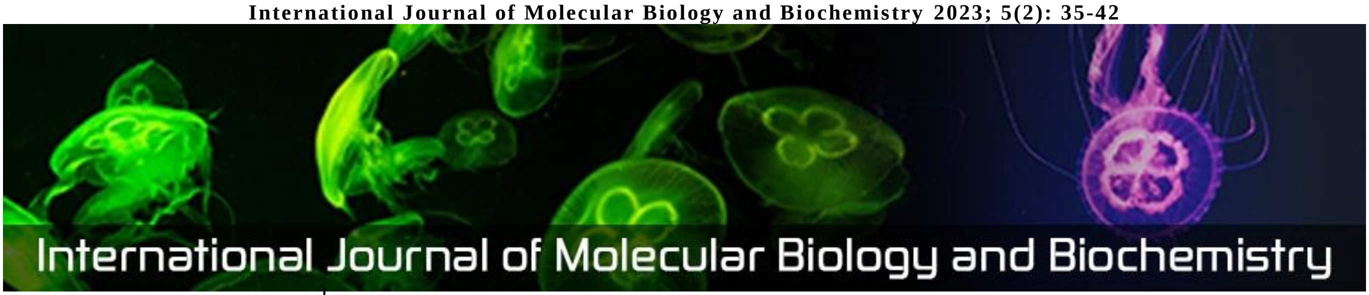

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Water quality from Ribeirão Santana downstream the rural area of São Luís de Montes Belos, Goiás, Brazil

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Abstract
Industrial waste thrown into waterways is a major problem in Brazil. On that premise, this work evaluated the water quality from Ribeirão Santana downstream the area of São Luís de Montes Belos, Goiás, Brazil throughout the year 2021. Physical-chemical and microbiological evaluation revealed inconsistencies ($p<0.05$) for turbidity, pH, total solids, conductivity, ammonia, biochemical oxygen demand, chemical oxygen demand, total phosphorus, nitrate, nitrite, total Kjeldahl nitrogen, fecal coliforms and total coliforms. Likewise, genotoxic and mutagenic analysis detected the presence of chromosomal abnormalities and micronuclei ($p<0.001$). Results raise concerns and highlight the necessity for constant water quality monitoring, as Ribeirão Santana directly participates in the municipality water supply.

Keywords: Water quality, industrial waste, Ribeirão Santana, São Luís de Montes Belos, Goiás, Brazil

1. Introduction
Water is a fundamental inorganic constituent for the maintenance of life. Brazil has a vast water heritage with about 12% of the total fresh water on the planet [1]. However, in certain regions, water does not have adequate physical-chemical and microbiological aspects to be considered consumable due to impacts mediated by anthropic actions such as industrialization and urbanization of cities with disposal of industrial waste in effluents [2].
Monitoring the quality of water in a watershed is essential for achieving environmental sustainability [3]. Industrial activities generate substantial amounts of waste with different pollution potentials and improper disposal can cause soil and water contamination by reason of the high organic load and the presence of various chemical substances, heavy metals, etc. [4].
In Brazil, CONAMA Resolution No. 357 of March 17, 2005 [5] establishes the criteria for pre-treatment of industrial waste before release for final disposal in a water course. Relatedly, Ministry of Health Ordinance No. 2,914 of December 12, 2011 [6] provides for control and surveillance procedures for the quality of water for human consumption and its portability standard. For the water to be adequate and released for consumption, it is established that the agencies responsible for supplying a given location carry out pre-established tests that prove its viability for consumption. Among the tests are those that evaluate physical-chemical and microbiological parameters, as well as genotoxicity and mutagenicity [7].
In this context, this work aimed to evaluate the water quality from Ribeirão Santana downstream the area of São Luís de Montes Belos, Goiás, Brazil throughout the year 2021 possibly influenced by the emission of industrial effluents. The realization of this research is justified by the absence of studies carried out in the Municipality to certify the quality of water found in the main local hydrographic basin.

2. Materials and Methods
2.1. Location and characterization of the study area
The municipality of São Luís de Montes Belos is located 120 km from the capital Goiânia, in the mesoregion of the center of Goiás and microregion 09 [8].

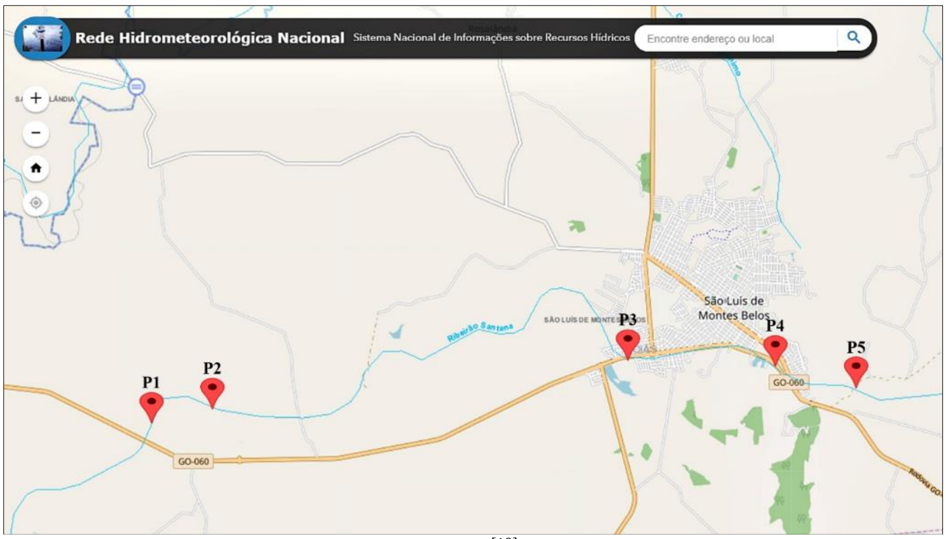
It has a total area of 825.999 km², altitude of 581.42 meters, tropical climate, 56.6% of sanitary sewage, population of 33,817 inhabitants, demographic density of 36.36 inhabitants per km² and Municipal Human Development Index (MHDI) of 0,731 (Figure 1) [9].

Source: IBGE, 2023 [9].

Fig 1: Geographic location of São Luís de Montes Belos, state of Goiás, Brazil.

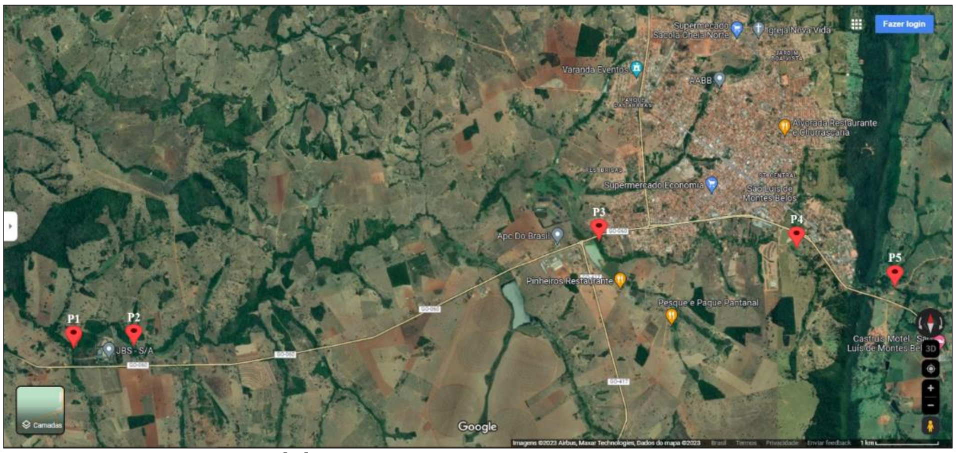
The research was carried out in a 12.4 km stretch of Ribeirão Santana (Tocantins Hydrographic Region) (Figure 2) that runs through the rural area of the municipality of São Luís de Montes Belos, downstream, at strategic fixed points corresponding to areas of effluents from a Food Industry (16°32'53.6"S 50°28'15.8"W), a Pharmaceuticals Manufacturing Industry (16°31'55.6"S 50°24'09.5"W) and a Dairy (16°31'45.8"S 50°22'20.2"W). For water analysis, 5 collection points (Figure 3) were established with the following classification:

- P_1 (control) - 1st collection point 300 meters before the 1st effluent dump site (16°32'50.0"S 50°28'33.0"W).
- P_2 - 2nd collection point 150 meters after the Food Industry effluent disposal site (16°32'50.4"S 50°28'01.1"W).
- P_3 - 3rd collection point at 150 meters after the effluent disposal site of the Pharmochemical Products Manufacturing Industry (16°31'53.0"S 50°23'47.5"W).
- P_4 - 4th collection point 150 meters after the Dairy Industry effluent disposal site (16°31'57.7"S 50°22'00.0"W).
- P_5 - 5th collection point at 1500 meters after the Dairy Industry effluent disposal site (16°32'19.0"S 50°21'06.3"W).



Source: Rede Hidrometeorológica Nacional, 2023 [10].

Fig 2: Ribeirão Santana stretch (Tocantins Hydrographic Region), municipality of São Luís de Montes Belos, state of Goiás, Brazil.



Source: Google Earth, 2023 [11].

Fig 3: Geographic location of water collection points from Ribeirão Santana, municipality of São Luís de Montes Belos, state of Goiás, Brazil.

2.2. Sampling

2.0 liters samples of surface class II water (Water that can be destined for human use) were collected quarterly throughout the year 2021, considering the seasonality of the dry and rainy season. Three collections were executed on the same day, in the afternoon of March 10th, July 10th and November 10th at the 5 sampling points discussed above. The observed precipitation on each collection day was ≤ 2.0 millimeters for March 10th, ≤2.0 millimeters for July 10th, and 26.1 millimeters for November 10th [12]. Water samples were collected approximately 20 cm below the surface in polyethylene containers with screw caps, previously degermed with phosphorus-free detergent, rinsed in deionized water and dried in an incubator at 35 ± 2 °C for 6 hours, sent and stored at 4 °C in the Laboratory of Physical Chemistry at the University Center Brasília de Goiás.

For the collection of water samples and putting into execution the genotoxicity, mutagenicity, physicochemical and microbiological evaluation, standards from CONAMA Resolution No. 357 of March 17, 2005 [5], CETESB, 2017 [7] and APHA 23rd [13] edition were followed.

2.3. Physical-chemical and microbiological evaluation

Physical-chemical and microbiological parameters were selected to provide a description for the collected water samples regarding the possible influence of effluents released along the rural area of the Ribeirão Santana, which surrounds the municipality of São Luís de Montes Belos. The determined parameters were: air temperature (ºC), water temperature (ºC), turbidity (UNT), pH, total solids (mg/L), conductivity (µS/cm), ammonia (mg/L), biochemical demand of oxygen (mgO_2/L), dissolved oxygen (mgO_2/L), chemical oxygen demand (mg/L), total phosphorus (mg/L), nitrate (mg/L), nitrite (mg/L), Total Kjeldahl nitrogen (mg/L), fecal coliforms (MPN/100 mL) and total coliforms (MPN/100 mL).

2.4. Genotoxic and Mutagenic analysis

For the evaluation of genotoxicity and mutagenicity, the *Allium cepa* test was developed based on Fiskesjö (1985) [14] and Zeyad *et al.* (2022) [15] with adaptations, in the Laboratory of Pharmacology and Toxicology at the University Center Brasília de Goiás. After collecting the water samples at the five points described above, 5 (five) *Allium cepa* bulbs arranged on 100 mL beakers were used to germinate with the root immersed directly in 50 mL of each sample at 24 ± 2 °C for 96 hours. Two more sequences with 5 (five) bulbs were used, one with deionized water as a negative control, and the other with potassium dichromate 0.006 mg/mL as a positive control, under the same conditions as the samples.

Subsequently, the germinated roots were collected, washed with distilled water and subjected to hydrolysis with 1N HCl at 60 °C for 10 minutes. Soon after, samples were cooled, washed once more with distilled water, subjected to slight pressure with cover slips to spread the cells on the slide and fixed with an ethanol-acetic acid solution (3:1, v/v) at 24 ± 2 °C for 24 hours. Fixed roots were then submitted to staining by the Quick Panoptic Kit® (0.1% triarylmethane, 0.1% xanthenes and 0.1% thiazines). Slides were immersed 5 (five) times for 1 second into each reagent of the Quick Panoptic Kit®, washed again with distilled water and dried at room temperature (24 ± 2 °C).

As for microscopic parameters, 500 (five hundred) cells were randomly quantified per slide, two slides for each sample, five samples for each collection group. For the genotoxic evaluation with the presence of chromosomal abnormalities (CA), chromosomal losses, delays and adhesions in distinct phases of the cell cycle were considered. For the mutagenic evaluation, the presence of micronuclei (MN) was numbered. Observation was performed under optical microscopy with 40X objective.

2.5. Statistical analysis

Windows version of the GraphPad Prism 5.01 software was used, and results were expressed as absolute values and/or mean ± standard deviation (SD). For the statistical treatment, one- or two-way ANOVA followed by Bonferroni post-tests were applied, with P values <0.05.

3. Results

Determinations results of physical-chemical and microbiological parameters in samples collected from Ribeirão Santana downstream the rural area of São Luís de Montes Belos, Goiás, Brazil; as well as the emission limits established by CONAMA Resolution No. 357 of March 17, 2005 [5] are demonstrated from Table 1 to Table 3.

It is observed in table 1 that before the effluent points, that is, in P_1, the quality of the flowing water collected on March 10, 2021 was fully within law parameters. However, significant change ($p<0.05$) was observed in water characteristics-especially in P_2 and P_3 -related to increase in turbidity, total solids, conductivity, ammonia, biochemical oxygen demand, chemical oxygen demand, total phosphorus, nitrate, nitrite, total Kjeldahl nitrogen, fecal coliforms and total coliforms. And, the pH and dissolved oxygen of the water collected in P_2, P_3 and P_4 was lower than that established by Brazilian legislation.

Table 1: Physical-chemical and microbiological evaluation of collected class II water samples on March 10, 2021 from Ribeirão Santana downstream the rural area of São Luís de Montes Belos, Goiás, Brazil.

Parameters	Standards[1]	Collection Points*				
		P_1	P_2	P_3	P_4	P_5
Air temperature (ºC)	-	29	29	30	30	29
Water temperature (ºC)	-	18.4	18.9	19.4	20.2	19.2
Turbidity (UNT)	100	10.6	28.4	63.8	41.3	31.1
pH	6.0 - 9.0	6.6	5.8	5.5	5.9	6.2
Total solids (mg/L)	500	14	283	564	491	118
Conductivity (µS/cm)	-	31	418	388	351	216
Ammonia (mg/L)	3.7	0.04	17.8	3.7	4.8	1.7
Biochemical oxygen demand (mgO_2/L)	5.0	1.93	369	272	208	111
Dissolved oxygen (mgO_2/L)	≥ 5.0	7.56	2.38	3.34	3.75	5.75
Chemical oxygen demand (mg/L)	-	14	131	789	454	28
Total phosphorus (mg/L)	0.1	0.0	4.2	0.5	0.2	0.2
Nitrate (mg/L)	10.0	0.2	2.88	6.16	4.51	2.18
Nitrite (mg/L)	1.0	0.003	0.76	0.77	0.16	0.15
Total Kjeldahl nitrogen (mg/L)	-	0.02	17.14	24.21	15.19	5.01
Fecal coliforms (MPN/100 mL)	1000	14	1619	1801	2201	433
Total coliforms (MPN/100 mL)	1000	52	4641	4670	5008	3199

[1] CONAMA Resolution No. 357 of March 17, 2005

*$p<0.05$. ANOVA and Bonferroni post-tests.

Table 2 also demonstrates that the collected water at P1 on July 10, 2021 was within parameters established by CONAMA Resolution No. 357 of March 17, 2005 [5]. Nevertheless, a marked deterioration ($p<0.05$) in water quality was observed as it received industrial waste from the Food Industry at P2 and from the Pharmochemical Products Manufacturing Industry at P_3, which were reflected in increased turbidity, total solids, conductivity, ammonia, biochemical oxygen demand, chemical oxygen demand, total phosphorus, nitrate, nitrite, total Kjeldahl nitrogen, fecal coliforms and total coliforms. In this same vein, water pH and dissolved oxygen were lower after effluents released along P_2, P_3, P_4 and P_5.

Table 2: Physical-chemical and microbiological evaluation of collected class II water samples on July 10, 2021 from Ribeirão Santana downstream the rural area of São Luís de Montes Belos, Goiás, Brazil.

Parameters	Standards[1]	Collection Points*				
		P_1	P_2	P_3	P_4	P_5
Air temperature (ºC)	-	30	30	31	30	29
Water temperature (ºC)	-	18.1	18.5	18.7	19.3	18.3
Turbidity (UNT)	100	7.1	14.2	18.5	75.1	7.7
pH	6.0 - 9.0	7.2	5.5	5.0	5.1	6.8
Total solids (mg/L)	500	23	418	662	1045	215
Conductivity (µS/cm)	-	77	571	108	222	112
Ammonia (mg/L)	3.7	0.01	23.1	14.6	4.3	1.1
Biochemical oxygen demand (mgO_2/L)	5.0	0.33	404	399	308	87
Dissolved oxygen (mgO_2/L)	≥ 5.0	7.89	2.18	3.55	3.94	5.37
Chemical oxygen demand (mg/L)	-	16	98	502	288	73
Total phosphorus (mg/L)	0.1	0.0	2.7	0.4	0.2	0.1
Nitrate (mg/L)	10.0	0.0	2.18	8.99	3.57	1.11
Nitrite (mg/L)	1.0	0.000	0.45	0.49	0.11	0.08
Total Kjeldahl nitrogen (mg/L)	-	0.00	11.31	18.08	3.14	2.44
Fecal coliforms (MPN/100 mL)	1000	24	1012	1141	1077	219
Total coliforms (MPN/100 mL)	1000	73	5104	3789	5211	2012

[1] CONAMA Resolution No. 357 of March 17, 2005

*$p<0.05$. ANOVA and Bonferroni post-tests.

In general, the water from Ribeirão Santana on November 10, 2021 was suitable for consumption up to P_1. From P_2 on a significant decrease ($p<0.05$) in water quality was observed, making it unsafe due to increases in turbidity, total solids, conductivity, ammonia, biochemical oxygen demand, chemical oxygen demand, total phosphorus, nitrate, nitrite, total Kjeldahl nitrogen, fecal coliforms and total coliforms. And, following the pattern of previous evaluations, the pH and dissolved oxygen were reduced after effluents thrown through the length of P_2, P_3, P_4 and P_5.

Table 3: Physical-chemical and microbiological evaluation of collected class II water samples on November 10, 2021 from Ribeirão Santana downstream the rural area of São Luís de Montes Belos, Goiás, Brazil.

Parameters	Standards[1]	Collection Points*				
		P_1	P_2	P_3	P_4	P_5
Air temperature (ºC)	-	28	28	28	29	28
Water temperature (ºC)	-	19.2	19.4	20.5	20.7	19.5
Turbidity (UNT)	100	81.2	85.1	89.7	88.1	80.9
pH	6.0 - 9.0	7.6	6.5	6.5	6.7	7.4
Total solids (mg/L)	500	341	489	512	565	360
Conductivity (µS/cm)	-	31	78	70	76	40
Ammonia (mg/L)	3.7	0.01	2.3	1.6	1.5	0.6
Biochemical oxygen demand (mgO_2/L)	5.0	2.33	4.18	4.16	4.18	3.01
Dissolved oxygen (mgO_2/L)	≥ 5.0	5.55	2.13	3.19	3.82	5.18
Chemical oxygen demand (mg/L)	-	77	93	114	112	90
Total phosphorus (mg/L)	0.1	0.0	1.6	0.6	0.1	0.0
Nitrate (mg/L)	10.0	0.0	1.14	2.08	1.09	0.77
Nitrite (mg/L)	1.0	0.000	0.44	0.31	0.11	0.04
Total Kjeldahl nitrogen (mg/L)	-	0.00	9.22	11.14	8.06	2.16
Fecal coliforms (MPN/100 mL)	1000	104	2045	2118	1965	804
Total coliforms (MPN/100 mL)	1000	211	2761	2913	2909	1414

[1] CONAMA Resolution No. 357 of March 17, 2005
**p*<0.05. ANOVA and Bonferroni post-tests.

Except for P_1 where no anthropic influence was observed, in general, genotoxic and mutagenic analysis by the *Allium cepa* test indicated significant levels (****p*<0.001) when the bulbs were exposed to samples from industrial waste effluents, especially in P_2, P_3 and P_4 (Table 4). Genotoxicity and mutagenicity were more significantly observed in July (Month of low rainfall) suggesting seasonal influence with greater accumulation of agents through the length of Ribeirão Santana.

Table 4: Genotoxic evaluation with the presence of chromosomal abnormalities (CA) and mutagenic evaluation with the presence of micronuclei (MN) developed by the *Allium cepa* test on water samples from Ribeirão Santana downstream the rural area of São Luís de Montes Belos, Goiás, Brazil.

		CA (%) Mean ± SD	MN (%) Mean ± SD
March 10th 2021	Negative control	0.97 ± 0.10	0.23 ± 0.03
	Positive control	4.37 ± 1.18	11.61 ± 2.77
	P_1	0.81 ± 0.59	0.56 ± 0.38
	P_2	4.18 ± 2.02***	6.14 ± 3.18***
	P_3	6.01 ± 2.64***	7.68 ± 3.90***
	P_4	2.05 ± 1.55***	4.06 ± 2.54***
	P_5	1.09 ± 0.38*	1.88 ± 0.89*
July 10th 2021	Negative control	0.61 ± 0.04	0.08 ± 0.02
	Positive control	6.64 ± 2.99	13.03 ± 2.19
	P_1	0.36 ± 0.18	0.69 ± 0.14
	P_2	4.69 ± 2.18***	8.69 ± 3.74***
	P_3	6.69 ± 3.14***	10.08 ± 2.55***
	P_4	2.62 ± 0.76***	6.06 ± 3.07***
	P_5	1.24 ± 0.54*	2.58 ± 1.12**
November 10th 2021	Negative control	0.82 ± 0.12	0.34 ± 0.04
	Positive control	4.66 ± 2.02	13.54 ± 3.48
	P_1	0.94 ± 0.83	0.88 ± 0.55
	P_2	3.06 ± 2.28***	5.50 ± 3.08***
	P_3	3.31 ± 1.77***	6.32 ± 3.84***
	P_4	2.11 ± 1.43***	3.81 ± 1.70***
	P_5	1.69 ± 0.79*	1.18 ± 0.67**

**p*<0.05, ***p*<0.01, ****p*<0.001. ANOVA and Bonferroni post-tests.

4. Discussion

Despite the small percentage of potable water available in the world, is notorious the anthropic action in recent decades through processes that alter its quality, such as bacteriological and chemical contamination, eutrophication and silting, mainly originated from the release of domestic and industrial waste into rivers and lakes [16]. In Brazil, CONAMA Resolution No. 357 of March 17, 2005 [5] provides for the classification of bodies of water and environmental guidelines for their classification, as well as establishes conditions and standards for the release of effluents and other measures.

The characteristics of a contaminated effluent can be expressed by three aspects: physical, chemical and biological, whereas the physical corresponds to the color, responsible for the water coloring and formed from dissolved solids; turbidity represents the degree of interference with the passage of light through the water, giving it a turbid appearance and formed by suspended solids. The chemical criteria depend on the effluent origin, involving the pH, which indicates water condition of acidity, neutrality or alkalinity and is formed by dissolved solids; nitrite nitrogen consists of suspended and dissolved solids, and acts in eutrophication and nitrification; biochemical oxygen demand and chemical oxygen demand determine the content of organic matter in contaminated water. Finally, the biological criteria involve the existence of wastewater microorganisms, such as pollution indicators (total coliforms and fecal coliforms), flow variations, among others [5].

Effluent treatment from Food Industries, Pharmochemical Products Manufacturing Industries and Dairy Industries are mostly of the biological type, with the sole objective of removing organic matter from the industrial waste through oxidation metabolism and cell synthesis. This is due to the large amount of biodegradable organic matter present in its composition [7]. In this work, the physical, chemical and microbiological data obtained from the Ribeirão Santana water revealed a deterioration in its quality as it received industrial waste compared to the point prior to disposal (Collection point P_1).

Average values of air temperature for the nine months sampled in 2021 are typical for the tropical climate of central Goiás and presented a minimum of 28 °C and a maximum of 31 °C. As for the water temperature, a small variation was observed, with data between 18.1 °C on July 10th, and 20.7 °C on November 10th. Water temperature is an important characteristic that determines trends in changing its quality and is also associated with climate variations, which can be seasonal and even daily [7]. Values for turbidity must not exceed 100 UNT for class II fresh waters [5]. The turbidity of a water sample refers to the degree of intensity attenuation that a beam of light suffers when crossing it, this occurs due to the presence of suspended solids such as organic matter, etc. In this parameter, all collected samples demonstrated agreement values, with an average of 48.18 UNT. It is worth mentioning that turbidity values were increased in the three collections after Ribeirão Santana received industrial waste.

Ecosystems are always subject to impacts caused by pH variation, as pH fluctuations in water bodies can affect the fauna and flora present in these environments [31]. Waters with very low pH values are corrosive and aggressive, which can inhibit bacterial growth; as well as high pH values can lead to an excessive bacteria proliferation [18]. CONAMA Resolution No. 357 of March 17, 2005 [5] establishes a pH range for class II water bodies between 6.0 and 9.0. In this study, six moments were observed in which results found disagreed with the Resolution, on March 10th at P_2, P_3 and P_4; and on July 10th also at P2, P3 and P4. It is suggested that the release of industrial effluents is directly responsible for altering pH values through the 12.4 km studied stretch of Ribeirão Santana.

Total solids correspond to all matter that remains as residue after a sample evaporation, drying, or calcination at a pre-established temperature during a fixed time. The presence of total solids in high concentration can cause damage to fish and aquatic life [7]. As with turbidity, the limit proposed by CONAMA Resolution No. 357 of March 17, 2005 [5] for class II freshwater environments -500 mg/L of total solids - was exceeded in the three collections at P_2, P_3 and P_4, corroborating that the industrial waste released negatively alter Ribeirão Santana's water quality. Regarding electrical conductivity, the sampled points showed values between 31 µS/cm and 571 µS/cm, with the highest means in P_2 and P_4, respectively 355.66 µS/cm and 216.33 µS/cm. These data were found above 100 µS/cm, level from which it describes impacted environments, being considered an indirect parameter of water pollution. Electrical conductivity is the way to numerically demonstrate the ability of water to conduct electrical currents, it depends on temperature and ion concentrations and indicates the presence of salts. Therefore, it indirectly represents the pollution in a body of water, that is, as more dissolved solids are added, the conductivity increases [19].

The evaluation of phosphorus (P) and nitrogen (N) contents in water bodies is important to estimate the eutrophication state. Eutrophication occurs when there is a high concentration of nutrients such as N and P. As they are essential for the growth of aquatic plants, they increase the degradation of the aquatic ecosystem that is exposed to this phenomenon [20]. The ammonia and total phosphorus parameters exhibited values above the standard in the three collections, especially at P_2; and total kjeldahl nitrogen particularly at P_3. However, nitrate and nitrite presented values accepted by the legislation at sampling points during the campaigns [5].

The biochemical oxygen demand indicates the amount of molecular oxygen required to oxidize organic matter by aerobic microbial decomposition to a stable inorganic form. Bodies of water that show a high value of biochemical oxygen demand indicate the presence of a large amount of organic matter in the environment, which can lead to a complete extinction of oxygen in the water and can cause the disappearance of fish and other forms of life water [7]. During the studied period, an alarming increase of up to eight thousand times the maximum allowed from P_2 onwards was observed in the first two collections in relation to what is recommended by the Brazilian legislation [5]. Dissolved oxygen is an indicator used in water pollution control and one of the most important dissolved gases for the characterization and dynamics of an aquatic ecosystem. The concentration of dissolved oxygen in a body of water varies as a function of temperature, altitude and water aeration and photosynthesis [13]. The determination of dissolved oxygen concentrations in a body of water is fundamental for controlling the water quality of this environment, since oxygen is involved in several chemical and biological processes [7]. In general, dissolved oxygen concentrations decreased from P_2 onwards [5], with greater depletion precisely at this point where the Ribeirão Santana watercourse receives effluents from the Food Industry. Finally, chemical oxygen demand also presented worrying data, with values well above the acceptable for potability and safe consumption of water from Ribeirão Santana, especially in P_3.

In the evaluation of water quality, the use of biological indicators of contamination is very important. Analyzes of fecal coliforms and total coliforms are considered the main indicators of biological contamination and are widely used as indicators of sanitary pollution [7]. Although not generally pathogenic, the presence of bacteria from the coliform group in water indicates the possibility of contamination by fecal matter and may therefore contain pathogenic microorganisms responsible for the transmission of waterborne diseases [13]. In general, the concentration of fecal and total coliforms in Ribeirão Santana intensely increased after effluents thrown through the length of P_2 and

P_3 [5], confirming that a large mass of *in natura* organic matter is released into Ribeirão Santana, making its water unfit for human and animal consumption.

The frequent release of industrial effluents without proper treatment into rivers, lakes and streams represent a significant risk to ecosystems, not only because of their substantial volume, but also because of their chemical complexity, as different toxins interact with each other and form complex mixtures with specific characteristics and genotoxic potential [21]. Genotoxic substances are capable of inducing damage in parental cells, resulting in chromosomal fragments that result in breaks that are not incorporated into the main nucleus of daughter cells after mitosis called micronuclei, containing small bodies of DNA located in the cytoplasm. Micronuclei appear in telophase and are the result of acentric chromosomal fragments, originating from isochromatid or chromatid breaks or mitotic spindle dysfunction, and may appear more than once per cell [22].

The *Allium cepa* test is widely applied as an environmental monitoring test [23]. The *Allium cepa* test-organism method is validated by the International Chemical Safety Program (IPCS, WHO) and the United Nations Environment Programme (UNEP) as an efficient test for *in situ* analysis and biomonitoring of cytotoxicity, genotoxicity and mutagenicity [24]. The induction of micronuclei may be related to chromosome delays during anaphase, due to spindle malfunction or the presence of acentric fragments derived from chromosomal breaks [22]. Complementarily, the presence of nuclear abnormalities shows alterations resulting from induction by cytogenotoxic agents, such as industrial waste [25]. Differences in the frequency of micronuclei between collection points in a river is conceivably to the volume of water (variation in the concentration of genotoxic agents) and/or the cumulative effect of diverse sources [26]. In this study, a high frequency of chromosomal abnormalities and micronuclei was observed in *Allium cepa* cells immersed into water samples from Ribeirão Santana, particularly those collected in P_2 and P_3 (Figure 4). Data from this work converge with several studies that demonstrated genotoxic and mutagenic potential in water samples from rivers and lakes that received industrial effluents [27, 28, 29, 30].

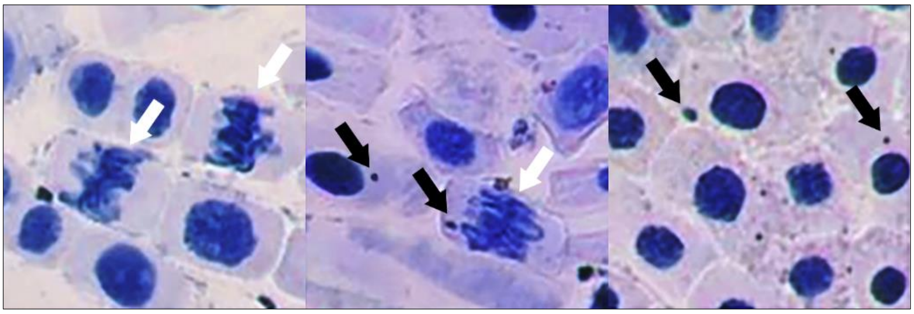


Fig 4: Chromosomal abnormalities (white arrows) and micronuclei (black arrows), magnification 40 times. *Allium cepa* test-organism method by Quick Panoptic Kit® staining on water samples from Ribeirão Santana downstream the rural area of São Luís de Montes Belos, Goiás, Brazil.

The expressive polluting potential at the evaluated points of industrial effluent emission (P_2 and P_3) can be attributed to the fact that they receive a high load of waste, maximizing the levels of contamination. The occurrence of mutagenicity and genotoxicity was observed in the three collections, with higher values in the month of July, possibly due to low rainfall and a decrease in river volume. Furthermore, it should be noted that this study did not determine toxic organic compounds and toxic metals, which could indicate additional results on degrees of toxicity.

5. Conclusion

In general, results obtained in this study describe a high polluting potential of effluents from the Food Industry, the Pharmaceuticals Manufacturing Industry and the Dairy through the analyzed stretch of Ribeirão Santana. Dangerous physical-chemical and microbiological effects, as well as high genotoxicity and mutagenicity observed by cytogenetic tests raise concerns and highlight the necessity for constant water quality monitoring, as Ribeirão Santana directly participates in the water supply of the municipality of São Luís de Montes Belos, Goiás, Brazil.

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7. Declaration of interest statement

Authors report no declarations of interest. Authors alone are responsible for paper content and writing.

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