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Quality of springs water and resistance profile of aerobic bacteria to antimicrobials under different land-use areas in the José Pereira River micro-basin (Itajubá – MG, Brazil) |

Qualidade de águas de nascentes e perfil de resistência de bactérias aeróbias a antimicrobianos, em áreas sob diferentes usos, na microbacia do Ribeirão José Pereira (Itajubá – MG, Brasil)

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Abstract: The indiscriminate use and disposal of antibiotics has led to an increase in reports of the presence of resistant bacteria in natural environments. In rural areas, the population often uses water from springs and other natural sources, but the quality of which is not always monitored. This study evaluated the physical, chemical and microbiological quality of water from 12 springs under different uses, in the José Pereira River micro-basin, Itajubá (MG), and investigated the resistance profile of aerobic bacteria to 10 antibiotics. Samples were collected during the dry and rainy seasons, and the results showed that none of the springs met the quality standards for human consumption. There was strong influence of season in microbial water quality, with higher average values for aerobic bacteria, *Escherichia coli* and total coliform bacteria for the rainy season. No relationship as observed between season and bacterial resistance to the antibiotics, despite more strains being resistant to penicillin, and fewer strains resistant to ciprofloxacin, chloramphenicol and gentamicin, independently of land-use surrounding the springs. These results highlight the need for continuous monitoring of water quality and more sustainable practices in antibiotic management to minimize risks to public health and the environment.
Keywords: Antibiotics, Coliforms, Heterotrophic bacteria, Water quality.

Resumo: O uso e descarte indiscriminado de antibióticos têm levado ao aumento de relatos sobre a presença de bactérias resistentes em ambientes naturais. Em áreas rurais, a população frequentemente utiliza água de nascentes e outras fontes naturais, cuja qualidade nem sempre é monitorada. Este estudo avaliou a qualidade física, química e microbiológica da água de 12 nascentes sob diferentes usos, na microbacia do rio José Pereira, Itajubá (MG), e investigou o perfil de resistência de bactérias aeróbias a 10 antibióticos. As amostras foram coletadas durante as estações seca e chuvosa, e os resultados mostraram que nenhuma nascente atendeu aos padrões de qualidade para consumo humano. Houve forte influência da estação na qualidade microbiana da água, com maiores valores para bactérias aeróbias, *Escherichia coli* e coliformes totais, na estação chuvosa. Não foi observada relação entre época de amostragem e resistência bacteriana aos antibióticos, apesar de mais cepas serem resistentes à penicilina e menos cepas resistentes à ciprofloxacina, cloranfenicol e gentamicina, independentemente do uso do solo no entorno das nascentes. Esses resultados ressaltam a necessidade de monitoramento contínuo da qualidade da água e de práticas mais sustentáveis no manejo de antibióticos para minimizar os riscos à saúde pública e ao meio ambiente.
Palavras-chave: Antibióticos, Bactérias heterotróficas, Coliformes, Qualidade de água.

6.1 Introduction

Antibiotic-resistant bacteria in natural environments have grown significantly due to inadequate practices for managing soils and water resources. Contamination with domestic, industrial, and hospital effluents containing high concentrations of antimicrobial drugs puts pressure on autochthonous strains from natural environments, resulting in resistant strains triggering (ALVARENGA; NICOLETTI, 2010; KING et al., 2021). According to Corrêa et al. (2019), resistance factors are increasingly present in the environment in light of human activity.

Water contamination resulting from these activities is of special importance since water is essential to sustain life. In some regions, local populations depend exclusively on water from local natural sources, like springs, and in most cases, they consume untreated water. Aquatic microbial communities reflect the conditions of the surrounding terrestrial environment, and the activities carried out there. Poorly managed human, agricultural, and livestock activities cause impacts on aquatic ecosystems, and result in changes to their communities and on water quality (FERREIRA, 2017).

Despite the importance of water resources for maintaining life, there is an ambiguous relationship between water use and the environment, since humans have degraded these resources and the areas where they are located, in the form of pollution and devastation (XAVIER; MEDEIROS, 2017; OKEREAFOR et al., 2020). In addition to deteriorated resources, there is increased consumption, and the problem of irregular water distribution among different regions on the planet.

These factors hinder access to good quality water, and many populations use alternative sources to supply their water needs. This is especially true in rural areas, where people frequently use water from springs and other natural sources, and the water quality is often not known (AQUOTTI et al., 2019). Alternative water source use is quite expressive, given the importance of consuming quality water. Regardless of the source, quality water control should be a primary objective of societies. There are several reports in the literature on isolated antibiotic-resistant and multi-resistant bacteria in public water sources and alternative water supplies (BORTOLOTI et al., 2018; GOMES FREITAS et al., 2017; ABERA et al., 2016).

Consolidation Ordinance GM/MS No. 888/2021 from the Ministry of Health (MS) (BRASIL, 2021) established control and surveillance procedures for water to regulate quality, regardless of origin, in addition to establishing potability standards, via reference values for physical, chemical, and microbiological attributes. Water for human consumption in any situation, including individual sources from wells, mines, springs, etc., must be free of coliform bacteria, being the *Escherichia coli* a good bioindicator. Another group of bacteria comprises those aerobic bacteria (BRASIL, 2021), microorganisms that require organic carbon as a source of nutrients, provide information about the bacteriological quality of water in a broad way. The determination includes the non-specific bacteria,

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whether of fecal origin, components of the natural microbiota of the water or resulting from the formation of biofilms in the delivery system. Therefore, it serves as an auxiliary indicator of water quality, by providing additional information on eventual failures in disinfection, colonization, and biofilm formation in the distribution system (DOMINGUES et al., 2007).

One other non-mandatory test is evaluating the bacterial resistance profile, and although this is an important and simple test to perform, it is not widely implemented. Bacterial resistance is defined as a bacteria ability to defeat the drugs designed to kill them (CDC, 2021). According to Reygaert et al. (2018), intrinsic bacterial resistance is the result of an evolutionary process, and represents the biochemical characteristic of an organism, while extrinsic or acquired resistance occurs via a process of genetic recombination, or less frequently, via mutation. Mutations are often related to anthropogenic factors, e.g., the presence of antibiotics in the environment, which may result in the inability to treat diseases caused by resistant bacteria. Some state that bacterial resistance could result in a step back towards a pre-antibiotic world. Microbial resistance is a global public health problem associated with several factors. It represents as one of the greatest threats to human health by the World Economic Forum's Global Risk Report (BLAIR et al., 2015). Lupo et al. (2012) suggest researching microorganisms as a water quality control mechanism, since high concentration levels of resistant strains in water pose dangers to populations, especially to those with depressed immune systems.

In this sense, the justification for this study is due to the fact that the assessment of water quality in rural environments, when carried out, does not involve monitoring and risks associated with the indiscriminate use of antibiotics, which ends up promoting the emergence of resistant microorganisms and their negative impacts on human health.

The objective of this study is to evaluate springs water quality in the José Pereira River micro basin, Itajubá (MG), in relation to its physical, chemical, and microbiological aspects, including the resistance profile of common aerobic bacteria to ten antimicrobials, and to associate water quality to land use systems in the surrounding area.

6.2 Materials and Methods

6.2.1 Site characterization and water sampling

The study was conducted in a rural area around Itajubá (MG), Brazil, within the hydrographic micro-basin of the José Pereira River, a tributary of the Sapucaí river in the middle region, located at 45° 27' 31" East and 45° 20' 57"West, and 22° 23' 18"North and 22° 26' 57"South. The area is approximately 40 km² and is limited on the south by the Pedra Amarela mountain range, on the east by the Água Limpa mountain range, on the north by the Juru and Toledos mountain ranges, and to the west by small dividers that separate it from the Sapucaí river basin (FLAUZINO et al., 2016). The Jose Pereira River micro basin is very important, since it provides water to rural populations. It also supplies water to the public water works in Itajubá, since the city's water catchment is located

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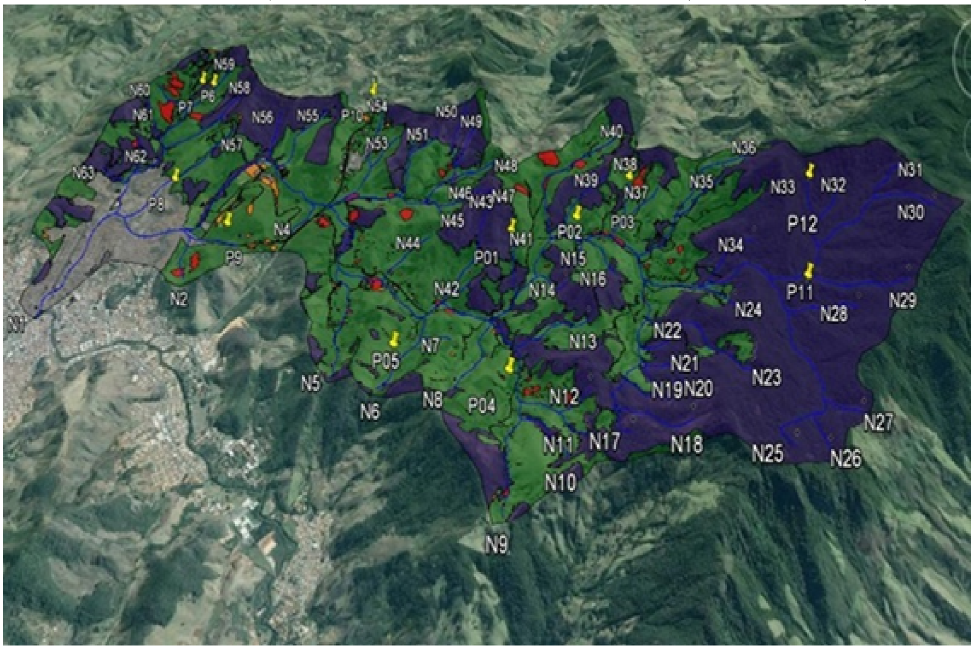
there. The José Pereira River micro-basin was defined for study given its economic and social importance, and the intense water use of its springs.

Twelve of sixty-three springs water were chosen according to the type of land use practices in the regions. The locations and descriptions of each spring are given in Figure 6.1 and Table 6.2, respectively. At the time of the study, water samples were collected in the rainy (December) and dry (June) seasons, using sterilized plastic bottles, three times per spring, and placed in a container for transport in laboratory and immediately treated for analyses.

Figure 6.1: Springs water of the Jose Pereira river micro basin (N1 to N63), Itajubá (MG, Brazil). Twelve springs/points were selected for the study (yellow markers)



Source: the authors.

6.2.2 Physical, chemical and microbial analysis

We analysed the physical and chemical properties of the water samples determining the hydrogenic potential (pH), conductivity (µS cm^{-1}), and dissolved oxygen (mg mL^{-1}) by Inolab portable multi-parameter device; turbidity (NTU-Nephelometric Turbidity Units) by Inolab analyser; acidity (mg L^{-1} $CaCO_3$), alkalinity (mg L^{-1} $CaCO_3$), and hardness (mg L^{-1} $CaCO_3$) by titration and total organic carbon (mg L^{-1}) by Analytikjena total organic carbon analyser. We then calculated the arithmetic mean for each attribute and water spring.

For microbial analysis and to access the general microbial quality, the water samples were used to evaluate the total density of aerobic bacteria, using the spread-plate method with inoculation of 0.1 mL of the original water sample in Petri dishes containing a Plate Count Agar (PCA) culture medium (APHA, 1999). To quantify the total coliforms and *Escherichia coli*, the Colilert® method was used by dissolving the reagent in 100 mL

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Table 6.2: Coordinates of the springs with their respective land uses.

Spring	Latitude (S)	Longitude (W)	Land use	Water Use
1	22°25'13,4'	45°24'03,5''	Overgrowth/pasture with cattle	Drinking water for animals
2 H	22°25'08,1'	45°23'35,1''	Overgrowth without cattle	Human consumption
3	22°24'50,1'	45°23'13,6''	Overgrowth without cattle	Energy production
4	22°26'11,0'	45°24'04,4''	Degraded pasture with cattle	Drinking water for animals
5 H	22°26'00,6'	45°24'49,4''	Pasture/Farming, eroded soil with cattle	Human consumption
6	22°23'47,2'	45°26'35,0''	Pasture, degraded soil with cattle	Drinking water for animals
7	22°23'45,0'	45°26'41,4''	Overgrowth/Pasture without cattle	-
8	22°24'40,2'	45°26'39,5''	Urban area, pasture with cattle	-
9 H	22°25'04,4'	45°26'08,9''	Pasture without cattle	Human/Animal consumption
10 H	22°23'54,1'	45°25'12,8''	Side of the road without cattle	Human consumption
11 H	22°25'45,9'	45°22'06,7''	Combination of Overgrowth/pasture with cattle	Human consumption
12	22°25'6,11'	45°22'2,98''	Serra dos Toledos Natural Reserve, possibly containing cattle	-

H (human consumption).

Source: the authors.

of each sample, then transferring them to a Quanti-Tray card, which was sealed and incubated in a bacteriological oven for 24 ± 3 h at 35 ºC ± 0.5 ºC (APHA, 1999). After the incubation period, we took measurements to determine the Most Probable Number (MPN) of bacteria, calculating the arithmetic mean for each spring.

Due to methodological limitations with respect to the total analysis of the colonies, each Petri dish was divided into four quadrants after counting the aerobic bacteria, and one bacterial colony from each quadrant was removed using a platinum loop, considering shape, chromogenesis, opacity and texture. Each colony was sub-cultured into individual tubes containing PCA, and grown in a bacteriological incubator for 48 ± 3 h at 35 ºC ± 0.5 ºC for isolation (CETESB, 2006).

Subsequently, the colonies were subjected to an antibiogram using the diffusion disk technique, also known as the Kirby-Bauer method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). The antibiotics tested were amoxicillin, aztreonam, cephalexin, cefepime, cefoxitin, ciprofloxacin, chloramphenicol, gentamicin, penicillin G, and vancomycin, selected according by class and medical importance. After the incubation period and growing the colony in the culture medium, an inhibition zone halo formed around each disk (mm), and was measured using a digital pachymeter. The diameters obtained were compared to standard values provided by the manufacturer (CECON) of the antibiotic discs (Table 6.3). The resistance profiles for each isolate were defined for the ten antimicrobials tested, using the following code sensitive (S), intermediate (I) or resistant (R), according to the inhibition zone diameter.

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Table 6.3: Antibiotics used in the antibiogram with their respective classifications and standard values for the determination of sensitivity.

Antibiotics	Classification	Resistant (R)	Intermediary (I)	Sensitive (S)
		----------	mm	----------
Amoxicillin AMO 10 µg	Penicilina*	≤ 13	14-16	≥ 17
Aztreonam ATM 30 µg	Monobactan*	≤ 17	18-20	≥ 21
Cephalexin CFE 30 µg	Cefalosporina* 1ª G	≤ 14	15-17	≥ 18
Cefepime CPM 30 µg	Cefalosporina* 4ª G	≤ 14	15-17	≥ 18
Cefoxitin CFO 30 µg	Cefalosporina* 2ª G	≤ 14	15-17	≥ 18
Ciprofloxacin CIP 5 µg	Quinolonas	≤ 15	16-20	≥ 21
Chloranphenicol CLO 30 µg	Cloranfenicol	≤ 12	13-17	≥ 18
Gentamicin GEN 10 µg	Aminiglicosídeo	≤ 12	13-14	≥ 15
Penicillin G PEN 10 UI	Penicilina*	≤ 14	-	≥ 15
Vancomycin VAN 30 µg	Glicopeptídeo	≤ 14	15-16	≥ 17

*β-lactam antibiotics (antibacterials causing cell wall alteration).

Source: the authors.

6.2.3 Principal Component Analysis

All arithmetic means of chemical, physical and microbiological attributes, and the percentage of bacterial resistance to ten antibiotics in water samples from twelve springs, collected during the dry and rainy season were submitted to multivariate analysis using the principal components technique by the software PC-ORD® (McCUNE; MEFFORD, 2011). This technique allows comparisons between attributes and sampling times in a broader way.

6.3 Results and Discussion

6.3.1 Physical and chemical analysis

The results of the arithmetic mean for the physical and chemical properties of the water samples from the different collection points, taken from the dry (D) and rainy (R) seasons, are shown in the Table 6.4. Specifically, collection springs 2, 5, 9, 10, and 11 were used for human consumption.

The samples with the highest average conductivity values, in both seasons, are those referring to point 5, and the samples that presented the lowest values correspond to point 12, in the dry season, and to points 2 and 12, in the rainy season, making it impossible to assess the influence of the sampling season. Point 5, where the highest average conductivity was recorded between the seasons (84.0 µS cm^{-1}), corresponds to a pasture area, with very degraded soil (Table 6.2), which, associated with the lack of protection of the spring, may have influenced the result, given that high values of conductivity in water bodies are related to the presence of contaminants. Point 12, where the lowest average for conductivity was recorded (15.4 µS cm^{-1}), corresponds to the spring inside the Serra dos Toledos Reserve, a well-preserved area not yet used for human consumption. Point 2, which refers to a source of significant nutritional use by humans, also showed a low conductivity value, especially in

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the rainy season, proving to be well protected. However, Brazilian current legislation does not establish limits for electrical conductivity in water intended for human consumption.

Table 6.4: Average of physical and chemical attributes for the water samples collected in the dry (D) and rainy (R) seasons.

	CE		OD		pH		TURB		ACID		ALCAL		DUR		TOC	
Spring	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R
1	26.2	38.9	8.4	7.7	7.1	7.1	2.1	5.9	5.3	13.3	8.0	25.3	20.0	18.4	5.1	0.0
2 H	27.9	12.8	7.9	7.7	7.1	7.8	16.3	2.9	5.3	8.0	5.3	6.7	26.1	13.8	5.5	0.4
3	53.4	64.8	9.5	7.3	7.0	7.6	52.8	362.7	6.7	9.3	14.7	41.3	41.5	27.7	3.8	0.0
4	37.7	34.7	9.7	6.9	7.6	7.4	2.0	3.9	4.0	6.7	6.7	22.7	32.3	15.4	3.4	0.5
5 H	86.4	81.6	9.5	6.3	7.5	6.1	2.4	0.5	8.0	18.7	14.7	49.3	56.8	35.3	2.2	0.0
6	42.7	35.7	7.0	8.3	7.0	7.0	0.4	1.8	4.0	10.7	9.3	26.7	12.3	16.9	3.8	0.0
7	36.5	46.2	6.9	8.5	7.5	7.6	1.4	1.6	5.3	10.7	8.0	33.3	18.4	20.0	3.9	0.0
8	53.8	70.2	5.9	8.2	7.1	6.7	4.1	4.3	5.3	18.7	12.0	38.7	24.6	26.1	0.0	0.0
9 H	64.5	36.8	5.6	8.1	6.2	6.5	1.4	5.8	10.7	20.0	13.3	25.3	18.4	35.3	2.1	0.0
10 H	49.4	50.4	6.2	8.2	6.2	7.1	1.9	3.5	8.0	10.7	9.3	29.3	39.9	29.2	2.9	0.0
11 H	32.6	42.4	6.7	7.6	7.5	7.7	4.1	6.0	4.0	9.3	9.3	29.3	18.4	16.9	0.9	1.5
12	15.7	15.1	6.8	7.5	7.6	6.9	2.9	3.9	5.3	10.7	4.0	12.0	20.0	6.1	0.8	4.5

CE (electrical conductivity - µS cm^{-1}), OD (dissolved oxygen - mg L^{-1}), TURB (turbidity - NTU), ACID (acidity - mg L^{-1} $CaCO_3$), ALCAL (alkalinity - mg L^{-1} $CaCO_3$), DUR (hardness - mg L^{-1} $CaCO_3$), TOC (total organic carbon - mg L^{-1}), H (human consumption)

Source: the authors.

The determination of dissolved oxygen in water is important to assess water conditions and identify possible environmental impacts such as eutrophication and organic pollution. In addition, dissolved oxygen is indicative of the preservation of aquatic life, and according to CONAMA Resolution 357/2005 (CONAMA, 2005), the minimum oxygen value to provide this preservation is 5.0 mg L^{-1}. All samples analysed showed good concentrations of dissolved oxygen, above the minimum value established by this Resolution, without influence of the time of collection.

As for the hydrogenic potential (pH), values between 6.2 and 7.8 were obtained, compatible with the balance and aquatic life (CONAMA, 2005), probably without influence on the water sampling season. Turbidity, on the other hand, indicates the presence of suspended particles in the water and is an aesthetic parameter that conditions the acceptance or rejection of water for different purposes. The maximum value allowed for water for nutritional purposes, to guarantee the microbiological quality of the water, in addition to the requirements relating to microbiological indicators, according to Ordinance GM/MS no. 888/2021 from the Ministry of Health (MS) is 5.0 NTU. As can be seen in table 6.4, the water samples referring to the rainy season showed higher turbidity values and, therefore, greater presence of suspended particles, resulting from the erosion of the surrounding soils. The waters at points 2 (dry season), 9 and 11 (rainy season), used for human nutritional consumption, exceeded the maximum allowed turbidity value (5.0 NTU)

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established by the aforementioned Ordinance. By the same Ordinance, water intended for human consumption must not exceed 300 mg L^{-1} $CaCO_3$ in hardness, as an organoleptic potability standard. As can be seen, all water samples collected, regardless of the season, showed low values for this attribute.

Finally, total organic carbon is related to the concentration of heterotrophic microorganisms in aquatic systems (ROCHA, 2007). As can be seen in Table 6.4, the dry season promoted the highest concentrations of organic carbon in the analysed waters, which may have occurred as a consequence of the decrease in water volume due to the low precipitation (30.2 mm) recorded in this period, causing a concentration of that component.

For a better understanding of the values obtained for these attributes, as well as their relationship with the microbiological aspects, the result of the principal components analysis will be presented later.

6.3.2 Microbiological analyses

The microbiological attributes, for the dry and rainy seasons, are presented in Table 6.5. The values of aerobic bacteria density can be interpreted as a general microbial contamination on evaluated springs. Based in these values, we can indicate that the springs 2, 9 and 10, from the dry season, and springs 9, 10 and 11, from the rainy season, presented lower microbial contamination, mainly the springs 9 and 10 in both seasons.

Table 6.5: Average aerobic bacteria density, total coliform group bacteria and *Escherichia coli* per 100 mL water sample, for the twelve collection points in the dry and rainy seasons.

	Dry season			Rainy season		
Spring	**Aerobic bacteria density**	**Total coliforms**	***E. coli***	**Aerobic bacteria density**	**Total coliforms**	***E. coli***
	CFU mL^{-1}	**MPN 100 mL^{-1}**		**CFU mL^{-1}**	**MPN 100 mL^{-1}**	
1	27	129.8	7.40	397	>2,419.2	2.77
2 H	73	19.2	11.00	13,510	>2,419.2	99.03
3	90	165.2	6.10	15,557	>2,419.2	127.80
4	60	165.2	29.50	16,150	>2,419.2	151.40
5 H	520	165.2	35.90	32,097	>2,419.2	7.97
6	290	> 2,419.2	2.00	2,237	>2,419.2	>200.5
7	517	> 2,419.2	11.10	953	>2,419.2	149.57
8	43	866.4	0.00	673	>2,419.2	44.57
9 H	10	104.30	0.00	107	1,337.63	7.27
10 H	40	49.50	0.00	193	1,986.08	31.87
11 H	1,060	> 2.419.2	83.1	383	>2,419.2	> 200.5
12	807	> 2.419.2	129.80	237	>2,419.2	122.93

H (human consumption), MPN (most probable number), CFU (colony-forming unit).
Source: the authors.

We observed that other springs, not used for human consumption, showed lower aerobic bacteria values, however, this alone is not sufficient for classifying potable water. In general, there was higher average aerobic bacteria density for the rainy season (6,874.45 CFU mL^{-1}), and lower average densities for the dry season (294.75 CFU mL^{-1}). Springs 11 and 12 (limit and inside the Reserve, respectively), were exceptions, and had higher average densities in the dry season. Other springs had higher aerobic bacteria values in the rainy season. This

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was possibly due to the springs' proximity to the Serra dos Toledos Natural Reserve, since climatic conditions do not vary greatly between dry and rainy seasons there. In a study by Amaral (2003), a similar result was found for aerobic bacteria density for different sampling periods. One hundred and eighty natural waters samples from 30 rural properties located in the Northeastern region of São Paulo were evaluated, for both dry and rainy seasons. They found that the average aerobic bacteria densities were higher in the samples from the rainy season. These data demonstrate the susceptibility to contamination that natural sources are exposed to, especially during the rainy season, when soil microorganisms flow quickly to water sources due to water percolation. These results show the risk that natural sources are subject to if measures are not applied to preserve water quality.

Total coliform counts are also important and are set out in Ordinance GM/MS no. 888/2021 from the Ministry of Health (MS), and even though not all representatives in this group come from fecal contamination, many are part of natural ecosystems, and are potential pathogens. In this way, since pathogenic microorganism detection in water is not routinely performed, groups of specific microorganisms can be more viably researched, including the main pathogens usually present in water, e.g., total coliforms.

According to the results in Table 6.5, the rainy season showed average total coliforms above 2,419.2 CFU mL^{-1}, higher than in the dry season. This result may be a reflection of higher precipitation levels during the rainy season, causing water source contamination. The highest average values obtained for the seasons came from spring 6 (pasture area with cattle and soil erosion), spring 7 (combination of forest and pasture land without cattle), spring 11 (combination of forest and pasture with cattle in the surrounding areas), and spring 12 (Serra dos Toledos Natural Reserve, possibly containing cattle-manure). Average values were above 2,419.2 CFU mL^{-1}, and the lowest average value for the seasons was for spring 9 (pasture with cattle), with an average of 720.97 CFU mL^{-1} bacteria in the total coliform group. In the dry season, when the lowest levels of total coliforms were found in water samples, spring 2 (forest without cattle) and spring 10 (side of the road without cattle) showed less than 50 CFU mL^{-1} total coliforms. As established by Ordinance GM/MS no. 888/2021 from the Ministry of Health (MS), water for human consumption must be free of bacteria from the total coliform group in a 100 mL sample. According to this rule, no spring was suitable for human consumption.

Finally, *E. coli* is the main microorganism associated with fecal material in water. According to Ordinance GM/MS no. 888/2021 from the Ministry of Health (MS), water for human consumption must be free of *E. coli.* in 100 mL samples. According to the results, only waters from springs 8 (pasture with cattle located in an urban area), spring 9 (pasture without cattle), and spring 10 (pasture on the MGC-383 highway, without cattle), from the dry season, met the standard established in the MS ordinance. Spring 8 (pasture with cattle located in urban areas), which is not used for human consumption, and spring number 2 (forest area with no cattle), spring 5 (pasture area with cattle, with soil erosion and small vegetation), and spring 11 (combination of forest and pasture with

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cattle), which are used for consumption, did not comply with the ordinance values. Higher average values were also registered for the rainy season.

We confirm that there was seasonal influence on water quality in the samples from the study areas, especially on the microbiological indicators, showing that it is important to conduct water quality studies at different seasons (DONADIO et al., 2005).

6.3.3 Antimicrobial resistance test

Figure 6.6 shows the resistance percentages for the isolates obtained from each of the twelve springs in the dry and rainy seasons. In total, 144 bacterial isolates were tested (12 from each spring) for the rainy season and 98 isolates for the dry season, due to lower bacterial growth. For the dry season (Figure 6.7A), spring 7 (combination of forest and pasture, without cattle), spring 10 (pasture with cattle located in urban areas), and spring 12 (Serra dos Toledos Natural Reserve, possibly containing cattle-manure), had higher percentage of resistance isolates equal to or greater than 50%. Springs 2 (forest with no cattle), spring 6 (pasture with cattle and soil erosion), and spring 11 (combination of forest and pasture with cattle), by contrast, had high percentage sensitivity strains with values above 70%, indicating that water consumption in the dry season is safer in terms of resistant microorganism presence that could potentially be harmful to human health.

In the rainy season (Figure 6.6B), only spring 3 (forest without cattle) had a resistance isolate percentage equal to 50%, while other springs had lower values. Spring 2 (forest without cattle), and spring 9 (pasture without cattle) had sensitivity percentages above 70%, indicating that these two springs have fewer resistant microorganisms in the rainy season. In general, there was no relationship between the sampling season and the percentage of bacterial resistance, i.e., the dry or rainy season did not interfere with water quality in terms of percentage of resistance for isolated strains against the tested antimicrobials.

Among the antibiotics tested (Figure 8.3), penicillin showed the highest percentage of resistance, which is due to its intense and indiscriminate use, which promoted a rapid selection of resistant strains in different environments (BRUNTON et al., 2007). An explanation for this finding may be related to the fact that penicillin, belonging to the β-lactam antimicrobial group, was the first antibiotic used in clinical practice from 1940 onwards (GUIMARÃES et al., 2010), and its use was trivialized both by health professionals and by self-medication, resulting in a large increase in bacterial resistance.

On the other hand, all bacterial strains obtained were sensitive to the antimicrobial ciprofloxacin, in both seasons. According to Lopes et al. (1998) this antimicrobial was introduced into clinical practice in the 80's when it proved to be very efficient, including against several bacteria resistant to other antimicrobials. However, currently, a growth of bacteria resistant to this drug has already been observed. All strains obtained in the dry season were sensitive to the antibiotic chloramphenicol and only 3% of the isolates from the rainy season showed resistance to this antimicrobial. Chloramphenicol has a broad spectrum of activity and easy penetration into the bacterial cell, such characteristics allow

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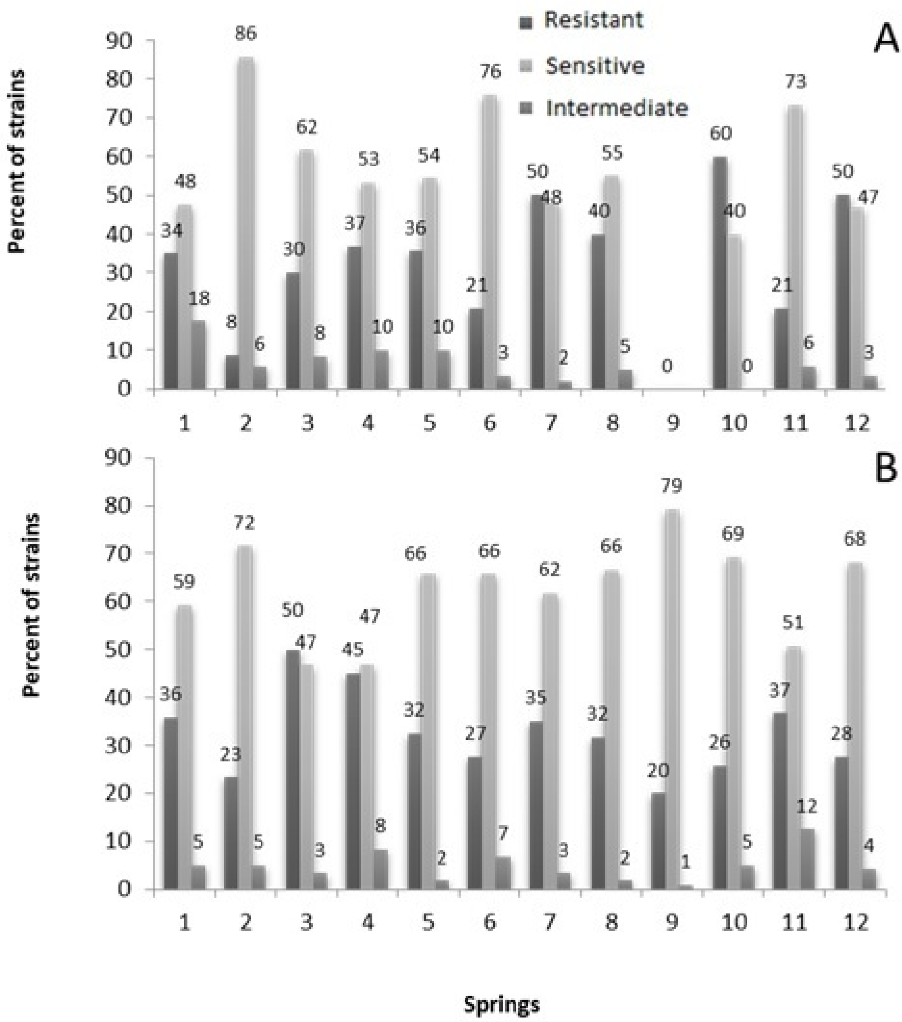
Figure 6.6: Percentage of sensitive (S), intermediate (I) or resistant (R) strains for each of the twelve springs in the dry (A) and rainy (B) season, according to ten antibiotics.



Source: the authors.

it to be used, even against some strains resistant to other drugs. Gentamicin also showed a low percentage of resistance, 2% only in the dry season and 3% in the rainy season. This drug, according to Virto et al. (2002), belongs to the class of aminoglycosides and has a broad spectrum of activity in addition to a low rate of pathogenic resistance. Cefepime also showed low resistance in the dry season (5%), against a higher value in the rainy season (15%). The other antimicrobials showed higher percentages of strains resistant to them.

6.3.4 Principal Component Analysis

For the dry season, the total variance was 68.5%. The first (CP1) explained 30.1%, and the second (CP2) explained 20.3% of the principal components. Of the main components that explained 72.4% of the total variance for the collection attributes in the rainy season,

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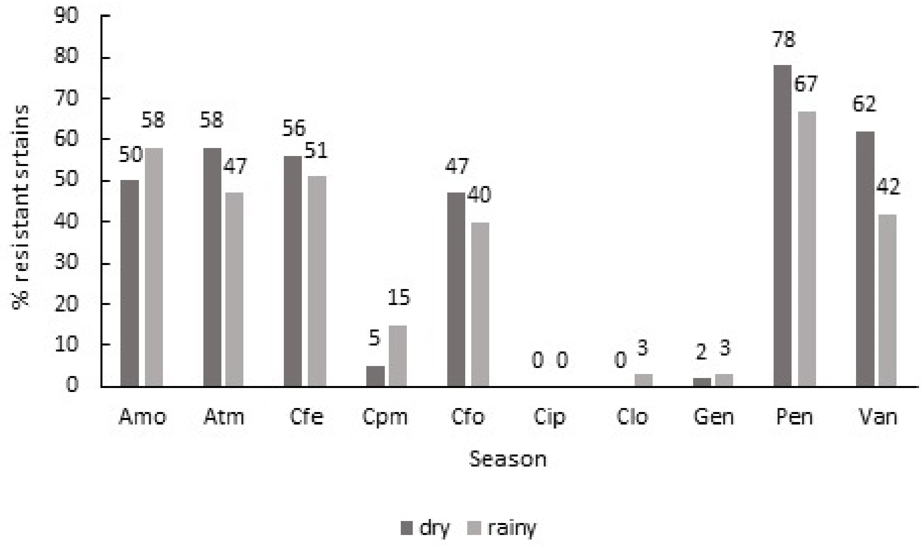
Figure 6.7: Percentage of strains of aerobic bacteria resistant to the antimicrobials tested, in samples of spring water, in the dry and rainy seasons. Amoxicillin (Amo), aztreonam (Atm), cephalexin (Cfe), cefepime (Cpm), cefoxitin (Cfo), ciprofloxacin (Cip), chloramphenicol (Clo), gentamicin (Gen), penicillin G (Pen), and vancomycin (Van).



Source: the authors.

the first (CP1) explained 39.1%, and the second (CP2) explained 24.2%. The results are shown in Figure 6.8.

For the dry season (Figure 6.8A) there was greater resistance to the tested antibiotics from bacterial isolates in water samples from springs 7, 8, 10, 11 and 12. Springs 1, 2, 3, 5, 6 and 9, by contrast, showed a small relationship with resistant bacteria in water from their respective springs, and had better microbiological aspects, since they had a small relationship with aerobic bacteria, total coliforms, and *E. coli*. However, water samples from springs 7, 11 and 12 had the greatest relationships with bacterial resistance, aerobic bacteria presence, total coliforms, and *E. coli*. We can verify that there was no relationship between the Total Organic Carbon (TOC) and aerobic bacteria density, contrary to results from Silva et al. (2006). These authors evaluated the correlation between TOC and aerobic bacteria density in water samples, and they concluded that high bacterial densities were obtained for TOC above 0.7 mg L^{-1}.

For the rainy season (Figure 6.8B), greater bacterial resistance was found for isolates from water springs 1, 3, 4, 7 and 11. Bacterial resistance, aerobic bacteria, *E. coli* and total coliforms were more related to samples from springs 11 (for the dry season), 3, and 4, and therefore, these were generally classified as lower quality water. Therefore, we can conclude that the best quality waters came from springs 2, 5, 6, 9, 10 and 12, since they were not strongly related to bacterial resistance, nor to aerobic spore-forming bacteria, total coliforms or *E. coli*.

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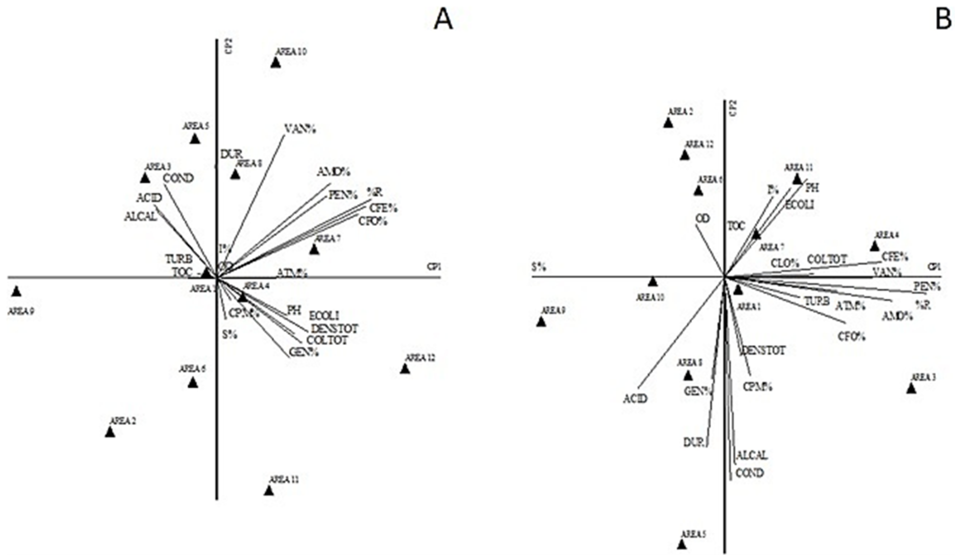
Figure 6.8: Main components CP1 x CP2 of the average chemical, physical and microbiological attributes, and the percentage of bacterial resistance to ten antibiotics in water samples from twelve springs/Areas (▲), collected during the dry (A) and rainy (B) season. CE (electrical conductivity), OD (dissolved oxygen), TURB (turbidity), ACID (acidity), ALCAL (alkalinity), DUR (hardness), TOC (total organic carbon). % Resistance to Amoxicillin (AMO), aztreonam (ATM), cephalexin (CFE), cefepime (CPM), cefoxitin (CFO), ciprofloxacin (CIP), chloramphenicol (CLO), gentamicin (GEN), penicillin G (PEN), and vancomycin (Van). ECOLI (*E. coli*), DESTOT (density of aerobic bacteria), COLTOT (total coliform bacteria).



Source: the authors.

6.4 Conclusions

The water samples of the different springs do not attempt the established standards for human consumption.

The sampling season had a strong influence on the physical and chemical attributes, and microbiological attributes, mainly for aerobic bacteria density, total coliform bacteria and *E. coli*, with emphasis on the rainy season. However, the season did not influence the percentage of resistant strains to the antimicrobials tested, despite higher percentage resistant strains to penicillin and lower percentage resistant strains for ciprofloxacin, chloramphenicol and gentamicin.

The land-use or soil conditions in the areas surrounding the springs do not affect the water quality by the attributes evaluated. More studies are needed for this association, mainly related to a more detailed environmental characterization of the study areas where the springs are located.

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6.6 References

ABERA, B.; KIBRET, M.; MULU, W. Extended Spectrum beta (β) – Lactamases and Antibiogram in Enterobacteriaceae from Clinical and Drinking Water Sources from Bahir Dar City, Ethiopia. PLoS One, Berkeley, v. 11, n. 11, p. e0166519, 2016. https://doi.org/10.1371/journal.pone.0166519

ALVARENGA, L. S. V.; NICOLETTI, M. A. Descarte doméstico de medicamentos e algumas considerações sobre o impacto ambiental decorrente. Revista Saúde, Guarulhos, v. 4, n. 3, p. 34-39, 2010. Disponível em: http://revistas.ung.br/index.php/saude/article/view/763/829. Acesso em: 17 fev. 2023.

AMARAL, L. A.; FILHO, N. F.; JUNIOR, O. D. R.; FERREIRA, F. L. A.; BARROS, L. S. S. Água de consumo humano como fator de risco à saúde em propriedades rurais. Saúde pública, Jaboticabal, v. 37, n. 4, p.510-514, 2003. https://doi.org/10.1590/S0034-89102003000400017

APHA, American Public Health Association. Standard Methods for the Examination of Water and Wastewater, 20th.ed. APHA, AWWA, WEF, 1999. 1120 p. Disponível em: https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/scientific/technical-documents/white-papers/apha-water-testing-standard-methods-introduction-white-paper.pdf. Acesso em: 7 fev. 2023.

AQUOTTI, N. C. F.; YAMAGUSHI, N. U.; GONÇALVES, J. E. Preservação e conservação de nascentes em propriedades rurais: impactos, ações e contradições. Enciclopédia biosfera, Jandaia, v. 16, n. 29, p. 1309-1323, 2019. Disponível em: https://www.conhecer.org.br/enciclop/2019a/agrar/preservacao.pdf. Acesso em: 6 fev. 2023.

BLAIR, J. M. A.; WEBBER, M. A.; BAYLAY, A. J.; OGBOLU, D. O.; PIDDOCK, L. J. V. Molecular Mechanisms of Antibiotic Resistance. Nature, London, v. 13, n. 1, p. 42-51, 2015. https://doi.org/10.1038/nrmicro3380

BORTOLOTI, K. C. S.; MELLONI, R.; MARQUES, P. S.; CARVALHO, B. M. F.; ANDRADE, M. C. Qualidade microbiológica de águas naturais quanto ao perfil de resistência de bactérias heterotróficas a antimicrobianos. Engenharia Sanitária e Ambiental, Rio de Janeiro, v. 23 n. 4, p. 717-725, 2018. https://doi.org/10.1590/S1413-41522018169903

BRASIL. Ministério da Saúde. Portaria de Consolidação 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: Brasília-DF, 07 maio 2021. 85. ed., seção 1, p. 127. Disponível em: https://bvsms.saude.gov.br/bvs/saudelegis/gm/2021/prt0888_07_05_2021.html. Acesso em: 24 fev. 2023.

DOI: 10.24979/ambiente.v18i1.1442

Ambiente (ISSN 1981-4127)
Vol. 18 nº 1. Jan/Abr 2025

BRUNTON, L. L., LAZO, J. S.; PARKER, K. L. Goodman & Gilman. As bases farmacológicas da terapêutica. 11. ed. Rio de Janeiro: McGraw-Hill Interamericana do Brasil, 2007. 1821 p.

CDC. Center for Disease Control and Prevention. About Antimicrobial Resistance. December, 2021. Disponível em: https://www.cdc.gov/drugresistance/about.html. Acesso em: 10 jan. 2023.

CETESB. Norma técnica L5 201, de janeiro de 2006. Contagem de bactérias heterotróficas: método de ensaio. São Paulo: CETESB, p. 14, 2006. Disponível em: https://cetesb.sp.gov.br/normas-tecnicas-cetesb/normas-tecnicas-vigentes/ . Acesso em: 7 fev. 2023.

CONAMA – Conselho Nacional de Meio Ambiente. Resolução 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. Disponível em: https://www.icmbio.gov.br/cepsul/images/stories/legislacao/Resolucao/2005/res_conama_357_2005_classificacao_corpos_agua_rtfcda_altrd_res_393_2007_397_2008_410_2009_430_2011.pdf . Acesso em: 7 fev. 2023.

CORRÊA, M. S. P. O.; SILVA, M. S.; MORAES, S. R.; CAVALCANTE, J. J. V. Bacterial ecology in the Araruama lagoon of Rio de Janeiro State. Semioses: Inovação, Desenvolvimento e Sustentabilidade, Curitiba, v. 13, n. 1, p. 47-59, 2019. Disponívem em: https://revistas.unisuam.edu.br/index.php/semioses/article/download/256/102/ . Acesso em: 3 jan. 2023.

DOMINGUES, V. O.; TAVARES, G. D.; STÜKEN, F.; MICHELOT, T. M.; REETZ, L. G. B.; BERTONCHELI, C. M.; HÖMER, R. Contagem de bactérias heterotróficas na água para consumo humano: comparação entre duas metodologias. Saúde, Santa Maria, v. 33, n.1, p.15-19, 2007. https://doi.org/10.5902/223658346458

DONADIO, N. M. M.; GALBIATTI, J. A.; PAULA, R. C. Qualidade da água de nascentes com diferentes usos do solo na bacia hidrográfica do córrego Rico, São Paulo, Brasil. Engenharia Agrícola, Jaboticabal, v. 25, n. 1, p. 115-125, 2005. https://doi.org/10.1590/S0100-69162005000100013

FERREIRA, E. P. B.; STONE, L. F.; MARTIN-DIDONET, C. C. G. População e atividade microbiana do solo em sistema agroecológico de produção. Revista Ciência Agronômica, Fortaleza, v. 48, n. 1, p. 22-31, 2017. https://doi.org/10.5935/1806-6690.20170003

FLAUZINO, B. K.; MELLONI, E. G. P.; PONS, N. A. D.; LIMA, O. Mapeamento da capacidade de uso da terra como contribuição ao planejamento de uso do solo em sub-bacia hidrográfica piloto no sul de Minas Gerais. Geociências, Rio Claro, v. 35, n. 2, p. 277-287, 2016. Disponível em: https:

DOI: 10.24979/ambiente.v18i1.1442

Ambiente (ISSN 1981-4127)
Vol. 18 nº 1. Jan/Abr 2025

//www.periodicos.rc.biblioteca.unesp.br/index.php/geociencias/article/view/11383. Acesso em: 8 fev. 2023.

GOMES, F. D.; SILVA R. D.; BATAUS L. A.; BARBOSA, M. S.; BITENCOURT BRAGA, C. A. S.; CARNEIRO L. C. Bacteriological water quality in school's drinking fountains and detection antibiotic resistance genes. Annals of Clinical Microbiology and Antimicrobials, London, v. 16, n. 5, 2017. https://doi.org/10.1186/s12941-016-0176-7

GUIMARÃES, O. D.; MOMESSO S. L.; PUPO T. M. Antibiotics: therapeutic importance and perspectives for the discovery and development of new agents. Química Nova, São Paulo, v. 33, n. 3, p. 667-679, 2010. https://doi.org/10.1590/S0100-40422010000300035

KING T.L.; SCHMIDT S.; THAKUR S.; FEDORKA-CRAY P.; KEELARA S.; HARDEN L.; ESSACK SY. Resistome of a carbapenemase-producing novel ST232 Klebsiella michiganensis isolate from urban hospital effluent in South Africa. Journal of Global Antimicrobial Resistance, v.24, p.321-324, 2021. https://doi.org/10.1016/j.jgar.2021.01.004

LOPES, A. A.; SALGADO, K.; MARTINELLI, R.; ROCHA, H. Aumento da frequência de resistência à norfloxacina e ciprofloxacina em bactérias isoladas em uroculturas. Associação Médica Brasileira, São Paulo, v. 44, n. 3, p. 196-200, 1998. https://doi.org/10.1590/S0104-42301998000300006

LUPO, A.; COYNE, S.; BERENDONK, T. U. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Frontiers in Microbiology, Lausanne, v.3, n. 18, 2012. https://doi.org/10.3389%2Ffmicb.2012.00018

McCUNE, B.; MEFFORD, M.J. PC-ORD. Multivariate analysis of ecological data. Gleneden Beach, Oregon: MjM Software, 2011.

NCCLS. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard - 8. ed., v. 23, n. 1, Wayne, NCCLS, 2003.

OKEREAFOR, U.; MAKHATHA, M.; MEKUTO, L.; UCHE-OKEREAFOR, N.; SEBOLA, T.; MAVUMENGWANA, V. Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. International Journal of Environmental Research and Public Health, v.17, n.7, p.2204, 2020. https://doi.org/10.3390/ijerph17072204

REYGAERT, W.C. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiology, v.4, n.3, p.482-50, 2018. https://doi.org/10.3934/microbiol.2018.3.482

VIRTO, M. R.; FRUTOS, P.; TORRADO, S.; FRUTOS, G. Gentamicin release from modified acrylic bone cements with lactose and hydroxypropylmethylcellulose. Biomaterials, Bethesda, v. 24, p.79-87, 2003. https://doi.org/10.1016/s0142-9612(02)00254-5

DOI: 10.24979/ambiente.v18i1.1442

Ambiente (ISSN 1981-4127)
Vol. 18 nº 1. Jan/Abr 2025

XAVIER, C. K. S., MEDEIROS, J. D. F. Degradação de recursos hídricos: água fator de saúde pública. Revista Uni-RN, Natal, v. 17, n. 1/2, p. 65-80, 2017. Disponível em: http://revistas.unirn.edu.br/index.php/revistaunirn/article/view/483. Acesso em: 4 jan. 2023.

DOI: 10.24979/ambiente.v18i1.1501