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PHYSICOCHEMICAL AND MICROBIOLOGICAL ANALYSES OF DRINKING WATER FOR DOMESTIC ANIMALS AT THE SANTA PAULA EXPERIMENTAL FARM – UFVJM

ANÁLISES FÍSICO-QUÍMICAS E MICROBIOLÓGICAS DA ÁGUA DE CONSUMO DE ANIMAIS DOMÉSTICOS NA FAZENDA EXPERIMENTAL SANTA PAULA – UFVJM

ANÁLISIS FISICOQUÍMICOS Y MICROBIOLÓGICOS DEL AGUA DE CONSUMO DE ANIMALES DOMÉSTICOS EN LA GRANJA EXPERIMENTAL SANTA PAULA – UFVJM

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ABSTRACT

Water is an essential element and an indispensable natural resource for the life of living beings. For animals, water performs several specific functions, and it is essential that it is available in the environment in adequate quantity and quality. This work aimed to carry out physical-chemical and microbiological analysis of the water present in animal drinking troughs installed at the Fazenda Experimental Santa Paula (FESP; Santa Paula Experimental Farm), Unaí-MG. The present study was developed considering the importance of evaluating water quality for animal consumption. The samples were collected in four FESP drinking fountains intended for different animal species. For each collection point, a methodology was developed based on the analysis of water quality using physical-chemical and microbiological parameters, in addition to the methylene blue (AM) technique. The physical-chemical results of the drinking fountains evaluated included temperature, pH, oxygen and electrical conductivity, which were within the values allowed by the CONAMA Resolutions. Regarding the AM technique, it was observed that in the second and fourth drinking fountains analyzed, it was used as an indicator of the quality of water intended for animals, especially in waters with high levels of organic matter. Furthermore, the results of the microbiological analyzes confirmed the presence of total and thermotolerant coliforms and Escherichia coli in the drinking fountains evaluated. Finally, it is concluded that this integrated approach of continuous monitoring of the quality and correct hygiene of drinking fountains contributes significantly to animal health and well-being.

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Keywords: Water Quality. Environmental Monitoring. Methylene Blue. Physicochemical Parameters.

RESUMO
A água é um elemento essencial e um recurso natural indispensável para a vida dos seres vivos. Para os animais, a água desempenha diversas funções específicas, sendo fundamental que esteja disponível no ambiente em quantidade e qualidade adequadas. Este trabalho teve como objetivo realizar análises físico-químicas e microbiológicas da água presente em bebedouros para animais instalados na Fazenda Experimental Santa Paula (FESP), em Unaí-MG. O estudo foi desenvolvido considerando a importância de avaliar a qualidade da água destinada ao consumo animal. As amostras foram coletadas em quatro bebedouros da FESP, destinados a diferentes espécies animais. Para cada ponto de coleta, adotou-se uma metodologia baseada na avaliação da qualidade da água por meio de parâmetros físico-químicos e microbiológicos, além da técnica do azul de metileno (AM). Os resultados físico-químicos dos bebedouros avaliados — incluindo temperatura, pH, oxigênio e condutividade elétrica — situaram-se dentro dos valores permitidos pelas Resoluções do CONAMA. Quanto à técnica do azul de metileno, observou-se que, no segundo e no quarto bebedouro analisados, ela serviu como um indicador da qualidade da água destinada aos animais, especialmente em casos de águas com altos teores de matéria orgânica. Ademais, os resultados das análises microbiológicas confirmaram a presença de coliformes totais, coliformes termotolerantes e *Escherichia coli* nos bebedouros avaliados. Por fim, conclui-se que essa abordagem integrada, que envolve o monitoramento contínuo da qualidade e a higienização adequada dos bebedouros, contribui significativamente para a saúde e o bem-estar animal.

Palavras-chave: Qualidade da Água. Monitoramento Ambiental. Azul de Metileno. Parâmetros Físico-químicos.

RESUMEN
El agua es un elemento esencial y un recurso natural indispensable para la vida de los seres vivos. Para los animales, el agua cumple diversas funciones específicas, por lo que resulta fundamental que esté disponible en el entorno en cantidad y calidad adecuadas. El objetivo de este trabajo fue realizar análisis fisicoquímicos y microbiológicos del agua presente en los bebederos para animales instalados en la Fazenda Experimental Santa Paula (FESP; Granja Experimental Santa Paula), en Unaí, estado de Minas Gerais (MG). El estudio se llevó a cabo considerando la importancia de evaluar la calidad del agua destinada al consumo animal. Las muestras se recolectaron en cuatro bebederos de la FESP, destinados a distintas especies animales. Para cada punto de muestreo, se aplicó una metodología basada en la evaluación de la calidad del agua mediante parámetros fisicoquímicos y microbiológicos, además de la técnica del azul de metileno (AM). Los resultados fisicoquímicos de los bebederos evaluados —que incluyeron temperatura, pH, oxígeno y conductividad eléctrica— se situaron dentro de los valores permitidos por las resoluciones del CONAMA. En cuanto a la técnica del AM, se observó que, en el segundo y cuarto bebedero analizados, esta sirvió como indicador de la calidad del agua destinada a los animales, especialmente en casos con altos niveles de materia orgánica. Asimismo, los resultados de los análisis microbiológicos confirmaron la presencia de coliformes totales y termotolerantes, así como de *Escherichia coli*, en los bebederos evaluados. Finalmente, se concluye que este enfoque integral, basado en el monitoreo continuo de la calidad y la correcta higiene de los bebederos, contribuye de manera significativa a la salud y el bienestar animal.

Palabras clave: Calidad del Agua. Monitoreo Ambiental. Azul de Metileno. Parámetros Fisicoquímicos.

1 INTRODUCTION

Water is essential for life and an indispensable natural resource for all living beings (WWF, 2006; Bacci; Pataca, 2008; Brazil, 2018; Silva *et al.*, 2019). This element is fundamental for the existence and maintenance of life, and therefore, it must be present in the environment in appropriate quantity and quality (Braga *et al.*, 2005). For animals, water performs several vital functions, such as regulating body temperature, transporting nutrients, removing metabolic waste, and lubricating joints. Its absence - or animal dehydration - can cause a variety of health problems, including kidney dysfunction, electrolyte imbalances, and even death.

Water quality is a crucial factor for animal health and immunity. Contaminated or poor-quality water may contain chemical agents, pathogens, and toxic substances, which can impair the immune system, production, and growth of animals. Consequently, the physical, chemical, and microbiological parameters of water must be evaluated according to established water quality assessment criteria.

Physicochemical evaluation provides detailed information regarding water composition and characteristics. Among the most frequently evaluated physical parameters are temperature, hydrogen potential (pH), dissolved oxygen, and electrical conductivity. Additionally, there are qualitative analyses that assist in assessing the level of water pollution, such as the methylene blue (MB) reduction test.

Microbiological analysis aims to identify the presence and quantity of pathogenic microorganisms and contamination indicators in water, which may pose risks to animal health. According to the National Health Foundation (Brazil, 2013), the traditionally accepted indicators of fecal contamination belong to a group of bacteria known as coliforms. The primary representative of this group is *Escherichia coli* (*E. coli*).

The quality of water provided to animals must meet the standards recommended by the World Health Organization (WHO, 2011), remaining adequate in its physical, chemical, and microbiological aspects. Therefore, to ensure the quality of water provided to livestock, it is essential to implement appropriate management practices, including protecting water sources from contamination, performing proper cleaning and maintenance of drinking troughs and water supply systems, and conducting regular water quality analyses.

2 OBJECTIVES

2.1 GENERAL OBJECTIVE

This study aimed to conduct physicochemical and microbiological analyses of the water in domestic animal drinking troughs located at the Fazenda Experimental Santa Paula (FESP; Santa Paula Experimental Farm) in Unaí, MG.

2.2 SPECIFIC OBJECTIVES

a) To analyze the physicochemical and microbiological aspects of the water in domestic animal drinking troughs;

b) To analyze the water from the drinking troughs using the methylene blue (MB) technique.

3 LITERATURE REVIEW

3.1 IMPORTANCE OF WATER QUALITY FOR ANIMAL HEALTH AND WELFARE

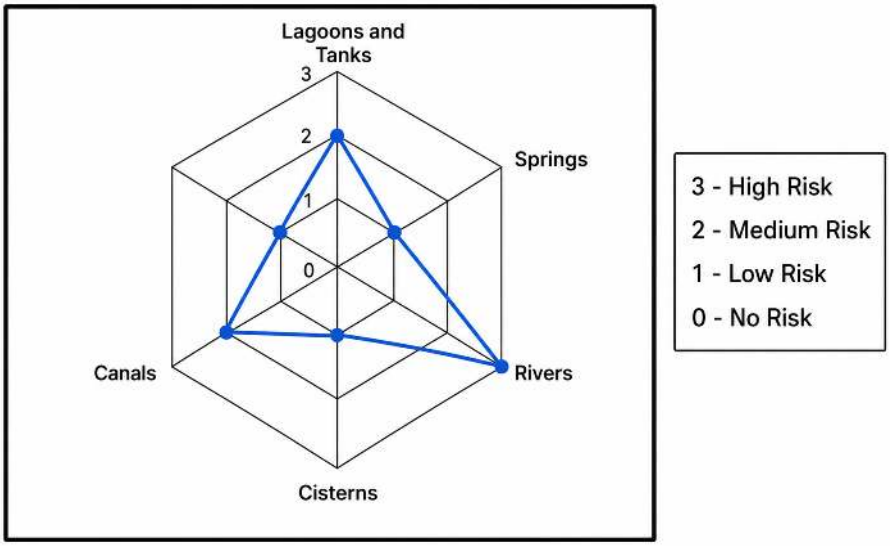
The water used for animal production and consumption must comply with the standards set by CONAMA Resolution No. 357, which classifies fresh to brackish waters and establishes minimum standards for Class 3 water intended for animal consumption, as well as CONAMA Resolution No. 396, which classifies groundwater (Brazil, 2005; Brazil, 2008). According to Portugal (2014), the standards for animal consumption are, in most cases, the same as those established for human beings, as observed in both Brazilian and international legislation. Two factors explain this: the scarcity of studies relating water quality to animal performance, which prevents the determination of specific standards; and the fact that the limit present in legislation represents the maximum concentration permitted for that specific class of use.

According to Palhares (2014), Figure 1 classifies water sources used for animal consumption regarding their quality risk. It is observed that no source is entirely risk-free, as water is constantly interacting with various factors and can have its quality altered by any human activity. Water quality is variable and can be affected by several environmental and human factors, such as rainfall and the use of fertilizers. Groundwater sources, such as wells, also require continuous monitoring. Rivers, meanwhile, are higher-risk sources due to interaction with human activities along their course, which may result in water that is unsuitable for animal consumption. In such cases, monthly analysis and, if possible, partial or total replacement with safer sources is recommended. Lakes, ponds, and canals present

a medium risk and should be fenced off to avoid direct contamination from animal movement and excreta.

Figure 1

Classification of sources based on water quality risk.



Fonte: Palhares (2014).

Water quality is inherently variable and can be influenced by various environmental and human factors, such as rainfall and the use of fertilizers. Groundwater sources, such as wells, also require continuous monitoring. Rivers are considered higher-risk sources due to their interaction with human activities along their course, which may result in water being unsuitable for animal consumption. In such cases, monthly analysis is recommended, alongside partial or total replacement with safer sources whenever possible. Lakes, ponds, and canals present a medium risk and should be isolated to prevent direct contamination from animal movement and excretion.

According to Portugal (2014), water quality control is the only way to verify if water is acceptable for livestock use - that is, whether it possesses adequate quality for animal feeding. Primary water sources should be evaluated periodically (annually), preferably at the beginning of summer to identify potential problems in each source. Any unusual situation related to water, such as changes in odor or color, or negative impacts on animal performance and health status, should be grounds for conducting a water analysis (Palhares, 2014). The presence of substances in concentrations above the permitted limits in the water consumed by livestock represents a risk to both animal health and food safety.

It is important to consider that if water quality is neglected and the evaluated parameters reach threshold values, humans may suffer direct or indirect consequences. Furthermore, poor water quality also affects animal intake, consequently limiting their feed consumption and overall health. Therefore, it is essential to ensure that water sources are sufficient in quantity, while also guaranteeing or improving their palatability and quality to achieve high levels of production, health, and animal welfare.

3.2 PHYSICOCHEMICAL PARAMETERS

Among the physicochemical parameters evaluated in the study were water temperature and electrical conductivity. Temperature is an important water-quality parameter because it influences physical, chemical, and biological processes, whereas electrical conductivity is related to the presence and concentration of dissolved ionic species in water (Von Sperling, 2014).

The chemical parameters analyzed in the study were pH and dissolved oxygen. According to Silva *et al.* (2021), these chemical compounds facilitate more specific water quality analyses in terms of cost and safety. These are inorganic chemical substances (such as minerals) or organic substances (carbon compounds, mainly of animal and plant origin) present in the water.

Water temperature is measured by heat intensity, which exerts a direct influence on aquatic organisms and the other parameters analyzed. Meanwhile, pH represents the concentration of hydrogen ions in a solution. The pH range varies from 0 to 14; values lower than 7 indicate acidic conditions, a pH of 7 represents neutrality, and values greater than 7 indicate basic conditions (Brazil, 2013). pH influences the degree of substance solubility, color intensity, and the organization of the free and ionized forms of various chemical compounds. Furthermore, it can determine the toxicity potential of various elements (Libânio, 2010).

Von Sperling (2014) stated in his study that dissolved oxygen is a baseline parameter for describing the effects of water pollution caused by organic waste. CONAMA Resolution No. 357 determines that dissolved oxygen levels should not be lower than 6 mg/L (Brazil, 2005). The ability of water to conduct an electric current is indicated by conductivity, which depends on the presence of substances that dissociate into cations and anions, such as manganese, iron, sodium, potassium, calcium, chloride, and magnesium ions.

Water's ability to conduct electric current is indicated by its conductivity, which depends on the presence of substances that dissociate into cations and anions - such as manganese,

iron, sodium, potassium, calcium, chloride, and magnesium ions. Conductivity is directly proportional to ionic concentration. Solutions with a high concentration of inorganic compounds exhibit high conductivity, whereas organic compounds that do not dissociate in aqueous solutions impede the flow of electric current (Libânio, 2010).

3.3 MICROBIOLOGICAL PARAMETERS

As established by the Ministry of Health (Brazil, 2021), water is considered potable from a microbiological standpoint when there is no presence of total and thermotolerant coliforms in a 100 mL water sample. Total coliforms correspond to a group of bacteria that includes non-spore-forming Gram-negative rods belonging to the family Enterobacteriaceae. According to Almeida *et al.* (2018), the main bacteria in this group belong to the genera *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella*. Therefore, the presence of total coliforms in water does not necessarily indicate fecal contamination or the presence of intestinal pathogens, as these microorganisms have the ability to multiply in extra-intestinal environments. Thus, their presence indicates inadequate sanitary conditions.

On the other hand, thermotolerant coliforms are indicators of aquatic contamination by fecal material, as they are present in the intestinal microbiota of some animals. *E. coli* is the most relevant bacterium in this group regarding its role as an indicator of fecal contamination. Of the four water samples analyzed by the MPN (Most Probable Number) method, all showed positive results for total and thermotolerant coliforms. CONAMA Resolution No. 396 determines that, for animal consumption, values are considered acceptable when lower than 200 MPN/100 mL (Brazil, 2008).

E. coli is a bacterium that tolerates acidic environments; its natural habitat is the intestinal tract of warm-blooded animals, making them thermotolerant. They are considered commensal bacteria when found in the intestine. Furthermore, they are classified as Gram-negative, facultative anaerobic, non-spore-forming, rod-shaped bacteria. They are mesophilic, with the ability to develop between 7ºC and 46ºC, with 37ºC being the optimal growth temperature (Costa, 2008; Silva *et al.*, 2021). Most *E. coli* strains do not cause disease, but there is evidence that others may be involved in pathologies such as urinary tract infections, pneumonia, and intestinal infections (Silva et al., 2021). For this reason, it is important to monitor and control the presence of this bacterium in water sources.

3.4 METHYLENE BLUE ANALYSIS

Considering the immeasurable importance of this natural asset – water - the relevance of monitoring programs for water systems is evident due to the need to determine the levels of contamination and water quality for different purposes (Al-Aboodi; Abbas; Ibrahim, 2018).

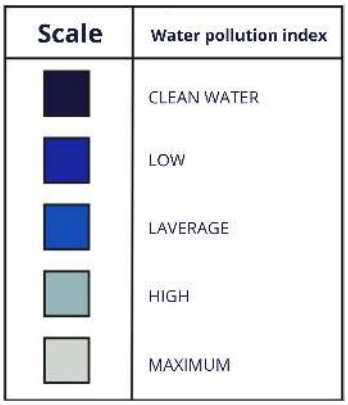
However, the use of products for the determination of contaminants is often high cost. Thus, the use of Methylene Blue (MB) has proven effective and more accessible. MB acts as a reducing agent through the anaerobic decomposition of organic substances, losing its color and, therefore, indicating the degree of organic matter pollution in the water bodies under study (Troppmair, 1988).

Research on MB and its action as an indicator of water resource quality, regarding pollution by organic material, has developed through the analysis of elements and experimentation, offering reliability in results (Monteiro; Viadana, 2009).

This is a visual analysis that uses color patterns: a dark blue shade signifies that the water is not contaminated or has a low concentration of organic matter; however, if the sample gradually loses its color—becoming light blue or transparent—it means that a chemical reaction has occurred due to a reduction process with the MB reagent, indicating that the water is contaminated with a high concentration of organic matter (Monteiro; Viadana, 2009). As shown in Figure 2, a scale in shades of blue was developed by Monteiro and Viadana (2009), serving as a basis for analyzing the water pollution index using the MB technique.

Figure 2

Color scale for the pollution index in samples with methylene blue.



Source: Monteiro; Viadana, 2009.

4 MATERIALS AND METHODS

4.1 STUDY AREA

The present study was conducted at the Fazenda Experimental Santa Paula (FESP; Santa Paula Experimental Farm) (latitude 16°26'11.1"S, longitude 46°53'52.8"W), belonging to the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), located in the municipality of Unaí, Minas Gerais, Brazil. FESP is an experimental farm affiliated with UFVJM, covering an area of 130 hectares, where teaching, research, and extension activities are carried out. The farm provides infrastructure for practical classes in undergraduate programs, including Agronomy, Agricultural and Environmental Engineering, Veterinary Medicine, and Animal Science, as well as for graduate students pursuing master's and doctoral degrees.

According to data from the Brazilian Institute of Geography and Statistics (IBGE, 2018), the municipality of Unaí (latitude 16.35194° S, longitude 46.90056° W, altitude 600 m) covers an area of 8,445.432 km². It is in the northwestern region of the state of Minas Gerais and exhibits environmental characteristics representative of the Brazilian Cerrado biome.

The region receives an average annual precipitation exceeding 1,300 mm, with rainfall concentrated between October and April, and the wettest period occurring from November to January. The average relative humidity ranges from 60% to 70%. The mean annual temperature is 24.4°C, with an average maximum temperature of 42.6°C and an average minimum temperature of 14.6°C (INMET, 2021).

4.2 SAMPLING SITES

The sampling sites were selected based on the location of each drinking trough distributed throughout the farm. Selection criteria included troughs used by cattle and horses between October and November 2023. These troughs are strategically positioned in various paddocks and facilities to ensure easy access for all animals and are supplied with artesian well water. This selection was based on the premise that these sites are subject to varying levels of contamination or fluctuations in water quality due to the materials used in the troughs, as well as interactions with the animals and environmental conditions.

The drinking troughs were mapped according to their location, the species, and the sex of the animals consuming water at each site - specifically those housed within the same area - as shown in Figure 3. The first drinking trough (B1) is in an isolated paddock and serves a single entire horse. The second drinking trough (B2) is situated in an area housing four

fistulated male cattle, used for research and undergraduate practical classes. The third drinking trough (B3) supplies water to a group of four cattle, consisting of both males and females of various ages. Finally, the fourth drinking trough (B4) is in a more remote area, accessible only to the 11 horses and 5 cattle kept at that location.

Figure 3

Mapping of drinking fountains located at FESP.



Source: Adapted from Google Earth (2024).

4.3 SAMPLE COLLECTION PROCEDURE

Water samples from the drinking troughs were collected on October 20, 2023, during the dry season, in order to minimize the dilution effects typically associated with the rainy season. Sampling was carried out between 7:30 a.m. and 8:30 a.m. to avoid potential interference in the results caused by the higher ambient temperatures that occur later in the day.

During the sampling process, photographic records were taken, and the geographic coordinates of each drinking trough were documented using Google Earth (Figure 3). From each drinking trough, a 1,000 mL water sample was collected and transferred into autoclaved plastic bottles. As shown in Figure 4, the samples were identified according to the location of each drinking trough and labeled as B1, B2, B3, and B4.

Figure 4

Water troughs used by the animals at FESP.



Source: Prepared by the authors (2024).

4.4 SAMPLE ANALYSIS PROCEDURES

The water samples collected from each drinking trough were analyzed to evaluate both physicochemical and microbiological parameters. These analyses were essential for assessing the quality of the drinking water and identifying potential impacts on the health and welfare of the animals.

4.4.1 Physicochemical analyses

Immediately after sample collection, *in situ* measurements of water temperature, pH, and dissolved oxygen (DO) were performed directly at each drinking trough. All analytical instruments were properly calibrated prior to use. Furthermore, sample preservation procedures and analytical holding times followed the recommendations established by CONAMA Resolutions No. 357 and No. 396 (Brazil, 2005; Brazil, 2008), ensuring the reliability of the measured parameters.

Following the field analyses, samples were transported to the Chemistry Laboratory at the UFVJM, Unaí Campus, where additional analyses were performed using appropriate laboratory equipment. These included the determination of physicochemical parameters,

such as electrical conductivity (APHA, AWWA, WEF, 2017), and the assessment of water pollution levels through the methylene blue (MB) technique (Troppmair, 1988).

Water temperature was measured immediately after the collection of each individual sample. The thermometer remained immersed in the water until the reading stabilized; the measurement was taken with the thermometer bulb submerged to minimize errors caused by exposure to ambient air (Brazil, 2013).

The pH was determined using a digital pH meter to ensure analytical precision (APHA, AWWA, WEF, 2017). Prior to each measurement, the instrument was calibrated according to the manufacturer's instructions. The electrodes were rinsed with distilled water and gently dried using soft absorbent paper before being immersed in the sample. Once calibrated, the electrodes were inserted into the sample, allowing the instrument to provide accurate pH reading. This procedure ensured reliable measurements of the hydrogen ion concentration, contributing to the characterization of the water's chemical properties and their environmental implications.

Dissolved oxygen (DO) was measured using an Instrutherm MO-900 dissolved oxygen meter. After activation, the probe was immersed in the sample, and the device recorded the measurement for approximately 30 seconds (Brazil, 2013). Results were displayed in mg/L.

Electrical conductivity (EC) was determined using a digital conductivity meter. Prior to each measurement, the instrument was calibrated with a standard conductivity solution. The sensor was rinsed with distilled water and dried with soft absorbent paper before and after each use. Subsequently, the sensor was immersed in the sample contained in a beaker, and the value was displayed on the instrument, providing information on the ionic concentration and conductive properties of the water sample (APHA, AWWA, WEF, 2017).

4.4.2 Microbiological analyses

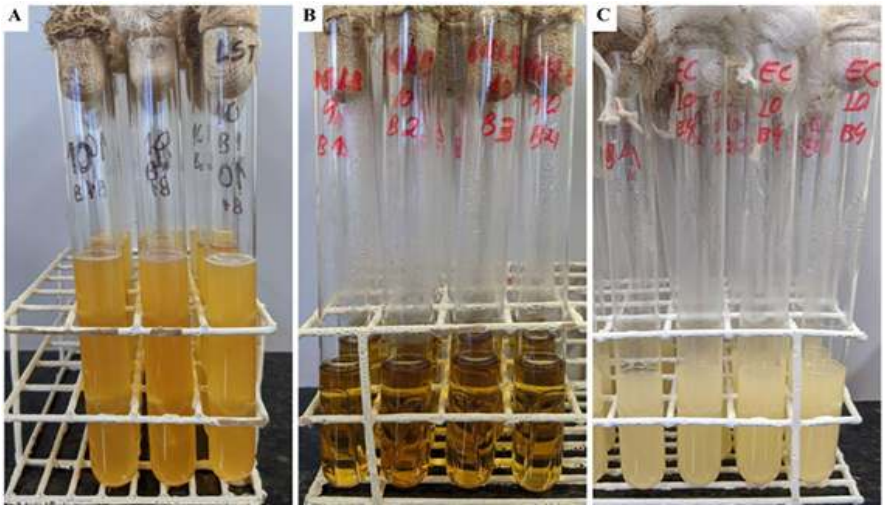
The microbiological quality of the water was evaluated using the multiple-tube fermentation technique, which estimates the bacterial density of coliforms by determining the Most Probable Number (MPN) (APHA, AWWA, WEF, 2017). Water samples were subjected to presumptive tests for the detection of coliform bacteria, followed by confirmatory tests to identify total coliforms, thermotolerant coliforms, and *Escherichia coli* (*E. coli*).

The methodology followed the procedures described in Brazil (2013) and standard international guidelines (APHA, AWWA, WEF, 2017), which recommend the multiple-tube technique for evaluating the microbiological quality of water intended for animal consumption.

Results were expressed as the Most Probable Number (MPN) per 100 mL of water. MPN values were determined using standard probability tables based on the combination of positive tubes obtained during the analysis (APHA, AWWA, WEF, 2017). For each sample, three tubes were inoculated for each of the following volumes: 10 mL, 1.0 mL, and 0.1 mL, as illustrated in Figure 5.

Figure 5

Detection of total coliforms, thermotolerant coliforms, and Escherichia coli.



*Detection of coliforms using LST culture medium (lactose broth) (A). Detection of total coliforms using BGLB culture medium (Brilliant Green Bile Lactose Broth) (B). Detection of thermotolerant coliforms using EC culture medium (*Escherichia coli*) (C).
Source: Prepared by the authors (2024).

According to the Companhia Ambiental do Estado de São Paulo (CETESB, 2018) and standard analytical guidelines (APHA, AWWA, WEF, 2017), the presumptive test for coliform detection (Figure 6A) consists of inoculating predetermined volumes of the water sample into a series of tubes containing Lauryl Sulfate Tryptose (LST) broth. The inoculated tubes are incubated at 35 ± 0.5°C for 24 - 48 hours to promote the growth of lactose-fermenting microorganisms. The production of gas and turbidity, resulting from lactose fermentation, is considered a presumptive positive result for the presence of coliform bacteria.

The confirmatory test for total coliforms (Figure 5B) is performed by transferring an aliquot from each presumptive positive LST tube into tubes containing Brilliant Green Lactose Bile (BGLB) broth, following established standard protocols (APHA, AWWA, WEF, 2017). These tubes are then incubated at 35 ± 0.5°C for 48 hours. Gas production in the BGLB medium confirms the presence of total coliform bacteria. This confirmation step reduces the likelihood of false-positive results, which may occur due to the activity of spore-forming

bacteria or Gram-positive lactose-fermenting microorganisms (CETESB, 2018).

According to the National Health Foundation (Brazil, 2013) and standard water examination procedures (APHA, AWWA, WEF, 2017), the detection of thermotolerant coliforms and *Escherichia coli* (*E. coli*) is carried out by transferring the inoculum from each positive LST tube into tubes containing EC broth, followed by incubation at 44.5 ± 0.2°C for 24 hours. The production of gas resulting from lactose fermentation in EC broth is interpreted as a positive result for thermotolerant coliforms (Figure 5C).

To identify *E. coli*, a flame-sterilized inoculating loop was inserted approximately 1 cm into each gas-positive EC tube. The inoculum was then streaked onto Eosin Methylene Blue (EMB) agar plates (Figure 6) using the streak plate method (APHA, AWWA, WEF, 2017). The plates were incubated at 35 ± 0.5°C for 24 hours. Following incubation, colonies exhibiting a characteristic metallic green sheen on the agar surface were identified as typical *E. coli*.

Figure 6

Preparation of materials for microbiological culture.



Source: Prepared by the authors (2024).

4.4.3 Methylene Blue Test

The Methylene Blue (MB) test is a qualitative method used to assess the level of organic pollution in water. According to Troppmair (1988), this technique is based on a reduction reaction, in which the decolorization of methylene blue serves as an indicator of the degree of water pollution associated with the presence of excess organic matter. This method complements conventional water quality analyses by providing additional information regarding the sanitary condition of water supplied to domestic animals.

As shown in Figure 7, a 50-mL aliquot of water from each drinking trough was

transferred into 50-mL plastic syringes, and 0.3 mL of a 1% (w/v) methylene blue solution was added to each sample. The syringes were sealed with plastic film to prevent oxygen ingress and minimize external interference. The samples were stored in the dark and monitored at 24-hour intervals over a 120-hour period to observe changes in color.

Figure 7

Use of the methylene blue technique on a water sample from the drinking fountains.



Source: Prepared by the authors (2024).

5 RESULTS AND DISCUSSION

As shown in Table 1, no substantial variation was observed in the water temperature among the drinking troughs. Water temperature remained well below the maximum value of 40°C, which complies with the standard established by CONAMA Resolution No. 357 (Brazil, 2005).

5.1 PHYSICOCHEMICAL PARAMETERS

As shown in Table 1, the results were obtained from physicochemical analyses performed on all samples. Drinking trough B3 exhibited the highest water temperature (27.0°C) compared with the other troughs. This result can be attributed to its Australian-style design and metal construction, since metal tends to absorb and retain more heat than materials such as concrete. In contrast, drinking trough B1, which is made of concrete, exhibited the lowest temperature (24.8°C).

Furthermore, drinking trough B2 is partially protected by a structure that reduces direct solar radiation on the water surface, thereby limiting temperature increases. According to Nogueira, Costa, and Pereira (2015), reduced solar exposure is one of the main factors contributing to lower water temperatures in aquatic environments. Among the four water samples analyzed (Table 1), the highest pH was recorded in B4 (8.76), whereas the lowest pH was observed in B3 (8.19), resulting in an mean pH of 8.52 across all drinking troughs.

These findings indicate that the pH values remained within the acceptable range established by CONAMA Resolution No. 357 (6.0–9.0), suggesting that the water was not affected by contamination from toxic substances (Brazil, 2005). Such contamination is more frequently associated with surface water sources, particularly rivers used for livestock watering. According to Portugal (2014), rivers present a greater risk of contamination because they are exposed to multiple anthropogenic activities along their course, including urbanization, industrial discharge, and agricultural practices, all of which may negatively affect water quality.

Table 1

Physicochemical parameters of water samples collected from FESP drinking fountains.

		Samples			
Parameters	**Expected values**	**B1**	**B2**	**B3**	**B4**
Temperature (°C)	< 40	24,80	25,50	27,00	26,70
pH	6,0 - 9,0	8,64	8,49	8,19	8,76
Dissolved Oxygen (mg/L)	> 6	21,50	21,70	14,20	22,20
Conductivity (µS/cm)	< 100	86,11	86,25	89,19	85,21

Source: Prepared by the authors (2024).

The highest dissolved oxygen (DO) concentration was recorded in sample B4 (22.2 mg/L), whereas the lowest was observed in B3 (14.2 mg/L). However, these concentrations are unusually high for freshwater at the measured temperatures, suggesting substantial oxygen supersaturation. Therefore, the reliability of these measurements should be verified by examining the instrument calibration, measurement procedures, and environmental conditions during sampling. Although the presence of aquatic vegetation and the refilling of the trough may have influenced DO concentrations, the available data are insufficient to establish these factors as the causes of the observed differences (Von Sperling, 2014).

The highest electrical conductivity (EC) values were observed in B2 (86.25 µS/cm) and B3 (89.19 µS/cm) (Table 1). A large accumulation of aquatic vegetation and organic sediments (biosolids) was observed at the bottom of B3. Similar findings were reported by

Souza Junior and Oliveira (2017), who suggested that increased electrical conductivity may be associated with the decomposition of submerged vegetation and the presence of organic residues, both of which increase the concentration of dissolved ions in the water.

As shown in Table 1, no substantial variation was observed in the water temperature among the drinking troughs. All recorded temperatures remained below 40°C, the maximum limit established by CONAMA Resolution No. 357 (Brazil, 2005). Drinking trough B3 exhibited a slightly higher temperature (27.0°C) than the other troughs because it is an Australian-style metal trough. Metal has a greater capacity to absorb and retain heat than concrete, resulting in higher water temperatures. In contrast, drinking trough B1, which is constructed of concrete, recorded the lowest temperature (24.8°C).

In addition, drinking trough B2 is partially shaded by a surrounding structure that reduces the incidence of direct sunlight on the water surface, thereby limiting water heating. According to Nogueira, Costa, and Pereira (2015), reduced solar radiation is one of the main factors contributing to lower water temperatures in aquatic environments. Among the four water samples analyzed, the highest pH was recorded in B4 (8.76), whereas the lowest pH was observed in B3 (8.19), with an overall mean pH of 8.52 across all drinking troughs.

These results indicate that the pH values of all sampled drinking troughs remained within the acceptable range (6.0–9.0) established by CONAMA Resolution No. 357 (Brazil, 2005), suggesting the absence of contamination by toxic substances. Such contamination is more commonly associated with surface water sources, particularly rivers used for livestock watering. According to Portugal (2014), rivers present a greater risk of contamination because they are influenced by a wide range of anthropogenic activities along their course, including urban development, industrial discharges, and agricultural practices, all of which may adversely affect water quality.

The highest dissolved oxygen (DO) concentration was recorded in drinking trough B4 (22.2 mg/L), whereas the lowest value was observed in B3 (14.2 mg/L) (Table 1). The comparatively lower DO concentration in B3 may be attributed to the fact that the trough was being refilled during sample collection, which likely interfered with the measurement. Furthermore, a significant amount of aquatic vegetation was observed within the trough. The metabolic activity and decomposition of these plants increase oxygen consumption, thereby reducing the dissolved oxygen concentration in the water (Von Sperling, 2014). These factors likely contributed to the lower DO level observed in this trough.

The highest electrical conductivity (EC) values were recorded in B2 (86.25 µS/cm) and B3 (89.19 µS/cm) (Table 1). A substantial accumulation of aquatic vegetation and biosolids was observed at the bottom of B3. Similar findings were reported by Souza Junior and Oliveira (2017), who suggested that elevated electrical conductivity may be associated with the decomposition of submerged vegetation and the presence of organic residues, both of which increase the concentration of dissolved ions in the water.

5.2 MICROBIOLOGICAL PARAMETERS

The microbiological analysis demonstrated that 100% of the water samples collected from the FESP drinking troughs tested positive for total coliforms. Although these troughs are cleaned weekly, it was observed that the cleaning process is inadequate. Furthermore, there is no water chlorination system in place, which would be highly effective for ensuring proper sanitization.

As shown in Figure 4, a significant amount of organic matter was observed inside drinking troughs B2 and B4. According to Palhares (2014) the presence of organic material, along with algae development, mineral deposition, and debris, creates a favorable environment for microorganisms to thrive and remain viable. Table 2 presents the results of the microbiological analyses of the water samples intended for animal consumption, collected from the FESP drinking troughs.

Table 2

Most probable number (MPN) of total and thermotolerant coliforms in samples of water from the drinking fountains at FESP.

Sampling sites	Presumptive test	MPN Method			Combinations	MPN per 100 mL
		3 tubes of 10 mL	3 tubes of 1 mL	3 tubes of 0,1 mL		
B1	Total coliforms	3	3	3	3-3-3	>1100
	Thermotolerant coliforms	3	3	2	3-3-2	1100
B2	Total coliforms	3	3	2	3-3-2	1100
	Thermotolerant coliforms	2	0	1	2-0-1	14
B3	Total coliforms	3	3	0	3-3-0	240
	Thermotolerant coliforms	3	2	1	3-2-1	150
B4	Total coliforms	3	3	3	3-3-3	>1100
	Thermotolerant coliforms	3	3	1	3-3-1	460

Source: Prepared by the author, 2024.

The CONAMA Resolution No. 396 (Brazil, 2008) was adopted as the reference standard for assessing microbiological contamination. As shown in Table 2, all drinking troughs were positive for total coliforms, with values exceeding the maximum acceptable limit of 200 Most Probable Number (MPN)/100 mL established by the regulation. In contrast, regarding thermotolerant coliforms and *Escherichia coli* indicators of fecal pollution (Von Sperling, 2014), 50% of the water samples failed to comply with the limits established by the current legislation. Only drinking troughs B2 and B3 showed low concentrations of thermotolerant coliforms, while B1 and B4 exceeded the recommended values, indicating fecal contamination of the water.

Table 3

Analysis of Escherichia coli in water samples from FESP drinking fountains.

COLLECTION POINTS	POSITIVE COMBINATIONS	MPN	*E. COLI*
B1	3-3-2	1100	Present
B2	2-0-0	9	Present
B3	3-3-1	460	Present
B4	3-2-3	210	Present

Source: Prepared by the author, 2024.

As observed in Table 3, the presence of *E. coli* (Figure 8) was confirmed in all analyzed samples (100%), which included drinking fountains B1, B2, B3, and B4. Ineffective maintenance of drinking fountains, alongside the lack of water chlorination - primary drivers for the proliferation of such pathogens in water systems (Von Sperling, 2014) - strongly suggests these factors as the main causes for the appearance of the aforementioned microorganisms.

Figure 8

Petri dish with the presence of Escherichia coli.



Source: Prepared by the authors (2024).

5.3 METHYLENE BLUE EVALUATION

The AM technique acts as a reducing agent for organic matter in water bodies. allowing the water quality to be assessed through a color indicator that varies from blue dark to transparent. As demonstrated in Figure 9, the realization of a standard sample (AP) is necessary so that it serves as a control and comparison for the others. This is composed only of distilled water and AM, in order to obtain a standard for coloring the A sample of clean water with no interaction with organic matter (Troppmair, 1988).

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Figure 9

Standard sample with distilled water.



Source: Prepared by the author, 2024.

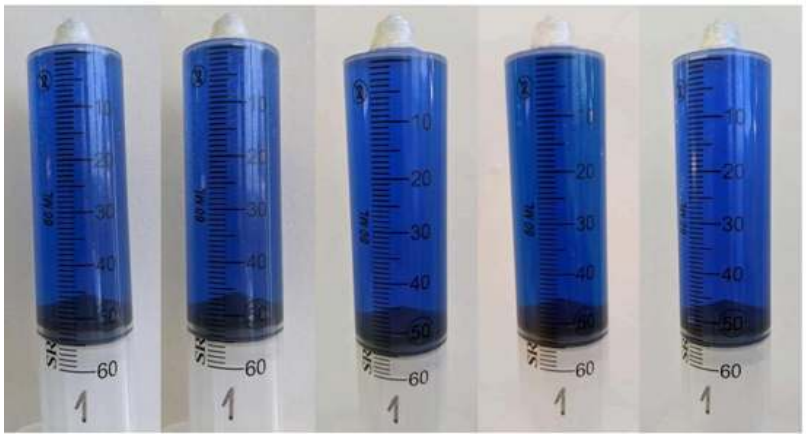
After the addition of methylene blue (MB), the water samples were monitored for 120 hours to evaluate possible changes in the characteristic blue coloration of the dye. At the end of the observation period, the sample collected from drinking trough B1 showed no visible change in color, as illustrated in Figure 10. This result indicates that the methylene blue remained stable and did not undergo anaerobic reduction, suggesting the absence of significant amounts of biodegradable organic matter in the water. Under the conditions of this study, the stability of the dye indicates that the sample was not subjected to anaerobic degradation processes.

Furthermore, drinking trough B1 showed no excessive accumulation of debris or organic material, which was corroborated by the MB test presented in Figure 10, indicating the absence of detectable organic pollution. According to Andrade et al. (2024a, 2024b), water quality assessments should consider not only the quantitative results obtained from laboratory analyses but also factors such as the strategic selection of sampling sites, pollution characteristics, environmental diversity, potential sources of contamination, sampling time, and prevailing climatic conditions, all of which may influence water quality.

Based on the methylene blue color scale proposed by Monteiro and Viadana (2009), the water from drinking trough B1 was classified as clean water.

Figure 10

Sample from the first drinking fountain.

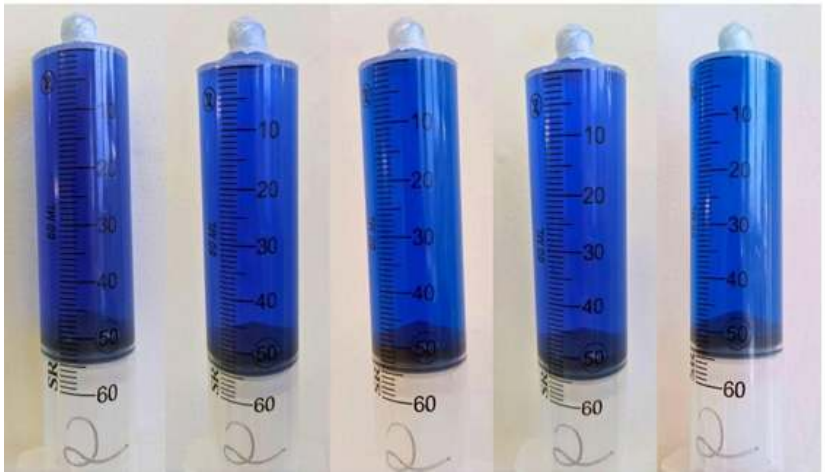


Source: Prepared by the author, 2024.

This finding is supported by Figure 11, where a lighter blue coloration in the methylene blue (MB) test indicates a moderate amount of biodegradable organic matter. Additionally, visual inspection revealed considerable debris and organic accumulation in drinking trough B2, classifying it as having moderate organic pollution based on the methylene blue scale, corroborating findings by Andrade et al. (2024a).

Figure 11

Sample from the second drinking fountain.



Source: Prepared by the author, 2024.

After the 120-hour observation period, the water sample from drinking trough B3 (Figure 12) retained the same blue coloration as the reference sample (AP), indicating the absence of organic pollution. This result is likely explained by the fact that B3 had been cleaned immediately before sample collection, resulting in a low concentration of organic

matter within the trough. Based on the methylene blue color scale, the water from drinking trough B3 was therefore classified as clean water, corroborating findings from previous studies on river water pollution analysis using methylene blue conducted by Andrade et al. (2024a).

It is important to emphasize that, according to Portugal (2014), livestock should have access not only to high-quality drinking water but also to water that is continuously available and located close to feeding areas. Therefore, regular monitoring and routine cleaning of drinking troughs are essential management practices to maintain water quality and promote the health and welfare of domestic animals.

Figure 12

Sample from the third drinking fountain.



Source: Prepared by the author, 2024.

As shown in Figure 13, at the end of the 120-hour observation period, the water sample from drinking trough B4 exhibited a slight loss of its original blue coloration, indicating the presence of a low concentration of organic matter. Because the color change was minimal, the sample was classified, according to the methylene blue pollution index proposed by Monteiro and Viadana (2009), as having a pollution level intermediate between clean water and low organic pollution (Figure 4).

Figure 13

Sample from the fourth drinking fountain.



Source: Prepared by the author, 2024.

6 FINAL CONSIDERATIONS

The physicochemical parameters evaluated in the drinking water demonstrated that water quality complied with the standards established by CONAMA Resolutions No. 357 and No. 396 (Brazil, 2005; Brazil, 2008). Therefore, these regulatory criteria can be adopted as reference standards when assessing the quality of water supplied to domestic animals.

The methylene blue (MB) test proved to be an applicable qualitative method for evaluating the quality of water supplied in animal drinking troughs, indicating evidence of organic contamination in drinking troughs B2 and B4. Likewise, the microbiological analyses confirmed the presence of total coliforms in 100% of the drinking troughs. Thermotolerant coliforms were detected in B1 and B4, whereas *Escherichia coli* (*E. coli*) was identified in B1, B3, and B4.

The analyses of total coliforms, thermotolerant coliforms, and *E. coli* enabled the identification of deficiencies in water quality and provided valuable information for implementing corrective measures aimed at maintaining a healthy environment for domestic animals. The findings of this study contribute to the development of appropriate water management strategies, including preventive and corrective actions to reduce microbiological contamination. Furthermore, the adoption of an integrated and continuous water quality monitoring program is essential for promoting animal health, welfare, and the sanitary safety of livestock production systems.

REFERENCES

AL-ABOODI, Ali H.; ABBAS, Sarmad A.; IBRAHIM, Husham T. Effect of Hartha and Najibia power plants on water quality indices of Shatt Al-Arab River, south of Iraq. *Applied Water Science*, v. 8, art. 64, 2018. DOI: 10.1007/s13201-018-0703-0.

ALMEIDA, Ananda Gomes et al. Análise microbiológica e físico-química da água de bebedouros em unidades de ensino no município de Ilhéus-BA. *SaBios: Revista de Saúde e Biologia*, v. 12, n. 2, p. 20–26, 2018.

AMERICAN PUBLIC HEALTH ASSOCIATION - APHA; AMERICAN WATER WORKS ASSOCIATION - AWWA; WATER ENVIRONMENT FEDERATION - WEF. **Standard Methods for the Examination of Water and Wastewater**. 23. ed. Washington, D.C.: APHA Press, 2017.

ANDRADE, Camila Silveira et al. Aplicação do azul de metileno na monitorização da poluição hídrica no Rio Preto em contraste com métodos convencionais. **In**: VIEIRA, Ariadna Faria; SILVA, Leonardo França da; JESUS, Fernanda Lamede Ferreira de (Org.). **O futuro das ciências agrárias: inovações e desafios 4**. Ponta Grossa: Atena Editora, 2024a. p. 1-10. Disponível em: https://doi.org/10.22533/at.ed.7282413111. Acesso em: 7 fev. 2026.

ANDRADE, Camila Silveira et al. Análise físico-química da qualidade da água utilizada na Fazenda Experimental Santa Paula (FESP) da UFVJM - Campus de Unaí. **In**: PEREIRA, Alécio Matos; LÔBO, Dhara Gabriella Silva; OLIVEIRA, Nayanne da Silva (Org.). **Bem-estar animal: práticas veterinárias, abordagens e cuidados 3**. Ponta Grossa: Atena Editora, 2024b. p. 38-43. Disponível em: https://doi.org/10.22533/at.ed.0672407115. Acesso em: 12 mar. 2026.

BACCI, Denise de La Corte; PATACA, Ermelinda Moutinho. Educação para a água. *Estudos Avançados*, São Paulo, v. 22, n. 63, p. 211–226, 2008. DOI: 10.1590/S0103-40142008000200014.

BRAGA, Benedito et al. *Introdução à engenharia ambiental: o desafio do desenvolvimento sustentável*. 2. ed. São Paulo: Pearson Prentice Hall, 2005.

BRASIL. Conselho Nacional do Meio Ambiente. Resolução CONAMA nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. *Diário Oficial da União*: Brasília, DF, 18 mar. 2005.

BRASIL. Conselho Nacional do Meio Ambiente. Resolução CONAMA nº 396, de 3 de abril de 2008. Dispõe sobre a classificação e diretrizes ambientais para o enquadramento das águas subterrâneas e dá outras providências. *Diário Oficial da União*: Brasília, DF, n. 66, p. 64–68, 7 abr. 2008.

BRASIL. Fundação Nacional de Saúde. **Manual prático de análise de água**. 4. ed. Brasília, DF: Funasa, 2013. 150 p.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância em Saúde Ambiental e Saúde do Trabalhador. *Qualidade da água para consumo humano: cartilha para promoção e proteção da saúde*. Brasília, DF: Ministério da Saúde, 2018. 51 p.

BRASIL. Ministério da Saúde. Portaria GM/MS nº 888, de 4 de maio de 2021. Altera o Anexo XX da Portaria de Consolidação GM/MS nº 5, de 28 de setembro de 2017, para dispor sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade. *Diário Oficial da União*: seção 1, Brasília, DF, n. 85, p. 112-144, 7 maio 2021.

COMPANHIA AMBIENTAL DO ESTADO DE SÃO PAULO. *L5.202: coliformes totais, coliformes termotolerantes e Escherichia coli: determinação pela técnica de tubos múltiplos*. 5. ed. São Paulo: CETESB, 2018. 29 p.

COSTA, Joice Vinhal. *Perfis de ERIC-PCR de Escherichia coli e E. coli O157:H7 em meias-carcaças bovinas*. 2008. 91 f. Dissertação (Mestrado em Ciência Animal) – Universidade Federal de Goiás, Goiânia, 2008.

GOOGLE. *Google Earth*. [S. l.]: Google, 2024. Available at: https://earth.google.com/. Accessed on: 7 Apr. 2026.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. *Perfil dos municípios brasileiros: saneamento básico: aspectos gerais da gestão da política de saneamento básico 2017*. Rio de Janeiro: IBGE, 2018.

LIBÂNIO, Marcelo**. Fundamentos de qualidade e tratamento de água**. 3. ed. rev. e ampl. Campinas: Átomo, 2010.

MONTEIRO, Adriano Bortolin; VIADANA, Adler Guilherme. Análise de poluição da água: a técnica do azul de metileno. In: **SIMPÓSIO BRASILEIRO DE RECURSOS HÍDRICOS**, 18., 2009, Campo Grande. *Anais [...]*. Campo Grande: ABRH, 2009.

NOGUEIRA, Fábio Fernandes; COSTA, Isabella Almeida; PEREIRA, Uendel Alves. **Análise de parâmetros físico-químicos da água e do uso e ocupação do solo na sub-bacia do Córrego da Água Branca no município de Nerópolis – Goiás**. 2015. Trabalho de Conclusão de Curso (Engenharia Ambiental e Sanitária) – Universidade Federal de Goiás, Goiânia, 2015.

PALHARES, Julio Cesar Pascale. *Qualidade da água na produção animal*. São Carlos, SP: Embrapa Pecuária Sudeste, 2014. 6 p. (Comunicado Técnico, 103).

PORTUGAL. Direção-Geral de Alimentação e Veterinária. *Água de qualidade adequada na alimentação animal*. Rev. 2. Lisboa: DGAV, 2014.

SILVA, Aldeni Barbosa da *et al.* Análise microbiológica da água de bebedouros nas escolas públicas da cidade de Esperança/PB. **South American Journal of Basic Education, Technical and Technological**, Rio Branco, v. 6, n. 1, p. 15–26, 2019.

SILVA, Neusely da; JUNQUEIRA, Valéria Christina Amstalden; SILVEIRA, Neliane Ferraz de Arruda; TANIWAKI, Marta Hiromi; GOMES, Renato Abeilar Romeiro; OKAZAKI, Margarete Midori; IAMANAKA, Beatriz Thie. *Manual de métodos de análise microbiológica de alimentos e água*. 6. ed. São Paulo: Blucher, 2021. 602 p.

SOUZA JUNIOR, Ednilson Gomes; OLIVEIRA, Vicente de Paulo Santos de. Qualidade da água do rio Itabapoana: análise dos parâmetros físico-químicos e microbiológicos e influência de empreendimentos hidrelétricos. **Boletim do Observatório Ambiental Alberto Ribeiro Lamego**, Campos dos Goytacazes, v. 11, n. 1, p. 29–41, 2017. DOI: 10.19180/2177-4560.v11n12017p29-41.

TROPPMAIR, Helmut. **Metodologias simples para pesquisar o meio ambiente.** Rio Claro: EMBRAPA-CNPDA, 1988. 232 p.

VON SPERLING, Marcos. **Introdução à qualidade das águas e ao tratamento de esgotos.** 4. ed. Belo Horizonte: Editora UFMG, 2014. 470 p. (Princípios do tratamento biológico de águas residuárias, v. 1).

WORLD HEALTH ORGANIZATION - WHO. **Guidelines for drinking-water quality**. 4. ed. Geneva: World Health Organization, 2011.

WWF-BRASIL. **Cadernos de educação ambiental Água para Vida, Água para Todos: livro das águas**. Texto de Andrée de Ridder Vieira; coordenação de Larissa Costa e Samuel Roiphe Barrêto. Brasília, DF: WWF-Brasil, 2006. 72 p.