unsatisfactory according to the current legal normative standards, Normative Instruction No. 161 National Health Surveillance Agency (ANVISA) of July 1, 2022.

In the returnable system, a 20 L carboy, the packaging is successively reused for water packaging, which makes it more complex compared to a disposable packaging system (one way) in which only preforms of PET origin are used. Thus, because the bottles are not owned by the bottling companies, they vary in manufacturers, time of use and material composition. These carboys must be standardized in order to adapt to automatic washing, filling and capping systems.

The ABNT NBR 14222:2005 standard establishes the minimum requirements and test methods for plastic carboys, regardless of the material, new and unused and with a capacity of 20 L.

According to the standard, the specified parameters are:

- Material: meet ANVISA specifications.
- Appearance: absence of defects internally and externally that favor the encrustation of dirt and the absence of undesirable odor is also required.
- Transparency: minimum specular light transmission of 60% at wavelengths 700 nm to 780 nm in the UV/VIS region.
- Mechanical resistance: evaluated by means of static compression tests, in which the bottled bottles must support 42 kg for 15 days at a temperature of 40ºC with a variation of more or less 3ºC, resistance to free fall at a height of 1.5 m and impact resistance using a mass of 12 kg, which evaluates the resistance of the carboy at the stage of application of the cap.

- Marking and identification: the standard establishes that the bottles must contain the following minimum words at the bottom and in high relief: recycling symbol, date of manufacture and expiration, manufacturer's name, mold number and the phrase "exclusive use for natural mineral water or water added with salts".

Compliance with ANVISA requirements aims to ensure that the packaging used in the packaging of bottled water is not a vehicle for contamination, taking into account that packaging materials are not totally inert and can transfer substances or microbiological contamination to the water.

All the water bottling companies from which the samples were collected and analyzed, 07 (seven), use reverse osmosis, which is a process that uses a semipermeable membrane, capable of retaining up to 99% of low molecular weight solutes, such as salts, viruses, bacteria and other contaminants, resulting in cleaner and safer water for consumption. Nowadays reverse osmosis is widely used in various sectors such as the pharmaceutical, food, chemical, petrochemical, desalination and drinking water and wastewater treatment industries.

CONCLUSION

This research of microbiological parameters showed the presence exclusively of total coliforms and *Pseudomonas aeruginosa in the* waters of the 20-liter carboys that were bottled in PP packages. According to the results obtained in this study and based on the standards established and in force in Normative Instruction No. 161 of the National Health Surveillance Agency (ANVISA) of July 1, 2022.

In all the samples analyzed, which were packaged in 20-liter PP-type bottles, there were amounts of total coliform bacteria and *Pseudomonas aeruginosa* above the acceptable limit by the legislation, in which it is noted that none of the bacteria standardized by IN No. 161 for this research is present in 250 mL of sample. Therefore, it is possible to understand that the water contained in carboys with this composition are unfit for human consumption.

It is important to note that there are no inspections in these companies that manufacture 20 L PP carboys and as a result we have a large number of packages that do not follow manufacturing standards appropriate to the standards in force in the country. It is essential to constantly monitor the good manufacturing practices of PP type carboys so that the microorganisms present that cause water contamination are eliminated. In addition, the proportional values of recycled and non-recycled (virgin) material inserted in the extrusion process until the final manufacture of the finished product must be identified and quantified.

Therefore, microbiological analyses were essential to investigate and raise an alert about the bottling of water for human consumption in PP type bottles with a capacity of 20 liters. The quality of the water available in this type of packaging, during the study period, was unsatisfactory for the purpose, which differs from the PET bottles that did not present any type of contamination in the research of microorganisms listed in IN No. 161 of ANVISA. Emphasizing: all water samples came from a reverse osmosis system in its manufacturing process, which leaves purified water.

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