ReGeo ISSN: 2177-3246

EVALUATION OF THE WATER QUALITY OF THE RIO PRETO (UNAÍ-MG) USING PHYSICOCHEMICAL PARAMETERS AND THE METHYLENE BLUE TEST

AVALIAÇÃO DA QUALIDADE DA ÁGUA DO RIO PRETO (UNAÍ-MG) POR MEIO DE PARÂMETROS FÍSICO-QUÍMICOS E DO TESTE DO AZUL DE METILENO

EVALUACIÓN DE LA CALIDAD DEL AGUA DEL RIO PRETO (UNAÍ-MG) MEDIANTE PARÁMETROS FISICOQUÍMICOS Y LA PRUEBA DE AZUL DE METILENO

https://doi.org/10.56238/revgeov17n11-012

Data de submissão: 03/10/2026 **Data de publicação:** 03/11/2026

Caroline Moura Soares Santos[1], Camila Martins Rodrigues[2], Paulo César Melquíades Santos[3], Mírian da Silva Costa Pereira[4]

ABSTRACT

The availability of drinking water and the preservation of its quality in the face of anthropogenic activities represent major contemporary challenges. The degradation of water bodies, accelerated by urbanization, agricultural expansion, and inadequate wastewater disposal, generates serious impacts on human health and aquatic ecosystems. This reality prompted the inclusion of Sustainable Development Goal (SDG) No. 6 in the UN 2030 Agenda, which seeks to ensure universal access to clean water and sanitation. In the Brazilian scenario, the National Water and Basic Sanitation Agency (ANA) coordinates the National Water Resources Policy to ensure rational use and combat pollution. The traditional assessment of this quality involves the analysis of essential physicochemical parameters, such as pH, dissolved oxygen, temperature, and electrical conductivity. However, the detailed characterization of organic matter demands complementary methods. In this sense, the methylene blue (MB) technique emerges as an innovative, simple, fast, and low-cost tool. Based on the decolorization of the dye by the activity of anaerobic decomposing microorganisms, it acts as an indirect indicator of organic pollutants and has a strong correlation with classic parameters such as BOD and TOC, proving to be ideal for preventive and field ecological monitoring

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

[1] Veterinary Medicine student. Institution: Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). E-mail: caroline.moura@ufvjm.edu.br Orcid: https://orcid.org/0009-0007-5336-7809 Lattes: http://lattes.cnpq.br/7154360091658474

[2] Veterinary Medicine student. Institution: Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). E-mail: camila.rodrigues@ufvjm.edu.br Orcid: https://orcid.org/0009-0008-7704-9191 Lattes: http://lattes.cnpq.br/4490881856125560

[3] Master in Electrochemistry/Electroanalytical Chemistry. Institution: Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). E-mail: paulo.melquiades@ufvjm.edu.br Orcid: https://orcid.org/0009-0007-9295-3755 Lattes: http://lattes.cnpq.br/3070244662665034

[4] PhD in Physical Chemistry. Institution: Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). E-mail: mirian.pereira@ufvjm.edu.br Orcid: https://orcid.org/0000-0002-3083-1505 Lattes: http://lattes.cnpq.br/1328127213991175

RESUMO
A disponibilidade de água potável e a preservação de sua qualidade diante das atividades antropogênicas representam grandes desafios contemporâneos. A degradação dos corpos d'água, acelerada pela urbanização, expansão agrícola e descarte inadequado de efluentes, gera sérios impactos na saúde humana e nos ecossistemas aquáticos. Essa realidade motivou a inclusão do Objetivo de Desenvolvimento Sustentável (ODS) nº 6 na Agenda 2030 da ONU, que busca assegurar o acesso universal à água potável e ao saneamento básico. No contexto brasileiro, a Agência Nacional de Águas e Saneamento Básico (ANA) coordena a Política Nacional de Recursos Hídricos para garantir o uso racional da água e combater a poluição. A avaliação tradicional dessa qualidade envolve a análise de parâmetros físico-químicos essenciais, como pH, oxigênio dissolvido, temperatura e condutividade elétrica. Contudo, a caracterização detalhada da matéria orgânica demanda métodos complementares. Nesse sentido, a técnica do azul de metileno (AM) surge como uma ferramenta inovadora, simples, rápida e de baixo custo. Baseado na descoloração do corante pela atividade de microrganismos anaeróbios decompositores, ele atua como um indicador indireto de poluentes orgânicos e apresenta forte correlação com parâmetros clássicos como DBO e COT, demonstrando ser ideal para monitoramento ecológico preventivo e de campo.

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

RESUMEN
La disponibilidad de agua potable y la preservación de su calidad frente a las actividades antrópicas representan grandes desafíos contemporáneos. La degradación de cuerpos de agua, acelerada por la urbanización, expansión agrícola y disposición inadecuada de aguas residuales, genera serios impactos en la salud humana y los ecosistemas acuáticos. Esta realidad motivó la inclusión del Objetivo de Desarrollo Sostenible (ODS) Nº 6 en la Agenda 2030 de las Naciones Unidas, que busca garantizar el acceso universal al agua potable y al saneamiento básico. En el escenario brasileño, la Agencia Nacional de Aguas y Saneamiento Básico (ANA) coordina la Política Nacional de Recursos Hídricos para asegurar el uso racional y el combate a la contaminación. La evaluación tradicional de esta calidad implica el análisis de parámetros físico-químicos esenciales, como pH, oxígeno disuelto, temperatura y conductividad eléctrica. Sin embargo, la caracterización detallada de la materia orgánica exige métodos complementarios. En este sentido, la técnica del azul de metileno (AM) surge como una herramienta innovadora, simple, rápida y de bajo costo. Basada en la decoloración del tinte por la actividad de microorganismos anaeróbicos descomponedores, actúa como indicador indirecto de contaminantes orgánicos y tiene una fuerte correlación con parámetros clásicos como DBO y COT, demostrando ser ideal para monitoreo ecológico preventivo y de campo

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

1 INTRODUCTION

The availability of water in adequate quantity and quality is indispensable for the survival of living beings and for the balanced functioning of ecosystems. In addition to playing a fundamental role in biological processes, this resource participates in economic, productive, and sanitary activities essential to society. In the human body, water represents a major part of body composition, acting in functions such as substance transport, temperature regulation, and the maintenance of homeostasis (Tundski, 2003).

The growing concern over the conservation of water resources has led international organizations to establish goals aimed at sustainable water management. In this context, the United Nations (UN) incorporated Sustainable Development Goal 6 (SDG 6) into the 2030 Agenda for Sustainable Development, which seeks to promote universal access to drinking water and basic sanitation, as well as encourage the responsible use of water resources (ONU, 2015).

In Brazil, water quality monitoring is carried out by the National Water and Basic Sanitation Agency (ANA), which coordinates actions destined for assessing the conditions of water resources in different regions of the country. Among the strategies used, the National Water Quality Monitoring Network (RNQA) stands out, which is responsible for obtaining standardized data that allows tracking the conditions of water bodies and supporting environmental management actions (Brazil, 2022).

To synthesize the information obtained during monitoring, the Water Quality Index (WQI) is used, a tool widely employed in environmental assessment. This index integrates different physicochemical and microbiological variables, such as temperature, pH, turbidity, dissolved oxygen, biochemical oxygen demand, nutrients, and thermotolerant coliforms, providing an overview of the quality of the analyzed water (CETESB, 2020).

According to the World Health Organization (WHO), water quality surveillance must be based on preventive measures and the identification of risk factors capable of compromising public health. The ingestion of contaminated water or contact with inadequate water sources can favor the transmission of various diseases, mainly in locations with insufficient sanitary infrastructure and unfavorable socioeconomic conditions (WHO, 2011).

The characterization of water quality depends on the determination of different physicochemical parameters. Among the most widely used are the hydrogen potential (pH), temperature, electrical conductivity, and dissolved oxygen concentration. Additionally, complementary tests can be employed to estimate the presence of organic matter, such as

the Methylene Blue assay, whose decolorization is associated with anaerobic microbiological activity and the degradation of organic compounds present in the sample (Von Sperling, 2014).

Among the analyzed indicators, dissolved oxygen is considered one of the most relevant for environmental assessment, since its concentration directly reflects the ecological conditions of the aquatic environment. Changes in its levels can indicate organic pollution processes and the impairment of aquatic life. Furthermore, factors such as temperature, atmospheric pressure, and salinity exert significant influence on its availability in water (Esteves, 2011).

Another widely used parameter is pH, which expresses the chemical characteristics of the medium and influences biological and microbiological processes. Values outside the recommended range can affect water potability, in addition to favoring corrosion or incrustation processes in distribution systems. In compliance with Ordinance GM/MS No. 888/2021, water intended for human consumption must present a pH between 6.0 and 9.0, also meeting the other quality standards established by current legislation to guarantee the sanitary safety of the population (Brazil, 2021).

2 MATERIALS AND METHODS

Water quality assessment was performed using the Methylene Blue (MB) reduction methodology described by Troppmair (1988), which is widely used as an indirect method to estimate organic matter concentration in aquatic environments. The principle of the method is based on the metabolic activity of anaerobic microorganisms during the decomposition of organic matter. In this process, the reduction of methylene blue occurs, promoting the loss of its characteristic blue coloration and indicating the presence of degradable organic compounds (Troppmair, 1988; Santos *et al.*, 2016).

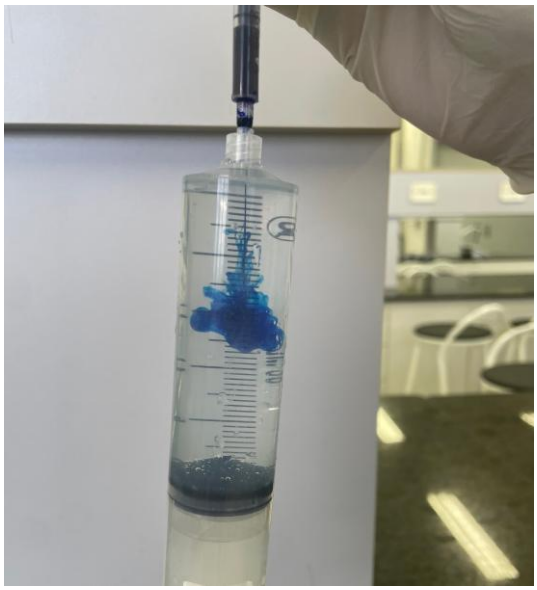
The study was conducted at three distinct sites along the Preto River, located in the urban area of the municipality of Unaí, Minas Gerais. Water samples were collected on September 16, 2025, between 11:00 a.m. and 1:00 p.m., using 1000 mL sterilized containers to avoid contamination that could interfere with the obtained results.

Laboratory analyses were carried out at the Chemistry Laboratory of the Institute of Agricultural Sciences of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), Unaí Campus. Initially, a 1% methylene blue solution was prepared by dissolving 1 g of the dye in 100 mL of distilled water. Subsequently, 50 mL of each sample was

transferred to 60 mL graduated syringes, and 0.3 mL of the previously prepared methylene blue solution was added. The syringes were kept in an environment protected from light incidence, since light exposure can influence redox reactions and compromise the interpretation of the results (Troppmair, 1988).

Figure 1

Application of the methylene blue technique to a sample



Source: Prepared by the authors, 2025.

Concurrently, physicochemical analyses of the samples were performed in triplicate to increase the reliability of the obtained data. The determinations occurred immediately after collection, considering that prolonged storage can cause alterations in sensitive parameters, especially in the dissolved oxygen concentration (Von Sperling, 2014).

To obtain the physicochemical parameters, an ASKO multiparameter meter, model AK88, was used to measure the hydrogen potential (pH), dissolved oxygen (DO), water temperature (°C), and electrical conductivity (µS/cm). During transport to the laboratory, the samples remained stored in properly sealed containers to minimize physical, chemical, and biological alterations and ensure the preservation of their original characteristics until the analyses were performed (APHA, 2017).

2.1 CHARACTERIZATION OF THE STUDY AREA AND SELECTION OF SAMPLING POINTS

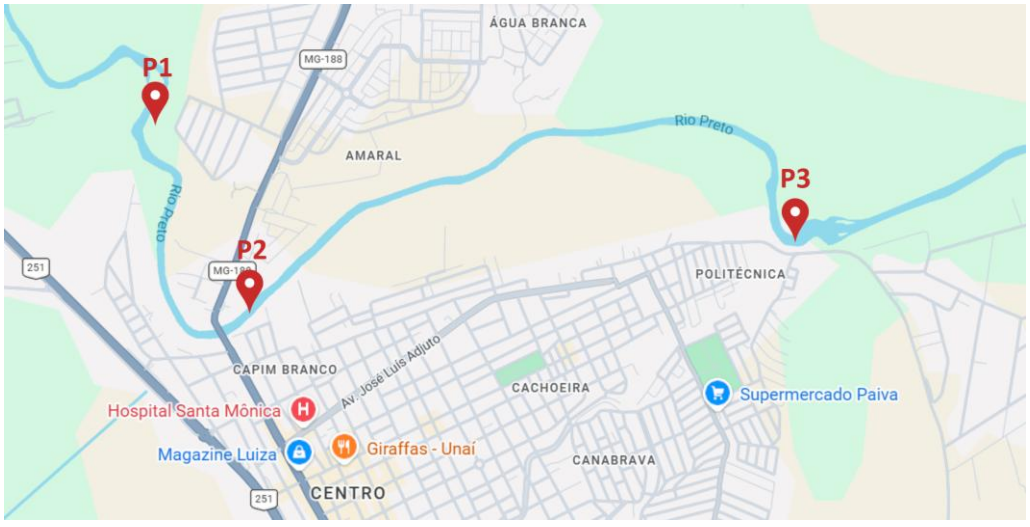
Aiming to perform a spatial analysis of water quality along the urban stretch of the Preto River, in the municipality of Unaí, Minas Gerais, three strategic sampling sites were defined and distributed longitudinally along the watercourse. This approach allowed for the assessment of potential alterations in water quality associated with the gradual accumulation of anthropogenic impacts resulting from urban occupation, wastewater discharge, and the different forms of land use and occupation across the studied area (Chapman, 1996; Von Sperling, 2014).

The sampling sites were selected to represent different environmental conditions and levels of influence from human activities in the river's surroundings. The sampled sites were:

- **Site 1 (P1):** near MTL Construtora, located at geographical coordinates 16°19'46.38" S and 46°54'57.61" W (-16.3295506; -46.9160028)
- **Site 2 (P2):** near Precol Aquapark, situated on José do Patrocínio Street, Capim Branco neighborhood, at coordinates 16°21'12.80" S and 46°54'24.66" W (-16.3535546; -46.9068504)
- **Site 3 (P3):** near SAAE Square, on Olarias Street, located at coordinates 16°20'58.52" S and 46°52'52.80" W (-16.3495902; -46.8813322)

Figure 2

Locations where samples were collected in the Rio Preto



Source: Google Maps, 2025.

The definition of the sampling sites considered criteria related to the environmental representativeness of the urban area, including the presence of residential zones, commercial enterprises, and proximity to basic sanitation infrastructure. In this manner, we sought to identify potential variations in water quality parameters as a function of the intensity of human occupation and the activities developed in the areas adjacent to the river.

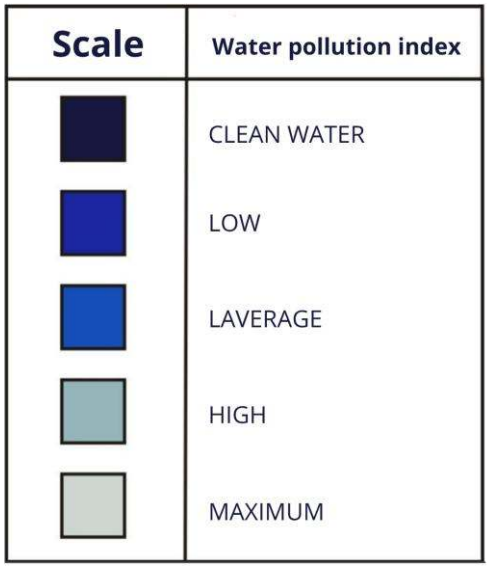
The use of sampling sites distributed along the watercourse constitutes a widely employed strategy in environmental monitoring studies, as it allows for the identification of potential pollution sources, understanding the dispersion dynamics of contaminants, and evaluating the natural self-purification capacity of aquatic ecosystems (Chapman, 1996; Von Sperling, 2014).

3 RESULTS AND DISCUSSION

The application of the Methylene Blue (MB) reduction technique allowed for an indirect evaluation of the presence of biodegradable organic matter in the samples collected along the urban stretch of the Preto River. The method is based on the ability of anaerobic microorganisms to utilize electrons derived from the degradation of organic matter, promoting the reduction of the dye and its subsequent loss of color. In this manner, the rate of decolorization is directly related to microbiological activity and the availability of degradable organic matter. Figure 1 presents the color scale used as a reference for interpreting the results (Troppmair, 1988; Andrade *et al.*, 2024a).

Figure 3

Color scale of Methylene Blue (MB) in water samples



Source: Monteiro; Viadanna, 2009.

The methylene blue (MB) solution was added to the water samples collected at three distinct sites along the Preto River, with the analyses being performed on the subsequent days 2, 3, 4, and 5, as illustrated in Figure 2. As a control, a standard syringe (DW) containing MB dissolved in distilled water was used to ensure the uniformity of the initial coloration. All samples were analyzed in duplicate, with observations made every 24 hours, always at 3:00 p.m., totaling five consecutive days of monitoring.

After adding the methylene blue solution to the samples collected at the three monitoring sites, daily observations were conducted for five consecutive days. Throughout the experimental period, no perceptible changes in the intensity of the blue coloration were observed when compared to the control containing distilled water. The maintenance of the original coloration suggests reduced activity of anaerobic bacteria and a low concentration of biodegradable organic matter in the analyzed environment, indicating favorable water quality conditions (ANDRADE *et al.*, 2024a).

The results obtained through the methylene blue assay corroborate the physicochemical data observed in the samples. The analyses were performed in triplicate, and the mean values obtained for pH, dissolved oxygen, temperature, and electrical conductivity remained within the limits established by Ordinance GM/MS No. 888/2021 and CONAMA Resolution No. 357/2005 for freshwater bodies classified as Class 2 (Brasil, 2021; Brazil, 2005).

Figure 4

Visual appearance of the Rio Preto River water samples subjected to the methylene blue (MB) assay: (a) day 1; (b) day 2; (c) day 4; and (d) day 5



Source: Prepared by the authors, 2025.

The spatial assessment of the three sampling sites revealed that, even in areas subject to the influence of urban activities, no significant changes in water quality were identified. The first sampling site, located in an urban-peri-urban transition region, presented characteristics compatible with low anthropogenic interference. The second site, situated in an urban area influenced by stormwater runoff, also showed no signs of significant impairment. The third site, located further downstream and potentially subject to the accumulation of impacts from the urban area, maintained parameters similar to those observed at the other locations, demonstrating the stability of environmental conditions along the studied stretch. The

physicochemical parameters obtained at three distinct sites (P1, P2, and P3) along the Preto River during Phase III are presented in Table 1.

The hydrogen potential (pH) values remained close to neutrality, ranging between 6.52 and 7.12. These results are within the range considered suitable for water intended for supply after conventional treatment, in addition to favoring the maintenance of natural biological and chemical processes in aquatic ecosystems (Brazil, 2021). According to Von Sperling (2014), aquatic environments with a pH close to neutrality tend to present greater ecological stability, providing favorable conditions for the development of aquatic biota.

Table 1

Physicochemical parameters at three distinct points (P1, P2 and P3) of the Rio Preto

Parameter	Expected values	P1	P2	P3
pH	6,0-9,0	6,52 ± 0,10	7,08 ± 0,02	7,12 ± 0,04
Dissolved oxygen (%)	> 60%	101,2 ± 7,20	93,5 ± 4,04	84,8 ± 0,65
Temperature (°C)	< 40	23,6 ± 0,00	24,2 ± 0,00	24,7 ± 0,00
Conductivity (ÿS/cm)	< 100	58,8 ± 3,96	66,3 ± 0,25	67,2 ± 0,19

Source: Prepared by the authors, 2025.

Dissolved oxygen (DO) presented high values at all evaluated sites, oscillating between 84.8% and 101.2%, with an overall mean above 90%. High concentrations of this parameter are frequently associated with low organic load and reduced biochemical oxygen demand, indicating environments with good capacity to support aquatic life (Esteves, 2011). Although a slight reduction in DO was observed at downstream sites, the values remained well above the minimum recommended limits. This behavior may be related to the influence of temperature on gas solubility in water, a phenomenon widely described in the literature (Yang *et al.*, 2021).

The temperatures recorded during monitoring ranged between 23.6 °C and 24.7 °C, showing a low thermal amplitude among the sampled sites. This stability can be attributed to regional climate characteristics and the river's hydrological conditions during the collection period. As described by Esteves (2011), temperatures in this range are common in tropical rivers and generally do not cause significant alterations in ecological processes or the dynamics of aquatic organisms.

Electrical conductivity presented values between 58.8 µS/cm and 67.2 µS/cm, remaining below the 100 µS/cm limit frequently used as a reference for water bodies with reduced anthropogenic influence. This parameter reflects the concentration of dissolved ions in water and, consequently, can indicate the presence of domestic wastewater, agricultural

runoff, or other sources of diffuse pollution. The observed results suggest a low concentration of dissolved salts and reduced interference from pollution sources in the analyzed stretch (Santos *et al.*, 2020).

The integration of the results obtained by the Methylene Blue technique with the physicochemical parameters reinforces the interpretation that the Preto River presents satisfactory environmental conditions in the studied urban stretch. The absence of dye decolorization, associated with high dissolved oxygen levels, suitable pH values, and low electrical conductivity, indicates a reduced organic load and limited influence of pollution sources on the aquatic system. Similar results were reported by Andrade *et al.* (2024b), who highlight the efficiency of the methylene blue method as a complementary tool for preliminary water quality assessments.

Furthermore, maintaining satisfactory standards across the different sampling sites suggests that the river still preserves its self-purification capacity against the anthropogenic pressures present in the urban area. According to Monteiro and Viadanna (2009), water bodies that present a balance between the input and degradation processes of organic matter tend to conserve better ecological and sanitary conditions.

In this manner, the obtained results demonstrate that the analyzed stretch of the Preto River presents characteristics compatible with aquatic environments of low organic pollution, highlighting the importance of continuing monitoring actions and water resources management. The use of simple and low-cost methodologies, such as the methylene blue assay, can significantly contribute to tracking environmental quality and to decision-making aimed at the conservation of aquatic ecosystems (Monteiro; Viadanna, 2009; Von Sperling, 2014).

4 CONCLUSION

The results obtained in this study demonstrated that the analyzed urban stretch of the Preto River presents environmental conditions compatible with a good-quality water body. The maintenance of the Methylene Blue coloration throughout the monitoring period indicated reduced activity of anaerobic microorganisms and a low concentration of biodegradable organic matter, evidencing a limited influence of organic pollution sources on the aquatic environment (Troppmair, 1988; Andrade *et al.*, 2024a).

Complementarily, the evaluated physicochemical parameters remained within the limits recommended by Brazilian legislation for freshwaters intended for multiple uses,

including supply after conventional treatment. The observed values of pH, dissolved oxygen, temperature, and electrical conductivity reinforce the interpretation that the river presents ecological balance and favorable conditions for the maintenance of aquatic biota (Brazil, 2021; Brazil, 2005; Esteves, 2011).

The joint analysis of the results suggests that the Preto River maintains a good environmental carrying capacity and reduced levels of degradation in the studied stretch. Considering its relevance for public supply, agricultural activities, leisure, and fishing in the municipality of Unaí, the preservation of its quality must remain a priority for management agencies and local society.

Finally, it is highlighted that the use of the Methylene Blue technique associated with physicochemical analyses proved to be an efficient, easy-to-apply, and low-cost strategy for preliminary water quality monitoring. The continuity of environmental tracking programs is fundamental to detect potential future alterations and to support actions for the conservation and sustainable management of water resources (Von Sperling, 2014; Monteiro; Viadanna, 2009).

ACKNOWLEDGMENTS

The authors thank the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM) for granting a scholarship linked to PIBIC/UFVJM (Brazil).

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