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Water Quality and Land Use Impacts in a Brazilian Conservation Unit with Speleological Heritage

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Abstract

Karst water systems are highly vulnerable to land use pressures, requiring integrated assessments to support conservation and management. This study evaluated the physicochemical, microbiological, and pesticide-related water quality in the Environmental Protection Area Nascentes do Rio Vermelho (APANRV), a karst conservation unit in the Brazilian Cerrado. Sixteen sampling sites (rivers, springs, and cave waters) were monitored during the dry (May 2024) and rainy (October 2024) seasons. Analyses included nutrients, major ions, *Escherichia coli*, and a broad spectrum of pesticides. The results showed marked spatial and seasonal variability, with elevated hardness and conductivity in karst areas due to carbonate dissolution. Nitrate and total phosphorus reached peak values of 13.59 and 0.132 mg L^{-1}, respectively, indicating localized nutrient enrichment. *E. coli* concentrations reached $\geq$2419.6 MPN 100 mL^{-1}, exceeding regulatory limits, particularly during the rainy season at recreational cave sites. Pesticides were detected in both seasons, with 11 compounds in the dry season and 8 in the rainy season, including atrazine degradation products, and maximum quantified concentrations up to 1.8 $\mu g\ L^{-1}$ (acephate). These findings highlight the combined influence of geology, seasonality, and land use on karst water quality and reinforce the need for continuous monitoring and targeted management strategies.

Keywords: karst; surface water quality; pesticide occurrence; land use impacts

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1. Introduction

Surface water is an essential resource that plays a strategic role in socioeconomic development and environmental sustainability [1]. In recent decades, increasing pressure on waterbodies, mainly driven by urban, industrial, and agricultural expansion, has compromised both the availability and quality of water in various regions of the world, especially in environmentally sensitive areas [2,3]. These pressures are commonly associated with increased inputs of nutrients, pesticides, and microbial contaminants, which can significantly alter the physicochemical and biological characteristics of aquatic systems [4].

In Brazil, Conservation Units (CUs) play a fundamental role in preserving the quality of natural resources, with particular emphasis on water resources [5]. By establishing

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protected areas and sustainable management strategies, CUs contribute to maintaining the integrity of aquatic ecosystems, such as rivers, lakes, and aquifers [6]. Furthermore, CUs act as natural filters, protecting water sources from pollution impacts and ensuring the availability of high-quality water resources [7].

In this context, the role of CUs located in the Cerrado biome stands out, a region of great ecological relevance and strategic importance for the maintenance of the country's water resources. The biome is recognized as one of the main biodiversity hotspots, hosting the richest flora among all savannas in the world. It covers approximately 23% of the Brazilian territory and supplies water to the country's major hydrographic regions [8,9]. Furthermore, the biome hosts one of the largest concentrations of natural subterranean cavities in Brazil, in terms of the number of registered caves [10]. Many of these systems are associated with karst aquifers, which are known for their high hydraulic connectivity and intrinsic vulnerability to contamination [11].

The Environmental Protection Area known as *Nascentes do Rio Vermelho* (*Área de Proteção Ambiental das Nascentes do Rio Vermelho*—APANRV), located in the northeastern portion of the state of Goiás (Midwest region of Brazil), is entirely situated within the Cerrado biome. It is a Sustainable Use Conservation Unit aimed at integrating nature and water protection, sustainable land use, and the conservation of speleological heritage [12,13]. The area hosts more than 180 cataloged caves and associated subterranean ecosystems, which play a key role in hydrological regulation and groundwater storage, making the preservation of water quality essential for maintaining both surface and subterranean ecological integrity [14,15].

The expansion of the agricultural frontier in Brazil, particularly since around the 2000s, marked by the advance of agribusiness in this region, has intensified pressures on the boundaries of the APANRV, particularly in areas adjacent to fields dominated by agriculture, livestock, and crop production (e.g., soybean, corn, beans and wheat) [16]. In karst environments, these inputs may rapidly reach waterbodies through surface runoff and subsurface flow, potentially leading to nutrient enrichment, pesticide occurrence, and microbiological contamination [17]. These factors may compromise the integrity of local ecosystems and negatively affect water quality, threatening the conservation of springs, karst aquifers, and a huge speleological heritage supposed to be protected by the Environmental Protection Area [18].

Despite the ecological and hydrological relevance of the APANRV, systematic studies addressing water quality and potential anthropogenic influences in this region are still scarce. Thus, considering all the above, this study aimed to evaluate surface water quality within the APANRV by addressing three main research questions: (i) how water quality parameters vary between the dry and rainy seasons; (ii) whether karst environments exhibit greater vulnerability to physicochemical, microbiological, and pesticide contamination compared to non-karst surface waters; and (iii) how surrounding agricultural land use influences nutrient concentrations, microbiological indicators, and pesticide occurrence in surface waters and cave rivers. By addressing these questions, this study seeks to clarify the combined effects of seasonality, geology, and land use on water quality to support management strategies for protecting karst water resources and speleological heritage.

2. Materials and Methods

2.1. Study Area and Sampling Sites

The APANRV is located in the northeastern mesoregion of the state of Goiás (Midwest region of Brazil), covering a total area of 176,159 hectares and encompassing four municipalities: Buritinópolis, Damianópolis, Mambaí, and Posse (Figure 1). Entirely situated in the Cerrado biome, the vegetation of the APANRV is diverse, and the climate is of the Central Brazil Tropical type, warm and semi-humid, with average temperatures above 18 °C throughout the year, an average annual rainfall of approximately 1300–1500 mm yr^{-1}, and well-defined dry and wet seasons. The dry season typically occurs from April to September, while the rainy season extends from October to March [19,20].

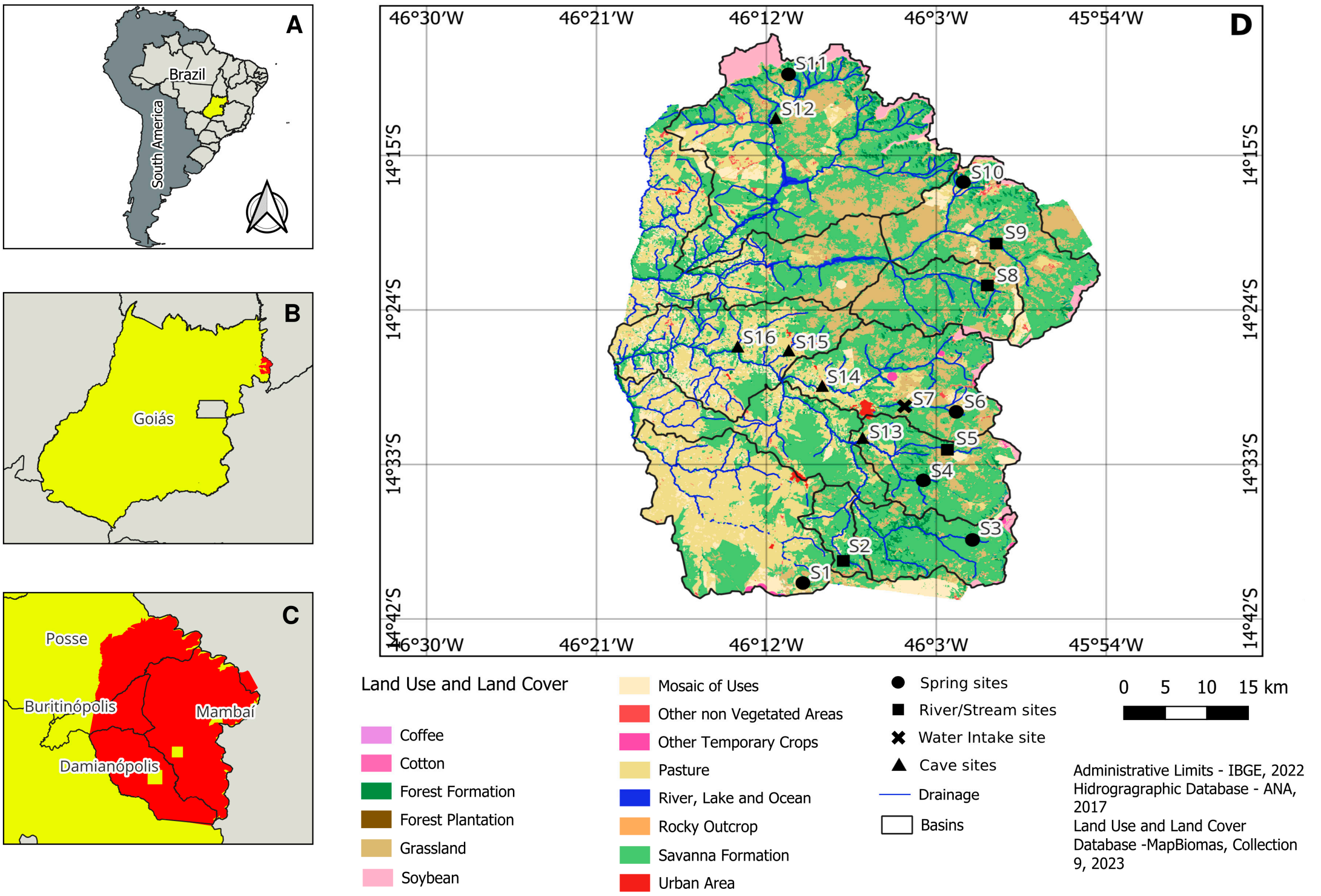

Figure 1. Study area location. (**A**) State of Goiás in Brazil. (**B**) Location of the APANRV in Goiás State. (**C**) APANRV boundaries and municipalities. (**D**) Location of the APANRV showing land use and land cover classes, hydrographic basins, drainage network, sampling sites, and administrative limits.

The geological context of the APANRV's speleological heritage is located in the Bambuí Group, within the Lagoa do Jacaré Formation, featuring colluvial-alluvial deposits and a small portion of the Urucuia Group with sandstones. The Lagoa do Jacaré Formation includes the presence of stromatolitic limestones, calcarenites, and dolomite, with karst geomorphology and associated caves [21,22].

The hydrography of the APA comprises a watershed containing several watercourses, like the Córrego Piracanjuba, Rio dos Buritis, Córrego da Onça, Rio Vermelho, Córrego Ventura, Córrego da Pedra, and Ribeirão São Vidal. The Rio Vermelho (Vermelho River) is of great importance to the region, as its headwaters originate within the Environmental Protection Area. All watercourses flow into the São Francisco and Tocantins-Araguaia hydrographic regions, two major Brazilian river basins [19]. The aquatic systems investigated in this study correspond to surface waters, including river stretches located both in open environments and within caves, where rivers flow through subterranean passages.

The Conservation Unit was established back in 2001, aiming at: regulate the occupation of areas influencing the local speleological heritage; supervise economic activities compatible with environmental conservation; emphasize environmental control and monitoring activities; promote environmental education, scientific research, and the conservation of cultural, historical, and archaeological values; ensure the sustainable nature of anthropogenic actions in the region, with particular focus on improving the living conditions and quality of life of communities within and around the Environmental Protection Area, and finally protect natural attributes, biological diversity, water resources, and speleological heritage [13].

Sixteen sampling sites were selected, taking into account access to the sampling points and the influence of land use and occupation in areas adjacent to the waterbodies (Figure 1). All samples were collected from surface water bodies, including rivers, streams, and cave rivers. Notably, one of the sites corresponds to the water intake point for the public supply of one of the largest municipalities within the APANRV (Mambaí). Table S1 (Supplementary Materials) presents the sampling sites, sub-watershed, waterbody, as well as the identification and geographic coordinates of all sites. To support the interpretation of the sampling design and site representativeness, a conceptual diagram is presented in Figure 2, illustrating the relationship between land use, potential anthropogenic sources, and contaminant transport pathways affecting surface waters and cave rivers within the karst system of the APANRV.

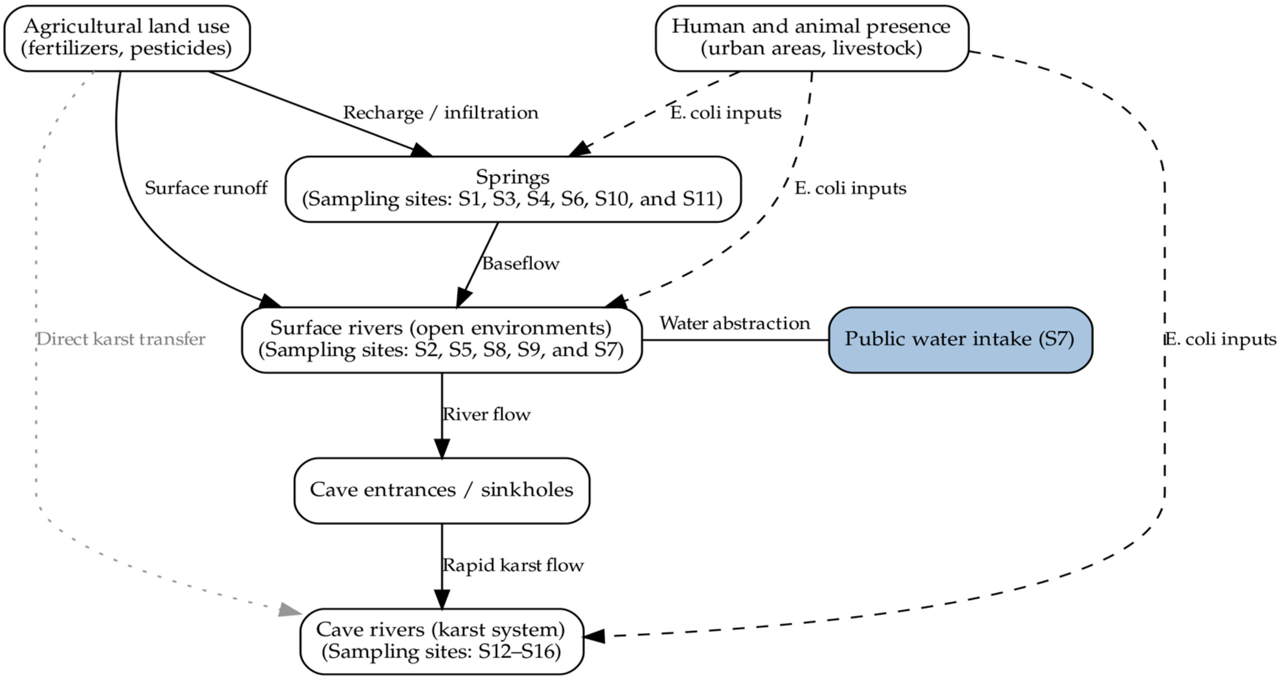

Figure 2. Conceptual diagram showing land use, anthropogenic pressures, contaminant pathways, and sampling sites in the karst system of the APANRV, including springs, surface rivers, cave rivers, and the public water intake.

2.2. Sampling, Physicochemical and Microbiological Analyses

Sampling was conducted during distinct hydrological periods: dry (May 2024) and rainy (October 2024) at the 16 sampling sites. All samples were collected manually from surface waters by immersing the sampling bottles at a depth of approximately 10–15 cm below the water surface. In cave environments, sampling was performed near cave entrances. Water samples for *Escherichia coli* analyses were collected in sterile 120 mL bottles. For physicochemical and metal analyses, samples were collected in separate 300 mL polyethylene bottles. For metal analysis, samples were preserved with HNO_3 (nitric acid) 1 + 1. Samples intended for ion analyses were filtered through 0.45 µm membrane filters prior to preservation and subsequent laboratory analysis. All samples were labeled and stored in a cooler, under proper refrigeration, protected from light, and preserved (acidification and/or freezing) according to the variables to be analyzed until the time of analysis.

The variables temperature, dissolved oxygen, pH, and electrical conductivity were determined in the field by using a portable multi-parameter meter, model HQ40d (Hach, Loveland, CO, USA). Turbidity was measured on the day of sampling by using a portable turbidimeter, model 2100P (Hach, Loveland, CO, USA). *Escherichia coli* analyses were performed immediately after sampling by using the Colilert® enzymatic substrate method (Idexx, Westbrook, ME, USA).

Chloride, fluoride, nitrate, nitrite, phosphate, sulfate, sodium, potassium, and ammonium ions were analyzed by ion chromatography using a 761 Compact IC Ion Chromatograph (Metrohm, Herisau, Switzerland) with a conductivity detector. A Metrosep A Supp 4/5 Guard pre-column and a Metrosep A Supp 5-100/4.0 column (Metrohm, Herisau, Switzerland) were used for anion analysis, and a Metrosep C4 Guard/4.0 pre-column and a Metrosep C4—150/4.0 column (Metrohm, Herisau, Switzerland) were used for cation analysis.

Total phosphorus was analyzed by using the ascorbic acid/colorimetric method with readings performed on a UV-Visible spectrophotometer, model UV-1800 (Shimadzu, Kyoto, Japan). Total nitrogen and total carbon contents were obtained by high-temperature catalytic combustion followed by non-dispersive infrared (NDIR) detection, using a total carbon analyzer, model Vario TOC Cube (Elementar, Langenselbold, Germany).

Total metal contents of aluminum, cadmium, lead, cobalt, copper, iron, manganese, nickel, and zinc were determined by using an inductively coupled argon plasma optical emission spectrometer (ICP-OES), model iCAP 6000 (Thermo Scientific, Waltham, MA, USA). Prior to analysis, water samples were acid-digested with concentrated nitric acid to solubilize dissolved and particulate metal fractions and subsequently filtered through 0.45 µm membrane filters.

All field instruments were calibrated daily and operated according to the manufacturers' instructions, using certified standard solutions when applicable, while methods not requiring calibration curves, such as the Colilert® enzymatic substrate method for *Escherichia coli*, were performed according to standardized protocols.

For analyses requiring instrumental quantification, external calibration curves were constructed within the validated linear working range of each analyte, with coefficients of determination (R^2) greater than 0.999. Linearity was further verified by inspection of calibration residuals. Limits of detection (LD) and quantification (LQ) were determined according to the respective methods. Analytical precision was assessed by replicate measurements.

The sampling, preservation, and analytical procedures followed the recommendations of the International Organization for Standardization (ISO) and the Standard Methods for the Examination of Water and Wastewater (SMEWW) [23,24]. Analyses were carried out both in the field and in the Water Analytical Chemistry Laboratory of Embrapa Cerrados (Brasília, DF, Brazil). Table S2 (Supplementary Materials) presents the variables, units, methods, and methodologies used in the analyses.

2.3. Pesticide Analyses

Water samples for pesticide analysis were prepared using a unified protocol comprising filtration (0.45 µm PTFE), freezing, and lyophilization. Aliquots of 10 mL (n = 3) were frozen at −21 °C, lyophilized (−70 °C, ≤50 mHg), and stored until analysis. Lyophilized samples were resuspended either in 50 mM ammonium formate (pH 2.9) for glyphosate, AMPA, and glufosinate, or in methanol–water (1:1, *v*/*v*) for multi-residue pesticides, followed by filtration. Analyses were performed within 2–4 months after lyophilization, and results represent the mean of three independent analyses per sample [25,26].

Analyses of the herbicide glyphosate and its degradation byproduct AMPA, as well as the herbicide glufosinate, were performed by using Ultra-High Performance Liquid Chromatography, model Acquity I-Class, coupled to a triple quadrupole mass detector XEVO TQ-S Micro (Waters, Milford, MA, USA), employing an Acquity Phenyl-Hexyl 2.1 × 100 mm column (Waters, Milford, MA, USA), and electrospray ionization (ESI) [25]. Multi-residue analyses of 76 pesticides from various classes, including their respective degradation byproducts, were conducted by using Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) on a QTRAP 6500+ (Sciex, Framingham, MA, USA), using a Luna Omega Polar C18 1.6 µm × 100 mm column (Phenomenex, Torrance, CA, USA) and electrospray ionization (ESI) [26]. Table S3 (Supplementary Materials) presents the analyzed pesticides as well as their respective limits of quantification (LQ) and detection (LD) for the methods employed. Pesticide analyses were carried out in the Toxicology Laboratory (LabTox) at the University of Brasília, in Brasília (Brazil).

2.4. Data Analysis and Visualization

The map of the study area and sampling sites (Figure 1) was produced using QGIS software version 3.32.2 (QGIS Development Team Beaverton, OR, USA). Data organization and descriptive analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Graphical representations of physicochemical variables (Figure 3) were generated in Excel. The conceptual diagram illustrating land use, contamination pathways, and sampling design (Figure 2), as well as the graphical representation of *Escherichia coli* results (Figure 4), were developed using Python version 3.12.12 (Python Software Foundation, Wilmington, DE, USA) programming language in the Google Colaboratory environment (Google LLC, Mountain View, CA, USA).

3. Results and Discussion

3.1. Physicochemical Analyses

The seasonal analysis of physicochemical variables in the APANRV revealed distinct patterns between the dry period (May 2024) and the rainy period (October 2024), reflecting both natural processes and anthropogenic influences. Figure 3 presents the data on water temperature (TEMP), dissolved oxygen (DO), pH, electrical conductivity (EC), turbidity (TURB), and total hardness (TH) from the 16 sampling points. The results were compared with the Brazilian National Environmental Council (CONAMA) Resolution No. 357/2005, which classifies surface waterbodies in Brazil into four classes according to their predominant uses and establishes limits for physicochemical and microbiological variables, with Class 1 being the most restrictive and Class 4 the least stringent [27].

TEMP remained relatively stable, ranging from 21.1 °C (S16, May 2024) to 30.4 °C (S11, October 2024), without marked seasonal differences. DO showed significant variation, with values during the dry period close to or below the minimum limit established for Class 4 (2.0 mg L^{-1}), while in the rainy period an increase was observed at some points, although values below the limits for the more restrictive classes persisted (Class 1: 6.0 mg L^{-1}).

The pH ranged from 3.98 (S3, October 2024) to 7.57 (S12, May 2024), with occurrences below the lower limit of 6.0 during the dry season, but without exceeding the upper limit of 9.0 established for freshwater. TH, in turn, showed higher values in the dry season, with the highest concentrations at points S4, S12, S13, S14, S15, and S16. EC exhibited a wide range, especially at points S4, S12, S15, and S16, indicating greater input of dissolved salts at sites located in the interior of caves.

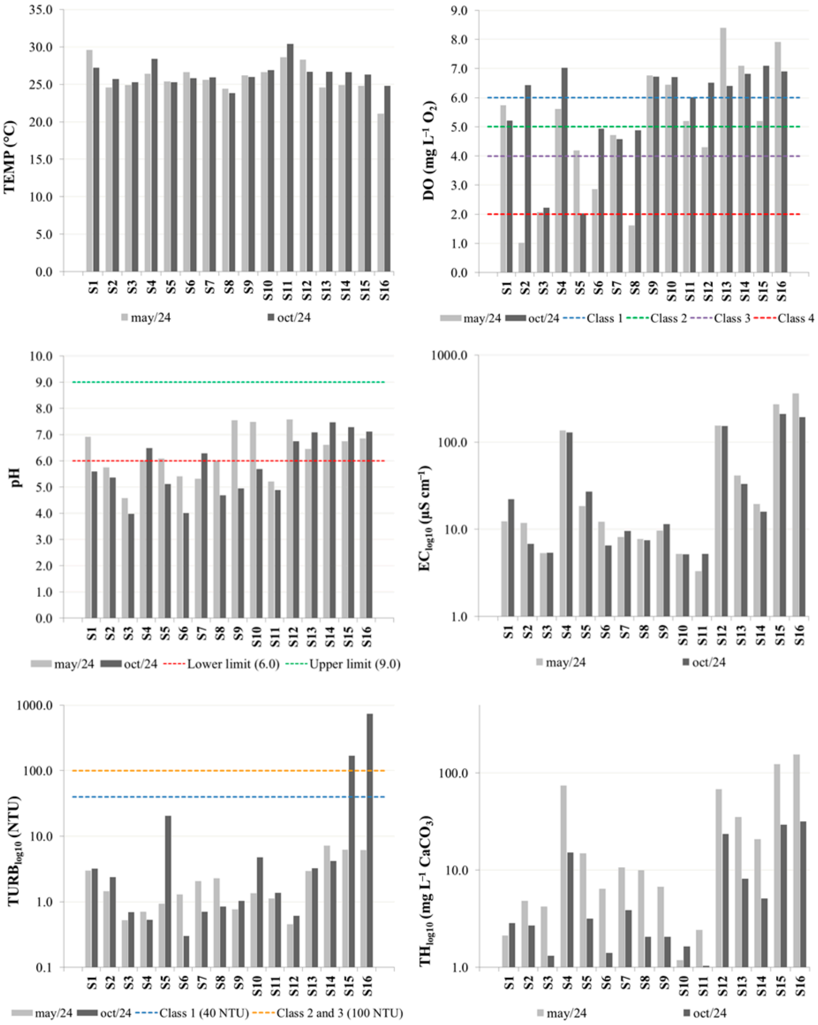

Figure 3. Seasonal variation (May 2024 and October 2024) of physicochemical variables in the APANRV. Dashed lines indicate the limits established by CONAMA Resolution No. 357/2005 for the different water quality classes.

The slightly acidic character of some samples reflects the natural acidity of Cerrado soils, which are highly weathered and acidic, while higher values of total hardness and electrical conductivity in cave water samples are related to the contact of water with minerals such as limestone and dolomite [22,28].

TURB increased during the rainy season at sites S5, S10, S15, and S16, with S15 (170 NTU) exceeding the Class 2 and 3 limit (100 NTU) by 1.7 times, and S16 (740 NTU) exceeding this limit by 7.4 times. These results indicate a strong influence of particle transport during rainfall events, particularly in karst environments [29].

The inorganic metals aluminum, cadmium, lead, cobalt, copper, manganese, nickel, and total zinc showed concentrations below the detection limit of the analytical method. The absence of detectable concentrations of most metals (with the exception of Fe) is consistent with the geological and geomorphological characteristics of the study area. The region is characterized by a fluvio-karstic system developed at the interface between the arenitic rocks of the Urucuia Group and the pelitic-carbonate formations of the Bambuí Group. This geomorphological setting favors the mobilization and redistribution of chemical elements from soils into the karst system through pluvial and fluvial processes. However, previous studies indicate that this transport is mainly associated with major oxides such as Fe_2O_3, MgO, TiO_2, Mn_2O_4, and SiO_2, rather than with trace metals [14].

Table 1 (dry season) and Table 2 (rainy season) present the values (in mg L^{-1}) of total carbon (TC), total nitrogen (TN), total phosphorus (TP), and the ions sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), ammonium ($NH_4{}^+$), nitrite ($NO_2{}^-$), nitrate ($NO_3{}^-$), chloride (Cl^-), fluoride (F^-), phosphate ($PO_4{}^{3-}$), sulfate ($SO_4{}^{2-}$), and iron (Fe).

Table 1. Chemical analyses of water (mg L^{-1}) at sampling points in the APANRV during the dry season (May 2024).

Point	TC	TN	TP	Na^+	K^+	Ca^{2+}	Mg^{2+}	$NH_4{}^+$	$NO_2{}^-$	$NO_3{}^-$	Cl^-	F^-	$PO_4{}^{3-}$	$SO_4{}^{2-}$	Fe
S1	7.35	0.67	1.235	1.415	1.851	0.420	0.215	0.167	<LD	0.534	1.269	0.041	0.806	0.046	<LD
S2	3.86	0.50	0.022	0.548	0.303	1.389	0.181	0.115	<LD	0.277	0.301	0.019	0.005	0.073	1.010
S3	1.88	0.34	0.009	0.184	0.185	1.122	0.224	0.161	<LD	0.287	0.165	0.010	0.001	0.060	0.207
S4	12.35	4.73	0.066	0.369	1.652	24.50	0.491	0.093	<LD	13.59	0.269	0.130	0.034	0.047	<LD
S5	2.22	0.58	0.013	0.357	3.269	4.950	0.076	0.110	<LD	0.294	2.739	0.057	0.003	0.059	<LD
S6	2.32	0.36	0.005	0.402	1.248	2.057	0.093	0.150	<LD	0.336	1.032	0.034	0.003	0.061	<LD
S7	2.99	0.50	0.012	0.266	0.300	2.281	0.968	0.165	<LD	0.327	0.188	0.022	0.002	0.062	0.156
S8	2.01	0.41	0.011	0.291	0.146	2.850	0.377	0.177	<LD	0.380	0.183	0.030	0.001	0.071	2.753
S9	2.70	10.60	0.021	0.167	0.909	2.084	0.153	0.189	<LD	1.953	1.610	<LD	0.004	0.069	<LD
S10	2.86	0.65	0.063	0.453	0.896	0.176	0.161	0.082	<LD	0.790	0.452	0.024	0.039	0.043	0.832
S11	2.04	0.37	0.006	0.261	0.004	0.531	0.212	0.113	<LD	0.293	0.127	<LD	0.003	0.062	<LD
S12	15.79	0.73	0.024	0.394	0.541	19.49	2.552	0.043	<LD	0.351	0.181	0.023	0.007	0.030	<LD
S13	6.78	0.33	0.006	0.310	0.833	11.19	0.558	0.082	<LD	0.474	0.484	<LD	0.005	0.056	0.589
S14	4.02	0.76	0.008	0.424	0.579	6.451	0.459	0.106	<LD	0.601	0.543	<LD	0.002	0.047	1.001
S15	23.05	0.65	0.047	2.623	0.734	35.96	4.790	0.195	<LD	1.106	0.839	0.264	0.009	5.410	0.109
S16	27.58	0.82	0.132	4.151	10.62	45.08	5.331	0.243	<LD	1.118	9.696	0.181	0.043	4.723	0.904

Note: <LD—below the limit of detection ($NO_2{}^-$ = 0.02 mg L^{-1}, F^- = 0.003 mg L^{-1}, Fe = 0.02 mg L^{-1}).

The chemical analyses performed in the APANRV indicated marked differences between the dry period (Table 1) and the rainy period (Table 2), reflecting seasonal influences on water quality linked to the local geological setting.

In the dry season, TC values were higher at points such as S15 (23.05 mg L^{-1}) and S16 (27.58 mg L^{-1}), both associated with karst areas, while in the rainy season, these points also stood out, reaching 28.25 mg L^{-1} and 33.50 mg L^{-1}, respectively. These results suggest that the dissolution of carbonate rocks is the main source of dissolved inorganic matter, being intensified by surface runoff during the rainy season, being intensified by surface runoff during the rainy season [22].

TN contents fluctuated widely between seasons. In the dry season, point S9 showed a concentration of 10.60 mg L^{-1}, while in the rainy season the same point remained high (8.31 mg L^{-1}), indicating a constant input of nitrogen possibly related to anthropogenic activities, such as agricultural use and diffuse discharge of effluents. In contrast, most sampling points showed values below 2.0 mg L^{-1}. TP showed low values in both periods, ranging from 0.005 mg L^{-1} (S6, dry season) to 0.132 mg L^{-1} (S16, dry season). Although CONAMA Resolution No. 357/2005 establishes a limit of 0.02 mg L^{-1} for lentic Class 2 environments, several sites exceeded this threshold, especially during the dry season (S1, S10, S16). This condition may indicate a potential risk of eutrophication in certain environments, particularly in areas more vulnerable to nutrient inputs [30].

Table 2. Chemical analyses of water (mg L^{-1}) at sampling points in the APANRV during the rainy season (October 2024).

Point	TC	TN	TP	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	NO_2^-	NO_3^-	Cl^-	F^-	PO_4^{3-}	SO_4^{2-}	Fe
S1	3.73	1.60	0.002	0.720	0.686	0.826	0.190	0.092	<LD	0.638	0.405	0.013	0.026	0.094	0.883
S2	3.69	1.65	0.003	0.264	0.073	0.697	0.230	0.111	<LD	0.597	0.174	0.009	0.003	0.023	<LD
S3	5.40	2.23	0.003	0.138	0.000	0.402	0.075	0.097	<LD	0.810	0.318	0.235	0.070	0.175	<LD
S4	19.08	1.58	0.006	0.235	0.483	5.624	0.294	0.057	<LD	0.703	2.669	0.068	0.011	0.114	<LD
S5	4.37	1.73	0.003	0.691	0.408	1.092	0.107	0.132	<LD	0.636	0.417	0.020	0.003	0.017	<LD
S6	3.74	1.89	0.004	0.221	0.107	0.472	0.056	0.088	<LD	0.554	0.511	0.015	0.002	0.016	<LD
S7	2.26	0.30	0.002	0.273	0.087	0.766	0.479	0.086	<LD	0.680	0.811	0.011	0.004	0.025	1.995
S8	2.28	0.36	0.001	0.149	0.093	0.494	0.199	0.091	<LD	1.778	0.234	<LD	0.015	0.032	<LD
S9	1.52	8.31	0.003	0.217	0.065	0.439	0.235	0.206	0.034	0.556	0.396	0.005	0.025	0.018	0.742
S10	2.31	0.47	0.001	0.400	0.085	0.447	0.127	0.167	<LD	0.548	0.661	0.281	0.009	0.022	<LD
S11	2.33	0.31	0.003	0.283	0.160	0.277	0.084	0.265	<LD	0.823	0.305	0.127	0.043	0.144	<LD
S12	18.26	0.25	0.001	0.308	0.225	6.528	1.764	0.115	<LD	0.759	0.679	0.008	0.051	0.027	0.231
S13	5.37	0.24	0.002	0.302	0.284	2.560	0.428	0.178	<LD	0.645	1.371	0.095	0.086	0.106	1.000
S14	3.27	0.35	0.003	0.392	0.214	1.263	0.481	0.228	<LD	1.037	3.549	0.304	0.020	4.737	1.154
S15	28.25	0.16	0.006	1.592	0.790	7.427	2.658	0.117	0.070	1.060	4.899	0.229	0.017	5.874	1.635
S16	33.50	0.32	0.079	1.955	1.255	8.642	2.457	0.112	<LD	0.638	0.405	0.013	0.026	0.094	0.883

Note: <LD—below the limit of detection (NO_2^- = 0.02 mg L^{-1}, F^- = 0.003 mg L^{-1}, Fe = 0.02 mg L^{-1}).

TH, expressed by calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, was markedly higher in caves and karst areas, such as S12, S15, and S16. At S16, Ca^{2+} and Mg^{2+} concentrations reached 45.08 mg L^{-1} and 5.33 mg L^{-1}, respectively, during the dry season, and although they decreased during the rainy season (8.64 mg L^{-1} of Ca^{2+} and 2.46 mg L^{-1} of Mg^{2+}), they remained elevated compared to other sampling sites. These patterns reflect the dissolution of limestone and dolomite, consistent with the local geological context. The higher occurrence of sulfate (SO_4^{2-}) at these sites further reinforces the influence of lithology on the ionic composition of the waters [31].

Regarding inorganic nitrogen compounds, nitrate (NO_3^-) concentrations ranged from <0.2 to 13.59 mg L^{-1} at site S4 during the dry season and reached up to 1.78 mg L^{-1} at site S9 in the rainy season. According to CONAMA Resolution No. 357/2005, the limit for Class 2 waters is 10.0 mg L^{-1}, indicating that only S4 exceeded this threshold during the dry period. This exceedance suggests a localized input of oxidized nitrogen, likely associated with nearby agricultural land use and reduced dilution under low-flow conditions, which may pose risks to aquatic ecosystems and, if persistent, to human health due to nitrate exposure [32].

Nitrite (NO_2^-) concentrations remained below the detection limit at nearly all sites. Chloride (Cl^-) and sodium (Na^+) ions showed slight variations, indicating low influence of anthropogenic saline pollution. In contrast, total iron (Fe) concentrations exceeded the Class 2 limit (0.3 mg L^{-1}) at S2 (1.01 mg L^{-1}, dry season) and S16 (1.64 mg L^{-1}, rainy season), which is consistent with the weathering of iron-rich lithologies in the region and the mobilization of particulate and dissolved iron during high-flow conditions. Elevated iron levels may affect water aesthetics, promote sediment deposition, and interfere with aquatic biota, reinforcing the importance of monitoring both geogenic and land use-related influences on water quality [33].

3.2. Microbiological Analyses

The analysis of *Escherichia coli* (Figure 4) revealed significant differences among the sampling sites and between the two evaluated periods (May and October 2024).

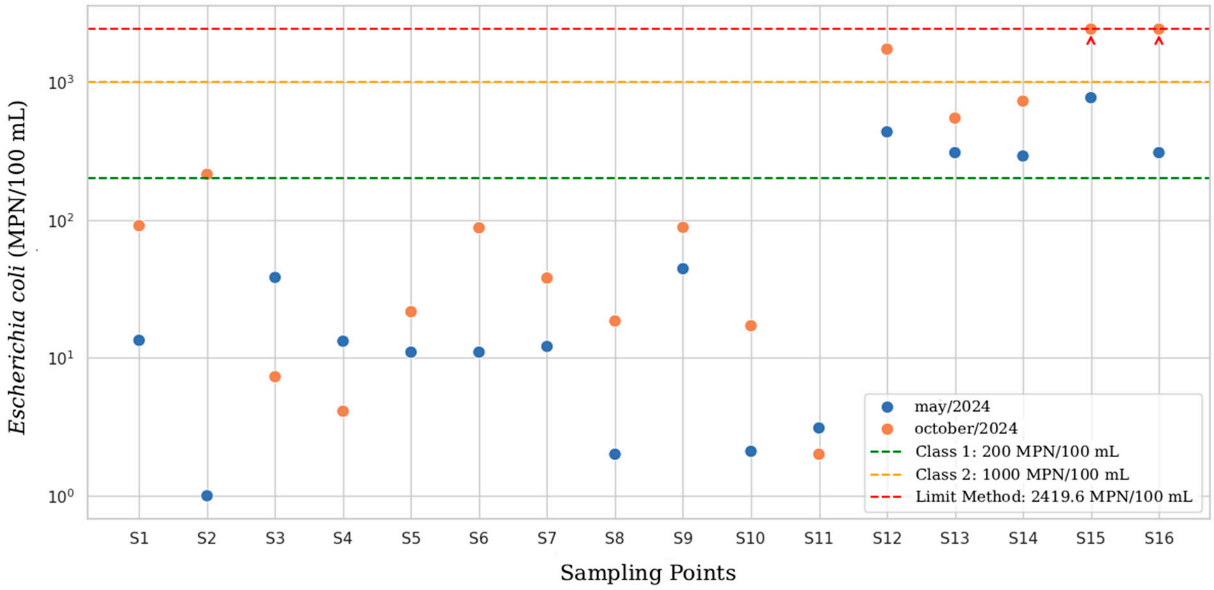

Figure 4. *Escherichia coli* (MPN/100 mL) at sampling sites S1 to S16 during May 2024 and October 2024 in APANRV. The symbol ˆ indicates values exceeding the method detection limit (2419.6 NMP/100 mL).

E. coli, a widely used indicator of fecal contamination in freshwater and a potential signal of enteric pathogens [34], exhibited pronounced spatial and seasonal variability across the APANRV sampling sites (Figure 4). During the dry season (May 2024), concentrations ranged from 1.0 to 770.1 MPN/100 mL, with a median of 13.3 MPN/100 mL, indicating generally low microbiological contamination at most locations. In this period, the majority of samples remained below the Class 1 limit (200 MPN/100 mL) established by CONAMA Resolution No. 357/2005 for freshwater intended for more restrictive uses [27]. Nevertheless, five sites (S12, S13, S14, S15, and S16) exceeded the Class 1 threshold, and site S15 presented values close to the Class 2 limit (1000 MPN/100 mL), suggesting localized fecal inputs even under lower hydrological connectivity.

In contrast, during the rainy season (October 2024), *E. coli* concentrations increased substantially, ranging from 2.0 to >2419.6 MPN/100 mL, with a median of approximately 88.0 MPN/100 mL. In this period, six sampling sites (S2, S12, S13, S14, S15, and S16) exceeded the Class 1 limit, and three sites (S12, S15, and S16) surpassed the Class 2 threshold, reaching the maximum detectable value of the applied method (>2419.6 MPN/100 mL) at S15 and S16. This marked increase in both median values and the number of exceedances highlights the strong influence of rainfall-driven surface runoff and particle transport on fecal contamination dynamics.

This scenario indicates fecal contamination at these sites, potentially associated with the discharge of untreated or inadequately treated domestic effluents, or contributions from animal waste in the drainage basins [35,36]. It is noteworthy that karst environments (such as sites S12 to S16) are highly vulnerable to pathogen contamination due to rapid water flows and the limited natural filtration capacity [37].

At site S7, which corresponds to the water intake point for the public supply of the municipality of Mambaí, part of APANRV, *E. coli* concentrations in both sampling periods remained within the maximum value allowed for Class 1 waters (which may be used for human consumption after conventional or advanced treatment), indicating low microbiological contamination (Figure 4). The low bacterial presence at this site suggests reduced microbiological risk and a lower burden on the treatment system, contributing to higher operational efficiency of public water supply [38,39]. Nevertheless, regular monitoring and verification of treatment efficiency are essential to ensure the continued safety of the public water supply, particularly during the rainy season.

Sites S13 (Borá II Cave), S14 (Funil Cave), and S16 (Penhasco Cave) are located in areas where primary recreational use consists of guided speleological tourism, involving direct water contact, and the high *E. coli* levels observed at these locations, especially in the rainy season (October), pose a potential health risk to tourists and local residents [40]. According to the bathing water quality criteria established in CONAMA Resolution No. 274/2000, the water sample from site S16 (Penhasco Cave) was classified as "unsuitable" for primary contact recreation, as *E. coli* concentrations exceeded 2000 MPN/100 mL, indicating a potential health risk to users [41].

Seasonality also appears to play a relevant role in the observed variation. In the rainy season (October), increased rainfall and surface runoff contribute to higher concentrations of pollutants in karst waters, including *E. coli* [42,43].

3.3. Pesticides

Among the 79 analyzed pesticides in total, only 11 were detected in the samples from points S1, S2, S4, S5, S9, S10, and S11 during the dry season (May 2024), and eight pesticides were detected at points S8, S15, and S16 during the rainy season (October 2024). The results of the analyses are presented in Tables 3 and 4.

Table 3. Results of pesticides in μg L^{-1} found in water samples from the dry season in APANRV.

Pesticide (μg L^{-1})			Sampling Points						
	LQ	**LD**	**S1**	**S2**	**S4**	**S5**	**S9**	**S10**	**S11**
Acephate ^{A,D}	3.000	0.015	<LQ	nd	nd	nd	nd	nd	nd
Aldicarb sulfone ^{*,A,E}	0.013	0.004	<LQ	<LQ	nd	nd	<LQ	nd	nd
Atrazine ^{B}	0.013	0.004	0.05	nd	nd	nd	nd	0.01	nd
Deethylatrazine ^{*,B}	0.100	0.030	0.02	nd	nd	nd	nd	0.03	nd
Deisopropylatrazine ^{*,B}	0.100	0.030	<LQ	nd	nd	nd	nd	<LQ	nd
Hydroxyatrazine ^{*,B}	0.100	0.030	0.08	nd	nd	nd	nd	0.08	nd
Azoxystrobin ^{C}	0.050	0.015	0.003	nd	nd	nd	nd	nd	nd
Carbofuran ^{A,D,E,F}	0.013	0.004	<LQ	nd	nd	nd	nd	nd	nd
Malaoxon ^{*,A}	Qualitative		<LQ	nd	<LQ	<LQ	nd	<LQ	<LQ
Methamidophos ^{A}	Qualitative		<LQ	nd	nd	nd	nd	nd	nd
Tebuconazole ^{C}	0.100	0.030	<LQ	nd	nd	nd	nd	nd	nd

Notes: A—insecticide; B—herbicide; C—fungicide; D—acaricide; E—nematicide; F—termiticide; * degradation products; LQ —limit of quantification; LD—limit of detection; <LQ—detected but not quantified (qualitative); nd—not detected (<LD and <LQ).

Table 4. Results of pesticides in μg L^{-1} found in water samples from the rainy season in APANRV.

Pesticide (μg L^{-1})			Sampling Points		
	LQ	**LD**	**S8**	**S15**	**S16**
2,4-D ^{B}	0.700	0.210	nd	<LQ	<LQ
Acephate ^{A,D}	0.013	0.004	1.8	nd	<LQ
Acetamiprid ^{A}	0.100	0.030	nd	nd	<LQ
Deethylatrazine ^{*,B}	0.100	0.030	nd	nd	<LQ
Hydroxyatrazine ^{*,B}	0.100	0.030	nd	<LQ	<LQ
Azoxystrobin ^{C}	0.050	0.015	nd	nd	<LQ
Carbendazim ^{C}	Qualitative		<LQ	<LQ	<LQ
Methomyl ^{A}	0.100	0.030	nd	nd	<LQ

Notes: A—insecticide; B—herbicide; C—fungicide; D—acaricide; * degradation products; LQ—limit of quantification; LD—limit of detection; <LQ—detected but not quantified (qualitative); nd—not detected (<LD and <LQ).

In the results obtained, <LQ indicates that the analyte was detected above the limit of detection but below the limit of quantification, making it impossible to assign a reliable numerical value; therefore, the result is reported only qualitatively [25]. At Point S1, during the dry season (May 2024), 11 pesticides were detected, including the herbicide atrazine (0.05 μg L^{-1}) and its degradation by-products: desethylatrazine (0.02 μg L^{-1}), hydroxyatrazine (0.08 μg L^{-1}), and deisopropylatrazine (qualitative). At Point S10, also in the dry season, atrazine (0.01 μg L^{-1}), desethylatrazine (0.03 μg L^{-1}), hydroxyatrazine (0.08 μg L^{-1}), and deisopropylatrazine (qualitative) were quantified.

Atrazine is one of the most widely used herbicides in Brazil, ranking third among pesticides applied in pre- and post-emergence stages of various crops, especially corn, soybean, wheat, cotton, sorghum, and sugarcane. According to CONAMA Resolution No. 357/2005, the maximum permissible limit of atrazine in Class 1 and 2 freshwater is 2.0 μg L^{-1} [27,44,45]. Atrazine degradation by-products (desethylatrazine and hydroxyatrazine) were also detected (qualitatively) at points S15 and S16 during the rainy season (Table 4). At point S1, during the dry season, methamidophos and carbofuran were detected, both pesticides of high acute toxicity, whose registration and commercialization were revoked in Brazil, and whose use was banned nationwide in 2011 and 2017, respectively [46,47].

These are highly toxic organophosphate insecticides that act as acetylcholinesterase inhibitors, affecting the nervous system of various aquatic organisms [48]. Their presence in water bodies can cause acute mortality in fish and invertebrates, as well as sublethal effects such as behavioral, reproductive, and growth alterations. Both exhibit high persistence in sensitive aquatic systems, representing a significant ecological risk [49–51].

In the present study, pesticides classified as less acutely toxic compared to highly hazardous compounds were also detected, such as 2,4-D, acephate, aldicarb sulfone (a degradation by-product of aldicarb), acetamiprid, azoxystrobin, methomyl, and tebuconazole. 2,4-D was detected at two sampling points during the rainy season (S15 and S16), while acephate had a detectable concentration of 1.8 μg L^{-1} at point S8, and below the LQ at S16, both in the rainy season. Acetamiprid and methomyl, insecticides of moderate acute toxicity, were qualitatively detected during the rainy season at S16 (Table 4).

Among the fungicides, carbendazim, azoxystrobin, and tebuconazole were detected at low concentrations or below the LQ, with restricted occurrence. Carbendazim, in particular, poses a risk to aquatic biota and human health due to its environmental persistence and toxic properties [52]. Despite being banned in Brazil since 2022, this fungicide was detected at points S8, S15, and S16 during the rainy season [53]. It is worth noting that points S1 and S10 correspond to two springs (Table S1—Supplementary Materials), environments highly vulnerable to pesticide contamination [54]. Point S1, located at the boundary of the APANRV, lies within a large rural property and is surrounded by areas of intensive agricultural cultivation (Figure 1). Points S15 and S16, on the other hand, are located in caves of a karst system, where rapid underground flows and low filtration capacity increase the risk of contaminant transport, including pesticides [18,55].

According to Brazilian legislation, the use of pesticides in Sustainable Use Conservation Units, such as APANRV, requires the establishment of specific rules and restrictions for their management. In these territories, the reduction in pesticide use and the adoption of agroecological practices are recommended [56]. These findings emphasize the need for targeted protection and management strategies, particularly at sampling sites used for public water supply and cave-based tourism activities, in order to minimize contamination risks and ensure the long-term sustainability of water resources within the conservation unit.

4. Conclusions

This study represents an initial integrated characterization of the chemical quality of water in the APANRV, providing support for understanding the influence of karst geology, land use and occupation, and seasonality on regional hydrochemistry. The results revealed that the dissolution of carbonate rocks is a key factor for the high water hardness in caves, while seasonal variation influenced nutrient and ion concentrations: in the dry season, there was a greater accumulation of salts and nutrients, whereas in the rainy season, the dilution effect prevailed, although accompanied by the mobilization of organic matter and iron.

Overall, most of the evaluated parameters are in accordance with the limits established by Brazilian legislation regarding the classification of surface freshwater bodies. However, some points showed elevated concentrations of phosphorus and nitrate, indicating possible anthropogenic influence. From a microbiological perspective, the presence of *Escherichia coli* at some sites constitutes an important warning, since this is the main indicator of fecal contamination in freshwater and might point to a sanitary risk associated with the presence of enteric pathogens, in addition to compromising recreational water uses.

Furthermore, the occurrence of pesticide residues in these environments, even at variable concentrations or below the limits of quantification in some samples, should be considered a preliminary warning, given the intensive use of these compounds in the region. Such substances, even at low levels, can affect aquatic biota and compromise water quality for human consumption.

The absence of historical data in the APANRV represents a limitation but also highlights the relevance of this work as an unprecedented contribution to environmental monitoring of the region. Thus, this study broadens scientific knowledge about hydrochemistry and risks associated with water quality in this Conservation Unit and its implications in terms of potential threats to its huge speleological heritage, providing essential technical support for managers and decision-makers.

Moreover, the expansion of the agricultural frontier due to the advancement of agribusiness near the conservation unit represents an ever-increasing pressure on water resources and the integrity of karst ecosystems, making the incorporation of these results into public policies even more crucial for the protection of water resources and ensuring both human supply and biodiversity conservation in these highly vulnerable karst systems.

Future research should focus on developing pollution vulnerability and risk maps, as well as defining protection perimeters for water intake points and cave systems, in order to support land use planning and strengthen preventive management strategies in the APANRV.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w18070799/s1. Table S1: Sampling points (S1–S16), indicating the sub-basin, water body, identification, and respective geographic coordinates within the study area; Table S2: Variables, abbreviations, units, methods and analytical methodologies of physicochemical analysis; Table S3: List of the 79 analyzed pesticides and their respective limit of quantification (LQ) as well as limit of detection (LD).

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Abbreviations

The following abbreviations are used in this manuscript:

2,4-D	2,4-Dichlorophenoxyacetic acid
AMPA	Aminomethylphosphonic acid
APANRV	Environmental Protection Area of the Nascentes do Rio Vermelho
Ca^{2+}	Calcium
Cl^{-}	Chloride
CONAMA	Brazilian National Environmental Council
DL	Detection Limit
DO	Dissolved Oxygen
EC	Electrical Conductivity
E. coli	Escherichia coli
F^{-}	Fluoride
Fe	Iron
HNO_3	Nitric Acid
IC	Ion Chromatograph
ICP-OES	Inductively coupled plasma optical emission spectrometer
ISO	International Organization for Standardization
K^{+}	Potassium
LQ	Limit of Quantification
LD	Limit of Detection
Mg^{2+}	Magnesium
MPN	More Probable Number
Na^{+}	Sodium
$NH_4{}^{+}$	Ammonium
$NO_2{}^{-}$	Nitrite
$NO_3{}^{-}$	Nitrate
NTU	Nephelometric Turbidity Unit
$PO_4{}^{3-}$	Phosphate
PTFE	Polytetrafluoroethylene
SMEWW	Standard Methods for the Examination of Water and Wastewater
$SO_4{}^{2-}$	Sulfate
TEMP	Water Temperature
TC	Total Carbon
TH	Total Hardness
TN	Total Nitrogen
TP	Total Phosphorus
TURB	Turbidity

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