Microbiological analysis of the waters of the Parnaíba river in the urban area of Floriano/ PI: a focus for environmental education

Análise microbiológica das águas do rio Parnaíba na zona urbana de Floriano/ PI: um foco para a educação ambiental

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ABSTRACT
The water plays an important role in the life of living beings, because it is through water that societies develop and grow. However, its quality has been affected on a daily basis. Thus, this research aims to evaluate the microbiological quality of the waters of the Parnaíba River in the urban area of Floriano city, through microbiological techniques for fecal and total coliforms and E. coli, besides making an environmental education work with students at Fauzer Bucar High School about the importance of water and the need to preserve it. The methodology used the Colillert system and also the reading of the Most Probable Number (MPN) through the technique of multiple tubes in cultures. The results showed that the waters of Parnaíba River do not meet the pre-established standards, because they showed in some points rates equal and above 1100 MPN. So, it was concluded that, due to the river contamination, it is necessary to take measures to improve the conditions of the place, joining public policies and the participation of the population in order to preserve this ecosystem.

Keywords: Microbiological Analysis, Environmental education, Quality of life.

RESUMO
A água desempenha um papel importante na vida dos seres vivos, porque é através dela que as sociedades se desenvolvem e crescem. No entanto, sua qualidade é afetada diariamente. Assim, esta pesquisa tem como objetivo avaliar a qualidade microbiológica das águas do Rio Parnaíba na área urbana da cidade de Floriano, através de técnicas microbiológicas para coliformes fecais e totais e E. coli, além de fazer uma educação ambiental, trabalhar com os alunos da Escola Fauzer Bucar sobre a importância da água e a necessidade de preservá-la. A metodologia utilizou o sistema Colillert e também a leitura do Número Mais Provável (NMP) através da técnica de tubos múltiplos em culturas. Os resultados mostraram que as águas do Rio Parnaíba não atendem aos padrões

pré-estabelecidos, pois apresentaram em alguns pontos taxas iguais e superiores a 1100 MPN. Assim, concluiu-se que, devido à contaminação do rio, é necessário tomar medidas para melhorar as condições do local, unindo políticas públicas e a participação da população para preservar esse ecossistema.

Palavras-chave: Análise microbiológica, Educação Ambiental, Qualidade de vida.

1 INTRODUCTION

The abundance of water in planet Earth creates a false impression that it is an inexhaustible product. However, according to many researches, most part of the existing water (95.1%) is salty, being improper for human consumption; and 4.75% are in glaciers or in underground regions of difficult access. Thus, only 0.147% is suitable for consumption and is located in lakes, river sources and underground water tables (PUPILE; CARVALHO; MONTEIRO, 2010).

Even being in small portions in the terrestrial environment, fresh water in its liquid form is fundamental to maintain life in Earth. So, it is necessary that the human beings reflect about this precious element (DOZZO, 2011). The anthropogenic action has caused environmental impacts and one of the main of concerns is the destruction of aquatic ecosystems, especially of fresh water. This creates a decrease and a loss of quality and biological diversity of water (HAMADA, 2004; MILLER, 2008).

The Parnaíba River plays an important role in the supply to Floriano and neighboring cities (SANTOS, 2008). However, nowadays it is suffering from degradation due to silting, deforestation of the riparian forest and the pollution through sewage and untreated galleries (BRASIL, 2013), being really affected by anthropic actions.

Water is a natural element, available in 71% of the Earth surface. It can be found in several environments, such as oceans, in salty water, and also in big aquifers, and in fresh water, and it plays a very important role in the life of living beings, because through it societies can develop and grow. Thus, water is a necessity for life (BUZANELLO, 2008).

This necessity occurs because it plays several functions, for example, acting as a temperature regulating property, diluting solids and carrying nutrients and residues throughout several organs, making it indispensable for life (DOZZO, 2011).

The beginning of the impacts on the aquatic ecosystems is due to many causes, resulting from anthropic actions, such as: deforestation of the riparian forest, silting, discharging of domestic and industrial waste, as well as untreated sewage and natural

processes, among them: the increase of population density, tide influence and rainfall in certain times of the year (CUNHA *et al*, 2004).

The rapid population growth has created improper occupation of places to live, causing a reduction in the quality of the water of rivers and wellsprings (RIBEIRO, 2009).

Researches show that the discharge of sewage is one of the most common ways of river pollution and has as consequences the microbiological contamination of the affected ecosystem, in addition to an alteration of the biodiversity, creating the eutrophication and the deposition of residues, what can generate diseases in the human beings that have contact with these waters (MARTINS; FROEHNER, 2008).

Therefore, the consumed water has to be within the appropriate standards of potability. This issue is relevant because through it, it is possible to have an effective action to prevent waterborne diseases (ALMEIDA *et al*, 2004). Many health problems have been associated with the consumption of water contaminated by biological or physical-chemical agents. Thus, some epidemics have the contaminated water as an important source of infection, and many of them have a high mortality rate in people with low immunity, especially elderlies and children under five years (SILVA; ARAÚJO, 2003).

Among the many functions of water there is the role it plays in health, economy and quality of life, making it important to maintain them. Besides, it also acts as part of the construction and growth of civilizations (SOUZA *et al*, 2014).

Being the main water source to supply most part of cities, rivers should receive extra attention about their quality. However, in many of them the water is contaminated because it receives a big amount of pollutants, mainly those that cross the urban perimeter, that has a closer relation with the local population (MADRUGA *et al*,2008 e CALISTTO *et al*, 2002).

A part from the pollution effluents, the constant unbalanced use of water resources and the discharge of hazardous products in these ecosystems have brought severe consequences. Issues like those have motivated monitoring aimed to evaluate and maintain the quality of these wellsprings (CUNHA *et al*, 2004).

Thus, the supply of good quality fresh water is needed for human beings and also for animals and vegetables (SIQUEIRA; APRILE; MIGUÉIS, 2012). According to Sousa (2014), the quality of water is indispensable for humans, because it has being suffering important restrictions resulting from natural and anthropic actions that cause alterations in quality and amount of water available for living beings in general.

The polluted water is the one that shows the presence of hazardous substances, detritus or agents that cause diseases, and thus, it is not suitable for human consumption (PUPILE; CARVALHO; MONTEIRO, 2010).

The quality of water is the combination of many processes that occur during the water course. The contamination in these environments proliferates not only by the transformation of urban and industrial societies but also during the production of consumer products that creates destruction and garbage (PEREIRA; LIMA, 2007). The aquatic ecosystems are usually contaminated by effluents and industrial or domestic waste that carry or not many pathogenic microorganisms that are used as pollution indicators and are labeled as heterotrophic bacterias (DOZZO, 2011).

This research aims to evaluate the microbiological quality of the water of the Parnaíba River in the urban area of Floriano city, through microbiological techniques for total and fecal coliforms and *E. coli*. It also aims, through environmental education, show to the students at Fauzer Bucar High School the importance of water and the necessity to preserve it.

2 METHODS

2.1 TYPES OF STUDY

This work adopted a descriptive and exploratory study because according to Cervo and Bervian (2002), this type of research allows the analysis and correlation of the facts without modifying them.

2.2 CHARACTERISATION OF THE RESEARCH FIELD

The research field focused on three points of the Parnaíba River located in the urban area of Floriano/PI, at a latitude of 60º46'01" south and longitude 43º01'21" west (IBGE, 2018). They were chosen for being points of bathing and for having galleries discharged nearby.

2.3 SAMPLES

The water samples were obtained through two collections carried out according to technical recommendations and asepsis. The first used the Colillert system and the second used culture medium techniques (Lactose Broth, Brilliant Green Bile Broth; EMB Agar and MacConkey Agar). In the first collection three sterilized plastic bottles were used, one for each point (Point 1- Bathing place 2 km from Pier riverside; Point 2

Pier riverside; Point 3- Bridge Floriano/Barão de Grajaú). In the second one, we used nine autoclavable Erlenmeyer flasks, three for each point (the same points of the first one).

2.4 INSTRUMENTS AND PROCEDURES OF DATA COLLECTION

The work implementation began with the descriptive and exploratory part and then the collections were taken. The first one happened at the end of the dry period (October 2017), in three specific points of the river, and the second was taken in the beginning of the first rains (January 2018).

After collecting the samples, the reagent Colilert was added to each bottle (system patented by IDEXX Laboratories) and then the samples were placed in a bacteriological incubator for 24 hours at 35º C. After that, the color of each bottle was observed to detect the presence or absence of yellowish total coliforms and *E. coli* through fluorescence in the presence of ultraviolet light at 365 nm.

When the first rains began, the second sample was taken, with 9 samples in erlenmeyer (3 in each point). Then, they were taken to the laboratory for microbiological analysis. The presumptive test was then done, inserting 1 ml of the sample in the tubes containing lactose broth, using the technique of multiple tubes. Next, the tubes were incubated in the incubator for 24 hours, and after this time, we watched how many of them showed positive reaction through cloudy aspect or formation of gas in Durhan tubes, and negative if there is no modification.

Then a confirmatory test was carried out, when the material of the tubes containing lactose broth was inserted in the tubes with Brilliant Green Bile Broth. Afterwards they were put into the incubator for 48 hours and the MPN was determined by the combination formed by the number of positive tubes.

For the selective testing and determination of *E. coli*, samples from the positive tubes with the Brilliant Green Bile Broth were inserted on a Petri dish containing EMB Agar and MacConkey Agar, that were placed in the incubator for 48 hours and then we verified a colony forming. After this process, we began the preparation and coloring of the bacteriological slides. When prepared, the slides were observed in the optical microscope with objective lens 40X and 100X.

Through the observation in the optical microscope, the following aspects were analysed: color (the Gram positive shows a blue or violet color and the Gram negative shows a red color), format (the ones with stick format are the bacillus and the round

ones are Coccus), and the disposition of the bacteria present in this sample. All the preparation and analysis processes were carried out in the health laboratories at College higher education of Floriano-FAESF.

The results obtained were used to perform an environmental education work with high school students at State School Fauzer Bucar, during a lecture about the importance of water and its conservation, when we showed the situation of the quality of the river and distributed brochures and produced a sign, because besides providing knowledge, we try to develop an environment where the students can deal with the information, understand, refute and elaborate it and then understand the world and act in it with autonomy (BRASIL, 2008).

Therefore, it will be possible to form sensitive and sympathetic individuals and citizens aware of the processes of the world and life, able to carry out practical actions, judge and make decisions (BRASIL, 2002).

3 RESULTS AND DISCUSSION

The results of the first collection were positive for total coliforms and *E. coli*, because in the samples containing the system Colilert (patented by IDEXX Laboratories), after 24 hours of incubation at 35º C, a change in color was observed. The sample produced a reaction of visible yellow color, that is considered as a positive reaction for total coliforms (IDEXX, 2016).

When examined with fluorescence using an ultraviolet long wavelength lamp (365nm), there was a fluorescence characteristic, determining *E. coli* positivity (IDEXX, 2016).

The results of the microbiological analysis of the samples in the points P1, P2 e P3 located in the urban area of Floriano, Piauí, show that some of them are similar or above the acceptable limit of contamination, that is, > 1100 (Table 1).

Table 1- Distribution of Most Probable Number (MPN) of organisms founded in the samples of the water of the Parnaíba River in the urban area of Floriano – PI

Samples	**Number of positive tubes Volume of product per tube (ml)**			**MPN per ml of the sample**
	10^{-1}	10^{-2}	10^{-3}	
P1	3	3	1	460
P2	3	3	2	1100
P3	3	3	3	> 1100

Comparing the indexes of MPN/100 ml obtained in the samples from the three points, it was noticed in all points the presence of fecal coliforms.

The results from point 1 showed an amount below the maximum limit allowed, what can be explained by the absence of discharging from galleries nearby, such as in the other analysed points.

On the other hand, points 2 and 3 had indexes that were equal or above 1,100 fecal coliforms/100 ml respectively, maximum count by Resolution 357 from CONAMA for bathing areas. The high presence of coliforms in the samples can be justified by the fact that these points are places of discharge of domestic and hospitalar waste from two galleries.

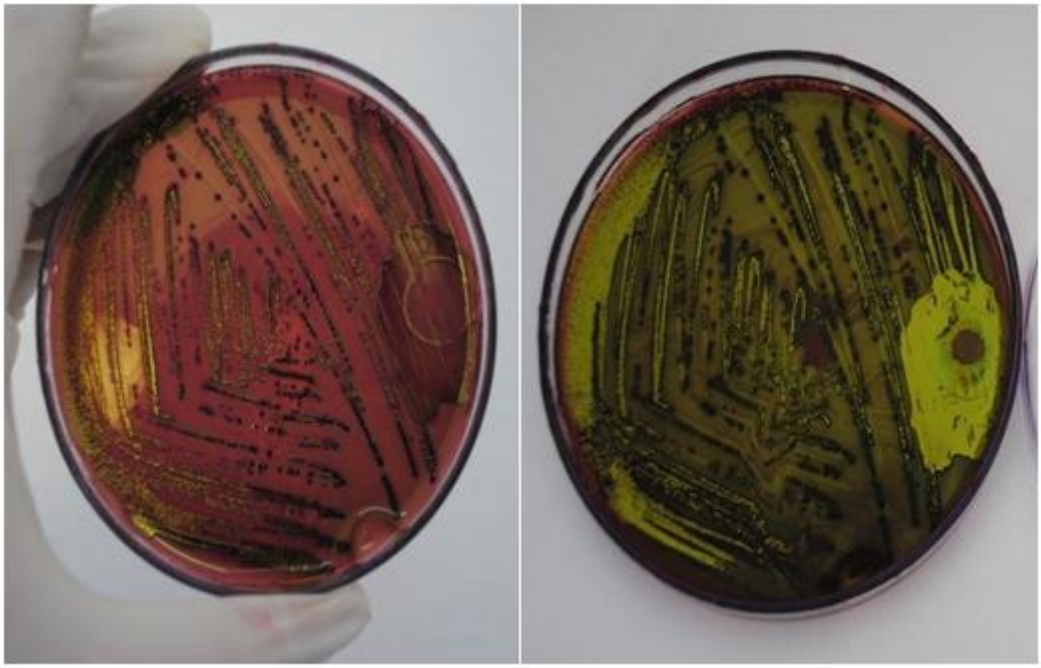
A research carried out by Castro (2012), also in the Parnaíba River, showed that in the analysed points the samples had a number of fecal coliforms/100ml above the allowed amount. It was also observed the presence of *E. coli*.

Results above the allowed limit were found in studies carried out in waters from other brazilian rivers. In this regard, Moura *et al.* (2009), when microbiologically monitoring the water from the Cascal River (PR), also showed high rates of contamination by total and thermotolerant coliforms. Rodrigues *et. al.* (2009), analysing 18 water samples from the Piracuama River, in Pindamonhangaba (SP), found high rates of contamination by total and thermotolerant coliforms.

We can also quote Almeida *et. al.* (2004) in the Stream Córrego Ribeirão dos Porcos (ES), where 24 water samples were taken in six different points --four collections in each point--, in duplicates. The microbiological analysis indicated high rates of total and fecal coliforms and he observed higher rates of pollution by fecal coliforms in the urban area where the stream receives domestic and industrial waste; Buzanello *et. al.* (2008) in a municipal lake in Cascavel (PA) also showed high results for fecal and total coliforms.

In the water samples from the Parnaíba River was also observed the microbial growth in EMB Agar dishes of small and greenish colonies, with metallic brightness and darker cores (Picture 1). This result, according to the criterias defined by Ribeiro *et al.* (2009), indicates the presence of *E. coli*. Besides that, it was showed during the evaluation by optical microscopy, a high presence of bacilli and cocco, Gram negative and Gram positive.

Picture 1 - Microbial growth in EMB Agar dishes



A project coordinated by Microbiology professor at Piauí State University (UESPI) Francisca Lúcia de Lima, in Teresina city, showed that the Parnaíba River is infested by bacterias resistant to disinfectants that come from the hospitalar network in Teresina and are discharged directly in the river through the untreated sewage system (LIMA, 2011).

The results found in this study show that the water from the Parnaíba River is improper for consumption, because, according to the Decree 518 of the Ministry of Health, the water for human consumption has to be free from *E. coli* or fecal coliforms --absence in 100 ml or maximum positivity of 5% for total coliforms.

Nonetheless, the results show that probably the households and commercial places, the polluting sources of the river, do not meet the standards of effluents discharge provided in article 24, chapter IV, Resolution 357/05 from CONAMA. It says that the effluents from any polluting source have to undergo a treatment, abiding conditions, standards and demands from the same resolution, before being discharged directly or indirectly in the water.

Corroborating these results, in other researches the waters from rivers analysed also showed strong impacts regarding quality, due to the discharge of domestic waste that raise the presence of total and fecal coliforms in these ecosystems. In this context, Oliveira *et al.* (2002), in São Lourenço do Sul city, RS and Vasconcelos *et al*. (2006),

also in São Lourenço, showed that the MPN of thermotolerant coliforms increases as the river receives effluents.

In addition, a microbiological analysis performed in River Mearim, in Bacabal-MA, showed 18,800 CFU / 100 mL total coliform and 4,800 CFU/100 mL of fecal coliform (JÚNIOR et al., 2014)

In a research conducted in the Parnaíba River, it was noticed through its results that its water need urgent measures to improve the treatment of the effluents discharged in the river, as well as to improve the inspection in treatment stations in the urban area of Teresina-PI, because some alterations were found in essential parameters, like *E.coli* and dissolved oxygen (CARVALHO, 2012).

4 CONCLUSIONS

This study was a preliminary diagnosis about the microbiological conditions of the Parnaíba River in the urban area of Floriano. Through the research, it was possible to show, initial hypothesis, that its water in certain points do not meet the pre-established standards because they showed rates above the minimum limit allowed for total and fecal coliforms, what makes the water improper for consumption.

The data obtained shows that the Parnaíba River has being impacted by untreated effluents, because laboratorial analysis proved the contamination from total and fecal coliforms, as well as *E. coli.*

In this context, it is necessary to preserve the water sources, avoiding the entrance of untreated sewage and also by the implementation of techniques to treat effluents in the city and the search for development of public policies aimed to monitor and inspect the preservation of the aquatic ecosystems. A part from that, it is necessary to work with environmental education, seeking the participation of the population to preserve this ecosystem.

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