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Assessment of biotic and abiotic variables in water samples from the Metropolitan region of Recife (Pernambuco, Brazil)

Avaliação de variáveis bióticas e abióticas em amostras de água da Região Metropolitana do Recife (Pernambuco, Brasil)

Evaluación de variables bióticas y abióticas en muestras de agua de la Región Metropolitana de Recife (Pernambuco, Brasil)

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Abstract
The monitoring of water resources is essential to meet the vital needs of human beings in addition to being a resource that feeds lines of agro-industrial production and domestic use. The care of this natural resource is subject to several abiotic and biotic changes in reaction to anthropic stimuli. In this study, physical-chemical and microbiological tests were carried out on water samples from homes in the Metropolitan Region of Recife, evaluating the influences of pH, turbidity, alkalinity and chlorides on the presence and density of the microbiota present. The presence of total coliforms, *Escherichia coli*, *Pseudomonas aeruginosa* and heterotrophic bacteria were detected. *Clostridium perfringens* was absent in all samples. The results indicate that the presence of *P. aeruginosa* showed a negative relationship with turbidity values and a direct relationship with pH, chlorides and alkalinity. Higher chloride concentrations positively and negatively influence the density of coliform bacteria and heterotrophic bacteria, respectively. On the other hand, alkalinity negatively influenced the development of coliforms. The results indicate that the presence of *P. aeruginosa* showed a negative relationship with turbidity values and a direct relationship with pH, chlorides and alkalinity. Higher chloride concentrations positively and negatively influence the density of coliform bacteria and heterotrophic bacteria, respectively. On the other hand, alkalinity negatively influenced the development of coliforms. Results indicate that the analyzed waters are unsuitable due to the presence of *P. aeruginosa*, which in turn indicates lack of sanitation in the reservoirs and insufficient chlorination.
Keywords: Water resource; Coliforms, Abiotic variables.

Resumo
O monitoramento dos recursos hídricos é imprescindível para atender às necessidades vitais dos seres humanos além de ser um recurso que alimenta linhas da produção agroindustrial e de uso doméstico. O cuidado com esse recurso natural está sujeito a diversas alterações abióticas e bióticas em reação a estímulos antrópicos. Nesse estudo, foram realizados ensaios físico-químicos e microbiológicos em amostras de águas em residências na Região Metropolitana do Recife, avaliando as influências de pH, turbidez, alcalinidade e cloretos sobre presença e densidade da microbiota presente. Foram detectados a presença de coliformes totais, *Escherichia coli*, *Pseudomonas aeruginosa* e bactérias heterotróficas. *Clostridium perfringens*, foi ausente em todas as amostras. Os resultados apontam que a presença de *P.*

aeruginosa apresentou relação negativa com valores de turbidez e relação direta com pH, cloretos e alcalinidade. Maiores concentrações de cloretos influenciam positivamente e negativamente a densidade de bactérias do grupo coliformes e bactérias heterotróficas, respectivamente. Por outro lado, a alcalinidade influenciou negativamente o desenvolvimento dos coliformes. Os resultados apontam que a presença de *P. aeruginosa* apresentou relação negativa com valores de turbidez e relação direta com pH, cloretos e alcalinidade. Maiores concentrações de cloretos influenciam positivamente e negativamente a densidade de bactérias do grupo coliformes e bactérias heterotróficas, respectivamente. Por outro lado, a alcalinidade influenciou negativamente o desenvolvimento dos coliformes. Resultados indicam que as águas analisadas mostram improprias devido à presença de *P. aeruginosa* que por sua vez indica falta de higienização dos reservatórios e cloração insuficiente.
Palavras-chave: Recurso hídrico; Coliformes, Variáveis abióticas.

Resumen
El monitoreo del recurso hídrico es fundamental para satisfacer las necesidades vitales del ser humano además de ser un recurso que alimenta renglones de producción agroindustrial y de uso doméstico. El cuidado de este recurso natural está sujeto a diversos cambios abióticos y bióticos en reacción a los estímulos antrópicos. En este estudio, se realizaron análisis físico-químicos y microbiológicos en muestras de agua de viviendas de la Región Metropolitana de Recife, evaluando las influencias del pH, la turbidez, la alcalinidad y los cloruros en la presencia y densidad de la microbiota presente. Se detectó la presencia de coliformes totales, *Escherichia coli*, *Pseudomonas aeruginosa* y bacterias heterótrofas. *Clostridium perfringens* estuvo ausente en todas las muestras. Los resultados indican que la presencia de *P. aeruginosa* mostró una relación negativa con los valores de turbidez y una relación directa con el pH, los cloruros y la alcalinidad. Las concentraciones más altas de cloruro influyen positiva y negativamente en la densidad de bacterias coliformes y bacterias heterótrofas, respectivamente. Por otro lado, la alcalinidad influyó negativamente en el desarrollo de coliformes. Los resultados indican que la presencia de *P. aeruginosa* mostró una relación negativa con los valores de turbidez y una relación directa con el pH, los cloruros y la alcalinidad. Las concentraciones más altas de cloruro influyen positiva y negativamente en la densidad de bacterias coliformes y bacterias heterótrofas, respectivamente. Por otro lado, la alcalinidad influyó negativamente en el desarrollo de coliformes. Los resultados indican que las aguas analizadas no son aptas por la presencia de *P. aeruginosa*, lo que a su vez indica falta de saneamiento en los embalses e insuficiente cloración.
Palabras clave: Fuente de agua; Coliformes, Variables abióticas.

1. Introduction

Society pressure on the protection of water resources is a global appeal, which is linked to increasing levels of eutrophication, both in urban and rural areas. The increase in agricultural and industrial production, associated with the lack of ecological awareness on the part of the population, are some of the factors that justify the commitment of water resources, putting at risk health and impacts on the economy, thus promoting social inequalities, since water is not a inexhaustible resource (Delgado-Munevar, 2015; Macedo et al., 2021)

In Brazil, the National Water Resources Policy, institutionalized in 1997, brought instruments for the management of aquatic resources, among which it allows the granting of use rights, under qualitative and quantitative control of water resources. The grant is a mechanism for rationalizing water resources, to guarantee human supply and protection of the aquatic ecosystem, especially in periods of water scarcity (Moreira et al., 2022).

Water can be an important vehicle for the transport and transmission of microorganisms, many of which are pathogenic to humans, such as enterobacteria (total coliforms, *Escherichia coli*, *Salmonella* spp), protozoa (*Entamoeba histolytica*, *Giardia* spp, *Cryptosporidium* spp) and viruses (Rotavirus, Hepatitis A). These microorganisms are responsible for the occurrence of severe enteric conditions, since they are potential pathogens, especially in immunosuppressed or immunosuppressed patients (Chaysiri et al., 2021). Recently, opportunistic pathogens, such as *Pseudomonas aeruginosa*, have become a leading source of waterborne disease outbreaks in several (Huo et al., 2021).

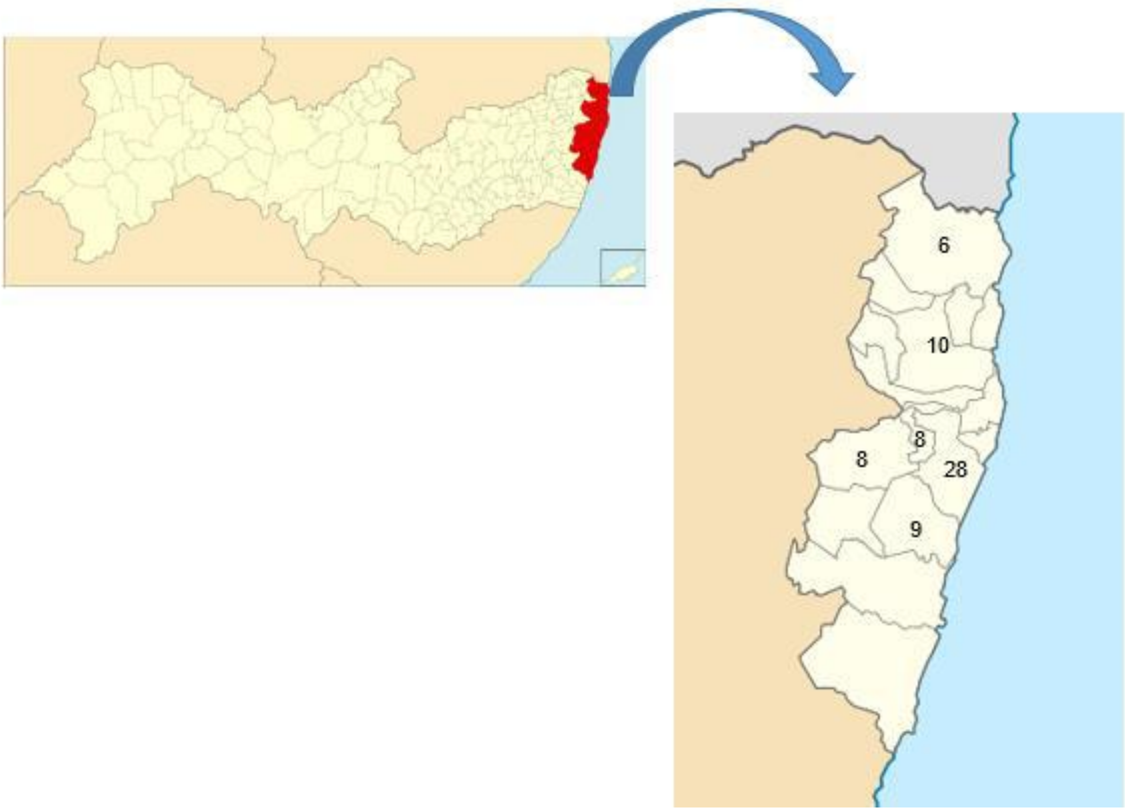
Several intrinsic and extrinsic biotic and abiotic factors regulate the multiplication of microorganisms (Egli, 2015), including aquatic ecosystems. According to Clercin and Druschel (2019), the eutrophication of surface and deep waters means enrichment by nutrients, mainly nitrogen and phosphorus. The use of physicochemical and biological parameters to assess water quality consists of evaluating the variables that are correlated with changes in the water resource, whether of human or natural origin, in order to better understand the impacts of eutrophication.

Considering that water is present in the daily life of human beings, whether in bathing, drinking water, preparing meals, crops, etc., it is important to understand the factors that influence the survival and development of microorganisms in aquatic ecosystems. Thus, this work aims to evaluate the influence of abiotic variables on the density of waterborne microorganisms.

2. Methodology

Water samples (n=69) from different treated water reservoirs located on the Metropolitan Region of Recife were sampled in the same day and in triplicate for physicochemical and bacteriological analyses and then stored and preserved, according to American Public Health Association (APHA 2017).

Figure 1. Map of the State of Pernambuco, highlighting the Metropolitan Region of Recife. Numbers represent the number of sampling (Source: https://pt.wikipedia.org).



Source: Authors.

The physicochemical variables of the analyzed water were turbidity (NTU), alkalinity (mg.L^{-1} $CaCO_3$), pH and chlorides mg.L^{-1}. Turbidity and pH were obtained through measurements with turbidimeter, colorimeter (Hach, USA), and potentiometer (Digimed, Brazil), respectively. The other variables were analyzed according to the methodologies described in APHA (2017).

The quantification of heterotrophic bacteria and determination of presence/absence of *P.aeruginosa* and *Clostridium perfrigens* was performed using the depth plating method (AOAC, 2000) and the results expressed in colony forming units per milliliter (CFU.mL^{-1}).

Regarding the quantification of total and thermotolerant (*E. coli*) coliforms, a presumptive coliform test was performed using Lauryl Sulfate Tryptose broth and confirmatory tests for coliforms were performed using Bright Green broth for total coliforms and EC broth for *E. coli*. The concentration of total and thermotolerant coliforms present in the sample were obtained in the confrontation with the Most Probable Number (MPN) table for tests with drinking water and the results expressed in MPN.100 mL^{-1} (APHA, 2017).

2.1 Data analysis

Abiotic and biotic matrices were constructed to perform the multivariate analyses, with biotic matrices using density data (total coliforms, thermotolerant coliforms and heterotrophic bacteria) and presence/absence (*P. aeruginosa* and *C. perfrigens*). Abiotic variables were standardized and biomass values were log transformed (x + 1). The relationship between physicochemical and bacteriological variables on water samples was evaluated using Redundancy Analysis (RDA), using the ordistep function to select variables.

Considering that the methodology used to detect *P. aeruginosa* and *C. perfrigens* in the samples allows the expression of the results in "presence" or "absence", a GLM analysis (Generalized Linear Method) was performed, applying the "binomial" family, in order to evaluate the relationship between the physicochemical variables analyzed and the presence or absence of these microorganisms. The model that presented the lowest value for the Akaike information criterion (AIC) was selected. This parameter is a metric that measures the quality of a statistical model, aiming at the comparison and selection of models, in which lower AIC values represent greater quality and simplicity.

All statistical analyses were performed using software R 3.1.1 (vegan package).

3. Results and Discussion

3.1 Physicochemical and bacteriological characterization of the water

The analyzed samples showed, on average, circumneutral values, with low values of turbidity and chlorides. Alkalinity presented minimum and maximum concentrations of 110 and 8 mg.L^{-1} $CaCO_3$, respectively. Regarding the bacteriological variables, an average density of total coliforms of 2.87 ± 1.38 MPN.100mL^{-1} was observed and an absence of thermotolerant coliforms were shown in 78.26% of the samples (Figure 1). Moreover, minimum and maximum densities of heterotrophic bacteria were 2 and 752 MNP.100mL^{-1}, respectively.

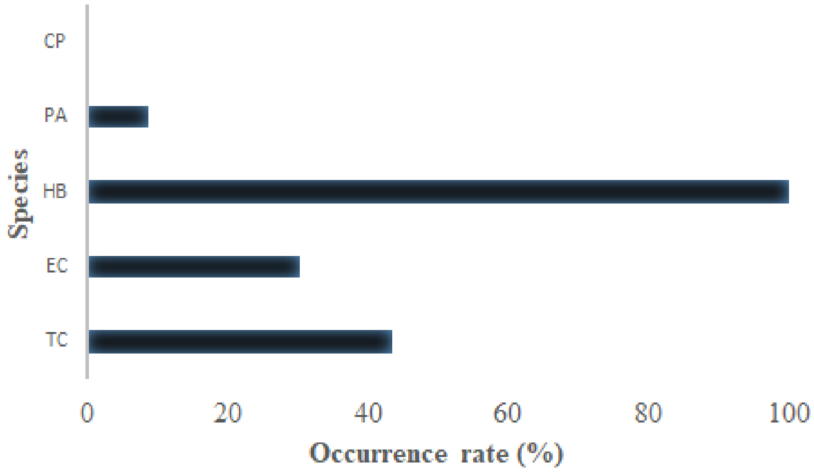
In 92% of the analyzed water samples (n = 63), *P. aeruginosa* was present, while the bacterium *C. perfrigens* was absent in all samples (Figure 1).

Table 1. Average and standard deviation values of physicochemical and bacteriological variables in water samples.

Variables	Average	Standard deviation
Physicochemical		
pH	6.43	0.68
Turbidity	2.98	1.34
Alkalinity (mg.L^{-1} $CaCO_3$)	31.68	21.86
Chlorides (mg.L^{-1})	0.05	0.04
Bacteriological		
Total coliforms (MPN.100mL^{-1})	2.87	1.38
Escherichia coli (MPN.100mL^{-1})	2.69	1.11
Heterotrophic bacteria (CFU.mL^{-1})	245.41	230.57

Source: Authors.

Figure 1. Occurrence rate of total coliforms (TC), *Escherichia coli* (EC), heterotrophic (HB), *Pseudomonas aeruginosa* (PA) and *Clostridium perfrigens* (CP) (n = 69) in water samples



Source: Authors.

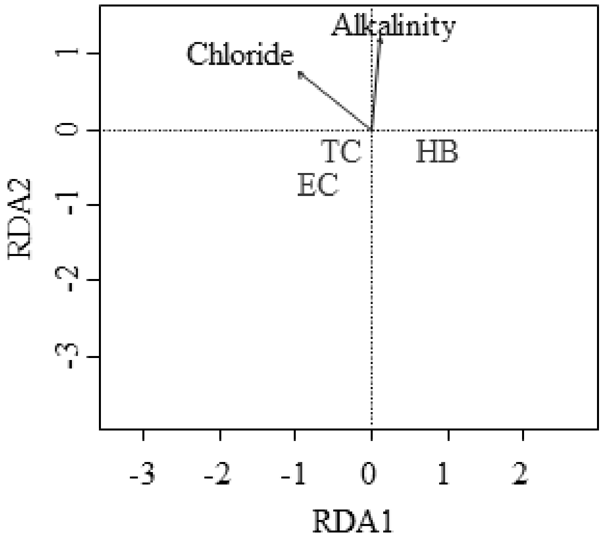
3.2 Relationship between water physicochemical and bacteriological variables

The RDA generated a significant model (p = 0.006), and it was possible to observe relationships between the abiotic and biotic variables. The eigenvalues of axes 1 and 2 explained 11.06% of the variation in the biological data, of which 86.50% were attributed to axis 1 and 13.50% to axis 2. In the ordination, only axes 1 significantly explained the relationships between abiotic and biotic variables (p = 0.011). Total and thermotolerant coliforms (*E. coli*) correlated negatively with axis 1 while heterotrophic bacteria group presented positive correlations with the same axis (Figure 2).

The predictive variables of bacteria densities were chloride (-0.79, axis 1) and alkalinity (0.09, axis 1). The force vector of the alkalinity is more strongly related to axis 2, however, as this axis did not show significance in the model, its relationship with axis 1 will be considered.

Thus, it is possible to infer that higher chloride concentrations positively influence the growth and development of coliform bacteria, while heterotrophic bacteria (except coliforms) is negatively influenced by this variable. Despite having a weak influence, it is important to note that alkalinity should favor the development of heterotrophic bacteria, but not coliforms.

Figure 2. Redundancy analysis for biotic and abiotic variables throughout the study in water samples TC: total coliforms; EC: *Escherichia coli* (thermotolerant coliforms); HB: heterotrophic bacteria.



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The GLM analysis to evaluate the relationship between the physicochemical variables of water and the presence or absence of *P. aeruginosa* resulted the most parsimonic model with AIC = 10 and showed a negative relationship between the density of this microorganism and the turbidity values (-91.40), besides a positive relationship with pH (302.05), chlorides (256.31) and alkalinity (2.18). The analysis for *C. perfrigens* was not performed, since this microorganism was not detected in any of the analyzed samples.

Chloride is an abundant anion on Earth and is present in several water samples. Study carried out by Roeßler et al. (2003) did not show requirement of chloride for growth of *E. coli* and other heterotrophic bacteria species. Nevertheless, in a hyperosmotic media containing high concentrations of Na^+, these microorganisms had a strict chloride dependence for growth or were significantly stimulated by chloride. Roeßler and Müller (2002) pointed out that chloride might be essential in Na^+ homeostasis, that is cytotoxic and all living cells tend to expel sodium ions from their cytoplasm via the chloride-sodium channels. Cesar et al. (2020) stated that for thriving under conditions of elevated osmolarity it is absolutely essential for the cells to measure external osmolarity and to adjust their metabolism accordingly, inducing the biosynthesis or uptake of osmolyte, such as chloride compounds. Salinity analyzes were not performed in this study, but the presented results may serve as a basis for further studies in order to corroborate this profile.

The influence of chloride ions on bacteria, mainly pathogens, is recognized by studies carried out by Tan (2021). According to the author, chloride can increases the expression of virulence factors in microbial pathogens, playing an important role in the bacterial growth and infection process.

Regarding to alkalinity, the Figure 2 shows negative relation between this variable and coliforms bacteria, although it has shown weak positive relation to heterotrophic bacteria group. Positive relation between this variable and *P. aeruginosa* can also be noted in GLM analysis. Tan et al. (2018) and van der Wall et al. (2011) pointed out that higher alkalinity levels can inhibit the growth of both Gram-positive and -negative bacteria through inactivating ATP synthesis and inducing oxidative stress. A classic study published in 1932 (Watkins and Winslow, 1932) show that higher alkalinity can inhibit the *E. coli* growth, mainly in young-age cultures, probably due to lack of power to form the protective substances or other factors associated with physiological youth. Van der Wall et al. (2011) found that high pH had limited bactericidal effect on *P.*

aeruginosa. Conversely, Harjai et al. (2005) relates that alkaline pH values increases the siderophores production and, consequently, the virulence of *P. aeruginosa*. Besides, according to the Buschell et al. (2019), low pH is a growth inhibitory for *P. aeruginosa*, as they are for many bacteria. These findings suggest that the influence of alkalinity and pH on bacteria, mainly *P. aeruginosa*, is unclear and needs further studies and our results can support some discussions.

According to Luján Roca (2014), the presence of *P. aeruginosa* is worrisome in terms of impact on public health, as it is one of the globally dominant nosocomial opportunistic pathogens in hospitals, causing severe impacts such as a range of infections in immunosuppressed patients, of bacteremia and is endowed with resistance mechanisms through mutation, against several available antimicrobials (Luján-Roca, 2014). Turbidity methods offer possibilities for generating data required for addressing microorganism indirect growth (Lindqvist 2006). According to this method, higher turbidity values accompanies higher bacterial concentrations. According to Huo et al. (2021), turbidity showed a great positive correlation with many opportunist bacteria including *P. aeruginosa*, possibly due to the inhibition of disinfecting agents by turbidity, allowing bacterial growth. Turbidity values means suspension material, such as cells and humic acids and studies show that for higher humic acids concentrations, have a negative impact on chlorine disinfection, allowing the bacterial growth (Léziart et al., 2019). In this work, the concentration of humic acid or other organic compounds was not determined, however, our results can be used to support future studies that show the influence of turbidity sources on microbial growth.

In the study, the GLM model showed a negative relationship between *P. aeruginosa* and this variable. It is known that this bacterium forms a biofilm (Ma et al. 2009), which adheres to the surface of a given substrate and the presence of *P. aeruginosa* in the water occurs when there is a detachment of the biofilm by action, for example, an increase in the flow of the water in the reservoir. Therefore, we believe that the negative relationship between *P. aeruginosa* growth and turbidity occurs by a mechanism that cannot be explained by the presented results.

According to Arroyave et al. (2012), the management of water resources and the issue of technical regulations are legal frameworks that contribute to reversing and protecting the environmental conditions of strategic ecosystems and the sources that generate these water resources. Environmental management is built by men and organized society so that together they promote the transformation and restoration of the environment, joint efforts that are not considered empty and fruitless actions. Among these actions by the government, monitoring by official bodies and research groups is foreseen, through biological, physical and chemical tests, which, when confronted with the parameters of the legislation, ensure that these aquatic resources are safe for human consumption.

4. Conclusion

The characterization of the water resource used by the population is relevant because it is related to public health. The present study showed that most samples are unfit for use. Our results show an influence of abiotic variables such as pH and alkalinity on the development of bacteria, especially in *P. aeruginosa*, but more studies need to be carried out to support these claims. The aquatic microbiota found in the water samples of this research for human consumption, indicate that the analyzed waters are shown to be unsuitable, due to the presence of *P. aeruginosa*, which in turn indicates a lack of unsatisfactory hygiene of the reservoirs and water tanks, combined with deficient chlorination.

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