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# Effect of anthropized water on eukaryotic organisms

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## ABSTRACT

This work explored whether anthropized environments have the potential to promote cellular alterations in eukaryotic organisms. Wild fish that are in constant contact with anthropized water were evaluated using the following variables: presence of nuclear alterations and micronuclei in erythrocytes using the Feulgen method; and tissue analysis of the gills and liver through histological sections using Hematoxylin and Eosin staining. Therefore, 200 individuals were analyzed over 24 months. The results show that these environments are promoters of significant changes in liver and gill tissue and induce nuclear changes in erythrocytes. The analyses were subjected to statistical tests at a significance level of 0.5%.

**Keywords:** aquatic environments, bioindicator, genotoxicity, histopathology.

# O efeito da água antropizada sobre organismos eucarióticos

## RESUMO

Os ambientes antropizados têm o potencial de promover alterações celulares em organismos eucarióticos? Esta questão impulsionou o objetivo deste trabalho. Os peixes selvagens que estão em constante contato com água antropizada foram avaliados nas seguintes variáveis: presença de alterações nucleares e micronúcleos em eritrócitos usando o método Feulgen; e análise de tecidos das brânquias e fígado através de secções histológicas usando Hematoxilina e coloração de Eosina. Por conseguinte, 200 indivíduos foram analisados durante 24 meses. Os resultados mostram que estes ambientes são promotores de alterações significativas no fígado e no tecido das brânquias e induzem alterações nucleares nos eritrócitos. As análises foram submetidas a testes estatísticos a um nível de significância de 0,5%.

**Palavras-chave:** ambientes aquáticos, bioindicador, genotoxicidade, histopatologia.

## 1. INTRODUCTION

Aquatic ecosystems are exposed to a variety of potentially polluting substances that originate from human activities. Domestic, industrial, agrochemical effluents are some of the main impacts of anthropization, and they are characterized by having a complex composition,

which includes organic and inorganic chemicals, metals, pathogenic microorganisms, as well as substances whose effects on the environment are still unknown.

Thus, studies have been carried out to evaluate the pollution levels of aquatic environments, including use of biological parameters based on the assessment of biological responses of organisms related to the environment where they live (Buss *et al*., 2003; Dieterich and Gaete, 2021; Lins *et al*., 2010; Kasper *et al.*, 2018; Lassen *et al*., 2021). Fish are considered excellent bioindicator organisms, since they are able to provide reliable information regarding the state of the environment, are present in nearly all rivers and lakes, and are easy to collect and handle (Moreira *et al*., 2010).

In fish, xenobiotics may cause cellular level damage, compromise the functioning of vital organs, as well as bioaccumulate and biomagnify (Simonato *et al.*, 2006). One of the tools used to verify the occurrence of these interactions is the histopathological evaluation of organs such as the liver and gills.

The gills perform vital functions in fish. Their activity promotes gas-exchange processes, osmoregulation, acid-base balance and excretion of nitrogen compounds (Lima *et al*., 2011; Machado and Fanta, 2003). They are affected by being in direct contact with substances through water and are one of the xenobiotics entry routes.

The liver, on the other hand, has a function similar to other vertebrates. It is responsible for the metabolism of proteins, lipids and carbohydrates, for the biotransformation of toxic substances in order to eliminate them from the body (Paris-Palacios *et al*., 2000), besides presenting bioaccumulation properties, being able to store toxic substances when they are present in high concentrations.

When reaching tissues, many xenobiotics have the ability to promote molecular harm, such as DNA damage, causing clastogenic and aneugenic effects (Ansari *et al*., 2011). Contaminants that cause DNA damage are known as genotoxic and there are efficient tests to evaluate its presence in aquatic environments, including the Micronucleus (MN) and Nuclear Abnormalities (AN) tests. The MN test was originally developed by Von Ledebur and Schmid (1973), with young rodent erythrocytes; later, the test was successfully adapted by Hooftman and Raat (1982) using fish peripheral blood. It is considered a low-cost test, has an easy methodology and demonstrates reliable results. AN, on the other hand, are conceptualized as changes in the morphology of the nuclear envelope, providing evidence of the presence of chemicals in water bodies that interact with the evaluated organism and result in genetic material alterations after mitosis (Fenech, 2020).

Therefore, this study aimed to evaluate the influence of environmental contaminants in anthropized areas of the Ijuí River through the histopathological responses in gills and livers of fish captured at five points of the river, as well as to perform genotoxic analysis, using the MN and AN tests in peripheral erythrocytes of these organisms.

## 2. MATERIAL AND METHODS

This research was approved by the Committee of Ethics on the Use of Animals - CEUA of Federal University of Fronteira Sul (UFFS), under protocol nº 23205.000642/2018-45.

### 2.1. Study area and sampling location

The water body used for this research was the Rio Ijuí, located in the southern region of Brazil, northwest of Rio Grande do Sul state, at the coordinates 27º45' and 26º15' south latitude and 53º15' and 56º45' west longitude, where five collection points were defined (Figure 1).

P1 is located at the coordinates 28º19’10.81” S and 53º58’54.52” W, close to urban and large agricultural monoculture areas; P2 is located at the coordinates 28º18’50.74” S and 54º18’38.39” W, and receives water from two rivers that cross the city of Santo Ângelo - RS,

that concentrates industries of several branches; P3 is located at the coordinates 28º11'53.20" S and 54º41'25.47" W which, precedes a hydroelectric dam and is surrounded by agricultural areas; P4 is located at the coordinates 28º08'13.35" S and 55º03'50.77" W, close to agricultural areas; and P5 is located at the coordinates 28º03'29,00" S and 55º07'39.00" W, close to the mouth of Ijuí River, where it flows into Uruguay River.

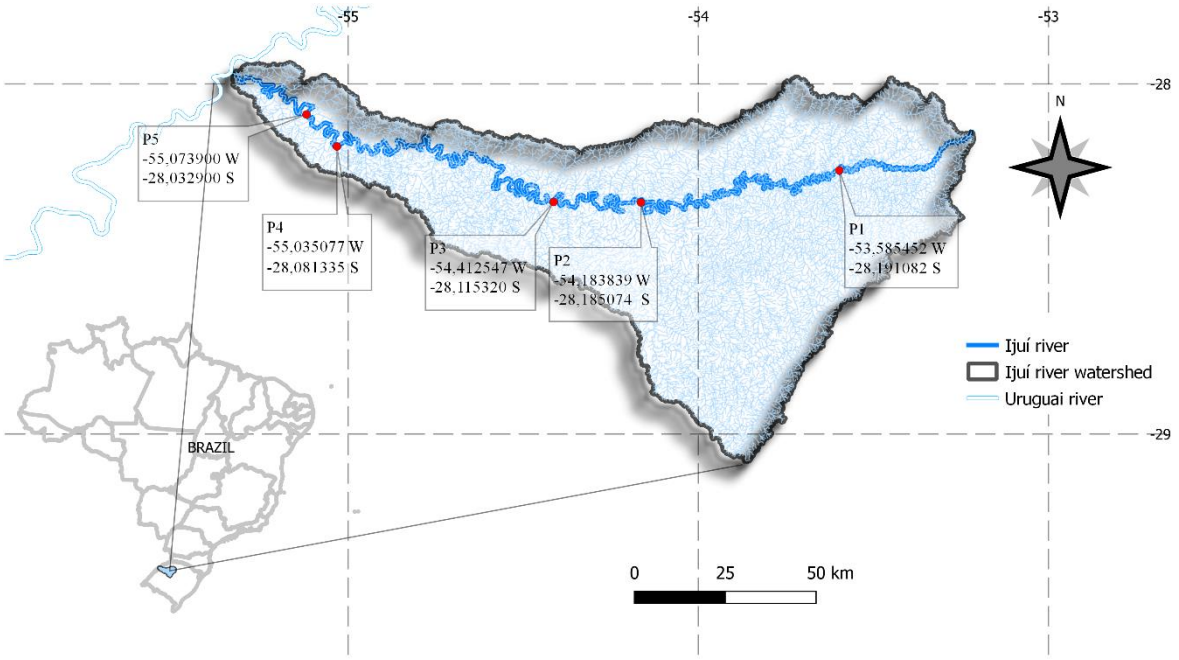


**Figure 1.** Location of the Ijuí River and the respective study points.

## 2.2. Fish collection

Four collections were made, one in each season of the year, comprising spring of 2017, summer, autumn and winter of 2018. Ten fish specimens were captured per season, totaling 40 fish per point, regardless of the species. The capture was made with fish hooks and live bait.

## 2.3. Physico-chemical parameters analysis

The physico-chemical parameters temperature (ºC), pH, dissolved oxygen (DO), conductivity (COND), total dissolved solids (TDS) and salinity (SAL) were measured using a multiparameter probe in all collections.

## 2.4. Smear slides

After capture, the fish were anesthetized by immersion in 3% Eugenol solution and immediately sacrificed by decapitation. The blood was extracted using a glass capillary containing 3% EDTA, to avoid coagulation.

Three blood smear slides per fish were made and left to dry at room temperature; and then, fixed in methanol for 15 minutes and stored in slide holders for transportation to the Genetics Laboratory of the Federal University of the Fronteira Sul - Campus Cerro Largo, RS. In the laboratory, the slides were subjected to hydrolysis in 5N HCl for 15 minutes at room temperature and washed in distilled water. Furthermore, nucleus staining with Schiff's reagent was carried out for 2 hours, followed by washing in distilled water and immersion in Fast-Green for 1 minute for counter staining.

## 2.5. Erythrocytes analysis

Using an trinocular microscope (Olympus® CX31), 1000 cells per slide were analyzed, totaling 3000 cells per fish, counting micronucleated cells (MN) and nuclear abnormalities

(AN) of the following types: “blebbed”, a nucleus that has a small invagination in the nuclear envelope; “Lobbed”, a nucleus with a greater invagination than the blebbed; “Notched”, nucleus with a notable cut in the content of the nuclear material; “Vacuolated”, nucleus that has a region resembling the vacuoles inside, “binu”, two well-defined nuclei; “Segmented”, two nuclei connected by a broad filament.

The data extracted from the counts were converted into frequencies of MN and AN using the Equation 1:

$$\text{Frequency} = \text{Total counted MN or AN X 100 total analyzed cells} \quad (1)$$

**2.6. Histological processing**

For histology, the gills and liver of the fish were extracted, cut in 2 x 2 cm blocks and stored for 48 hours in 10% formaldehyde for fixation. After fixation, the gills were decalcified in a solution of ethylenediaminetetraacetic acid (EDTA 10%) for 96 hours. Posteriorly, the samples were subjected to the following stages: alcoholic dehydration at increasing concentrations of ethanol (70% to 100%), immersion in Xylol, and paraffin impregnation. Then, the blocks were cut in a microtome with thickness of 5 µm, placed on slides and stained with hematoxylin and eosin (HE). The slides were analyzed using a trinocular optical microscope (Olympus® CX31).

**2.7. Histological analysis**

Histological changes in liver and gill tissue were analyzed following the classification proposed by Poleksic e Mitrovic-Tutundzic (1994), according to the severity stage, in which, Stage I corresponds to changes considered mild, that don’t alter tissue function; Stage II corresponds to moderate changes, that partially alter the tissue function; while Stage III represents the severe alterations, that completely alter the tissue function (Table 1).

**Table 1.** Classification of histopathological changes in the liver and gills by the severity of the lesions, according to Poleksic e Mitrovic-Tutundzic (1994) with adaptations.

| Stages | Histological changes in liver | Histological changes in Gills |
|---|---|---|
| **I** | Nucleus on the periphery of the cell<br>Cell contour deformation<br>Nucleus contour deformation<br>Cellular hypertrophy<br>Nuclear hypertrophy<br>Cellular atrophy<br>Nuclear atrophy<br>Melanomacrophage centers<br>Cytoplasmic vacuolization | Lamellar epithelium hypertrophy<br>Elevation of the lamellar epithelium<br>Lamellar epithelium hyperplasia<br>Lamellar disarrangement<br>Dilatation of the blood sinus<br>Blood sinus constriction<br>Vascular congestion |
| **II** | Nuclear vacuolization<br>Cytoplasmic degeneration<br>Nuclear degeneration<br>Cell disruption<br>Biliary stagnation<br>Hyperemia | Incomplete lamella fusion<br>Complete fusion of some lamellae<br>Complete fusion of several lamellae<br>Epithelial rupture<br>Lamellar aneurysm |
| **III** | Focal necrosis | Destruction of the lamellar epithelium<br>Vascular congestion<br>Necrosis |

Based on this classification, the Histological Changes Index (HCI) was calculated using

the following equation: HCI = 100. ∑SI +101. ∑SII +102. ∑SIII where, ∑SI, ∑SII and ∑SIII = sum the of changes in stages I, II and III; 100 = multiple of phase I; 101 = multiple of phase II; 102 = multiple of Phase III.

Once the HCI values were obtained, organ lesions were classified in four categories: normal functioning - HCI from 0 to 10; mild changes - HCI from 11 to 20; moderate changes - HCI from 21 to 50; severe lesions - HCI from 51 to 100.

### 2.8. Statistical analysis

For statistical analysis, the frequencies between points, and between seasons of the year, were submitted to the Kruskal-Wallis test. The means were submitted to the Tukey test of multiple comparisons between the points and the seasons.

The means of the HCI values were compared between points and between seasons, using the t-test to compare independent samples, adopting $p \leq 0.05$ as significant.

## 3. RESULTS AND DISCUSSION

The state of Rio Grande do Sul is the third largest consumer of pesticides in Brazil (IBGE, 2017; Pignati, 2017) and Ijuí River is located within an area where its use is intensive, besides being close to cities that discharge urban and industrial effluents into tributary rivers or directly into Ijuí River. The presence of agricultural areas is evident at all points, with a predominance of monoculture and livestock, that advances over the riparian forest to the banks of the river.

The riparian forest works as a natural filter for water bodies, holding contaminants carried by rainwater and soil through processes such as erosion and aggradation, besides being the habitat of countless species of birds, reptiles and mammals that use the river as a source of food (Lowrance *et al*., 1997).

The five collection points established for this study were strategically selected with the aim to comprise the most diverse environments and their potential impactors. All points show signs of anthropization, characterized by the large agricultural areas due to the release of domestic and industrial effluents, with worse conditions at P1 and P2.

### 3.1. Collected fish species

For the current study, 200 specimens of native fish belonging to five genus were captured. These were *Astyanax, Steindachnerina, Hypostomus, Galeocharax and Pimelodus.*

### 3.2. Physico-chemical parameters analysis

The physico-chemical parameters (Table 2) of the four seasonal collections, at the five points, meet resolution nº 357 (CONAMA, 2005), being classified as Class 2 fresh water.

**Table 2.** Average and standard deviation of the physico-chemical parameters of the four seasons of the year, at the five collection points.

| | **Means and standard deviation of the physico-chemical parameters** | | | | | |
|---|---|---|---|---|---|---|
| **Point** | **DO mg/L** | **pH** | **COND µS/cm** | **TDS mg/L** | **SAL %** | **T ºC** |
| **P1** | 6.1±0.3 | 7.18±0.1 | 87.6±10.1 | 60.85±9.2 | 0.04±0.01 | 20.1±2.3 |
| **P2** | 6.6±0.8 | 7.24±0.3 | 115.1±12.0 | 81.2±5.9 | 0.06±0.01 | 17.5±5.9 |
| **P3** | 6.5±0.9 | 6.8±0.01 | 124.5±2.6 | 90.3±15.1 | 0.06±0.01 | 19.2±9.5 |
| **P4** | 6.3±1.7 | 7.4±0.1 | 119.4±17.6 | 83±20.6 | 0.06±0.01 | 17.6±8.1 |
| **P5** | 6.3±1.6 | 7.4±0.05 | 194±1.6 | 128.9±7.1 | 0.09±0.04 | 17.6±10.3 |
| **CONAMA 357/2005** | >5.0 | 6.0 ≥ 9.0 | 60> | <500 | <0.5 | |

* DO = dissolved oxygen; COND = conductivity; TDS = total dissolved solids; SAL = salinity.

### 3.3. Erythrocytes analysis

In the present study, the presence of MN (Figure 2) and AN of the types "blebbed", "lobbed", "notched", "vacuolated", "binucleo", "segmented" (Figure 3) were observed.

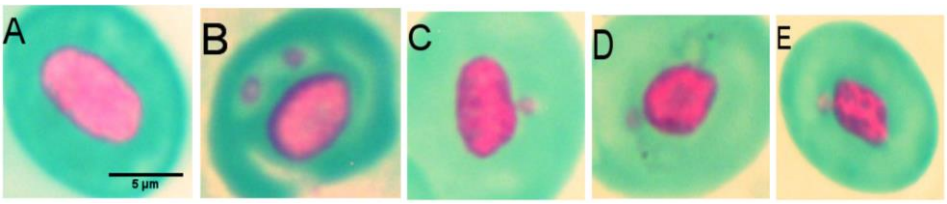


**Figure 2.** Normal erythrocyte (A) and micronucleated erythrocytes (B, C, D and E) observed in a 100x objective lens.

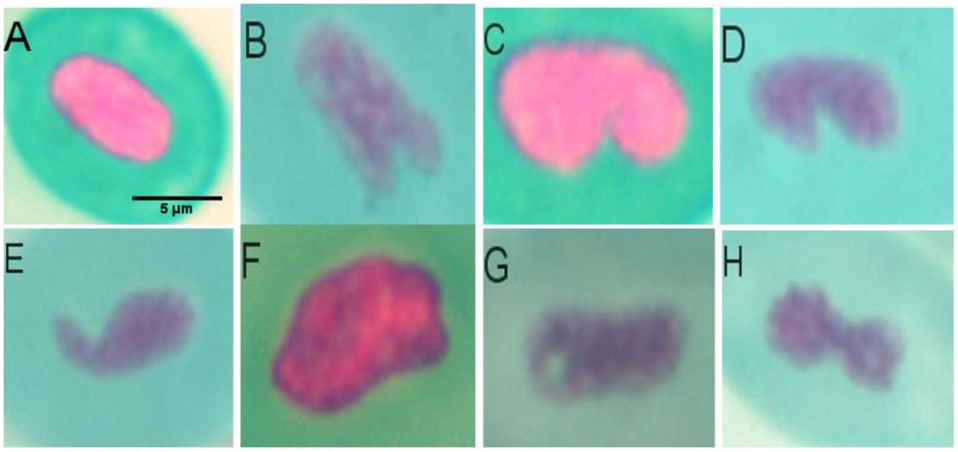


**Figure 3.** Erythrocytes from the analyzed fish with AN: A - normal; B and C - blebbed; D and E - lobbed; F - notched; G - vacuolated; and H – segmented.

The fish collected at the five points of Ijuí River, in general, did not exhibit significant presence of MN, except for Point 3 in the spring (Figure 4A), where the index was higher than expected, which is 0.2%. The data collected from cells with AN, showed higher values than the index of cells with MN, with a greater increase in fish from P2 in the spring, summer and autumn seasons (Figures 4A, B, C).

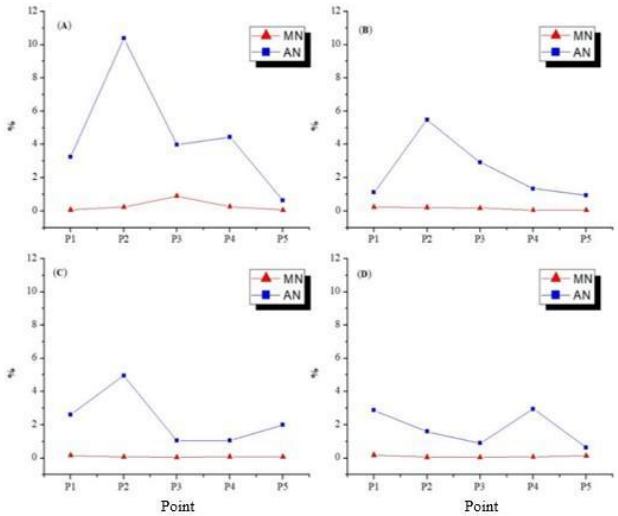


**Figure 4.** Frequency (%) of MN and AN in 10 fish/season, being A (Spring) B (Summer) C (Autumn) and D (Winter).

The data submitted to the Kruskal-Wallis test for the frequencies of MN do not differ between seasons and between points, since p-value > 0.05 for both. Furthermore, the means of MN and AN were submitted to the Tukey test for multiple comparison (Table 3), and the results corroborate with the Kruskal-Wallis test.

**Table 3.** Tukey test of the means of MN and AN in 40 fish/season (3,000 analyzed cells per fish).

| Season | MN* mean | AN* mean |
|---|---|---|
| Spring | 91.4 a | 1532 a |
| Summer | 45 a | 894 a |
| Autumn | 26.4 a | 827.2 a |
| Winter | 26.4 a | 585.8 a |

* Means followed by the same letter do not differ at a 5% significance level.

The analyzed fish present non-significant levels of MN, with the exception of Point 3 in the spring, which has an index higher than the expected, which is 0.2%, considered a level of spontaneous formation of MN (Mañas *et al*., 2009). This higher frequency of MN in P3 reflects the conservation status of the area that is characterized by the absence of riparian forest, presence of pig farming activities and agricultural monocultures. In addition, the spring season includes a rainy period that increases the process of runoff and deposition of waste on stream beds, which may explain this increase in comparison to the other analyzed periods.

According to Heddle *et al*. (1991), the MN are short-term responses to a genotoxic substance, hence, their occurrence depends on the intensity of exposure to a xenobiotic and does not depend on the duration of that exposure. Another factor to be considered is that the maximum induction of MN occurs between one and five days of exposure, which converges with the results found by Al-Sabti e Metcalfe (1995); (Grisolia and Cordeiro, 2000) that obtained higher levels of MN induction between two and seven days of exposure to mutagens and a decrease in the frequency after the fourteenth day of exposure.

Thus, the obtained results may reflect a time window in which the caught fish do not present significant levels of MN. We can also point out that the frequency of MN within a cell population is highly dependent on the kinetics of cell proliferation. This kinetics may vary according to the species and the evaluated tissue. Thus, it is not possible to establish an ideal time for an exposure and the consequent appearance of MN. Due to these variables, the joint analysis of AN is important (Grisolia and Cordeiro, 2000; Fenech, 2020).

It can be observed that the presence of AN was superior to the presence of micronuclei, with increased frequency at the point that is close to the urban area (P2) in the spring, summer and autumn seasons (Figure 4A; 4B; 4C), providing evidence that seasonality can increase the levels of xenobiotics and contaminants in periods of rain due to runoff, and in periods of drought, increasing their concentration (Carrola *et al.*, 2014).

The increase in AN is consistent with other studies that demonstrated the same behavior when these tests are applied, as in the findings by Carrola *et al.* (2014) that identified a higher frequency of AN than MN in fish erythrocytes from three rivers in Portugal. Grisolia *et al*., (2009) evaluated the presence of MN and AN in erythrocytes from different species of fish from the Paranoá Lake - DF and observed a higher frequency of AN. Matozo *et al.* (2015), verified that the fungicide Ridomil, when in contact with fish, induces a higher frequency of AN than MN.

Ayllon and Garcia-Vasquez (2001) suggested that AN should be included in fish genotoxicity analyzes to make the study more reliable, because bioassay tests with fish can present different responses to different compounds.

AN are changes caused by the same agents that induce MN; however, the molecular mechanisms that cause the changes are little known (Palhares and Grisolia, 2002). However, it has been reported that they are related to errors that occur during mitotic processes, and that after these errors occur, the repair mechanisms are activated and try to reorganize the nucleus by giving conditions for the cells to finish division (Fenech, 2000).

The data obtained in this research show that compounds present in the waters are capable of interacting with the DNA of fish, inducing errors in the mitotic processes and, in face of that, the repair mechanisms are activated, minimizing the appearance of MN and increasing AN.

### 3.4. Histological analysis

The gills' HCI showed that the lesions were mild to severe (Figure 5). Severe injuries were observed in P1 in summer season and in P5 in winter season.

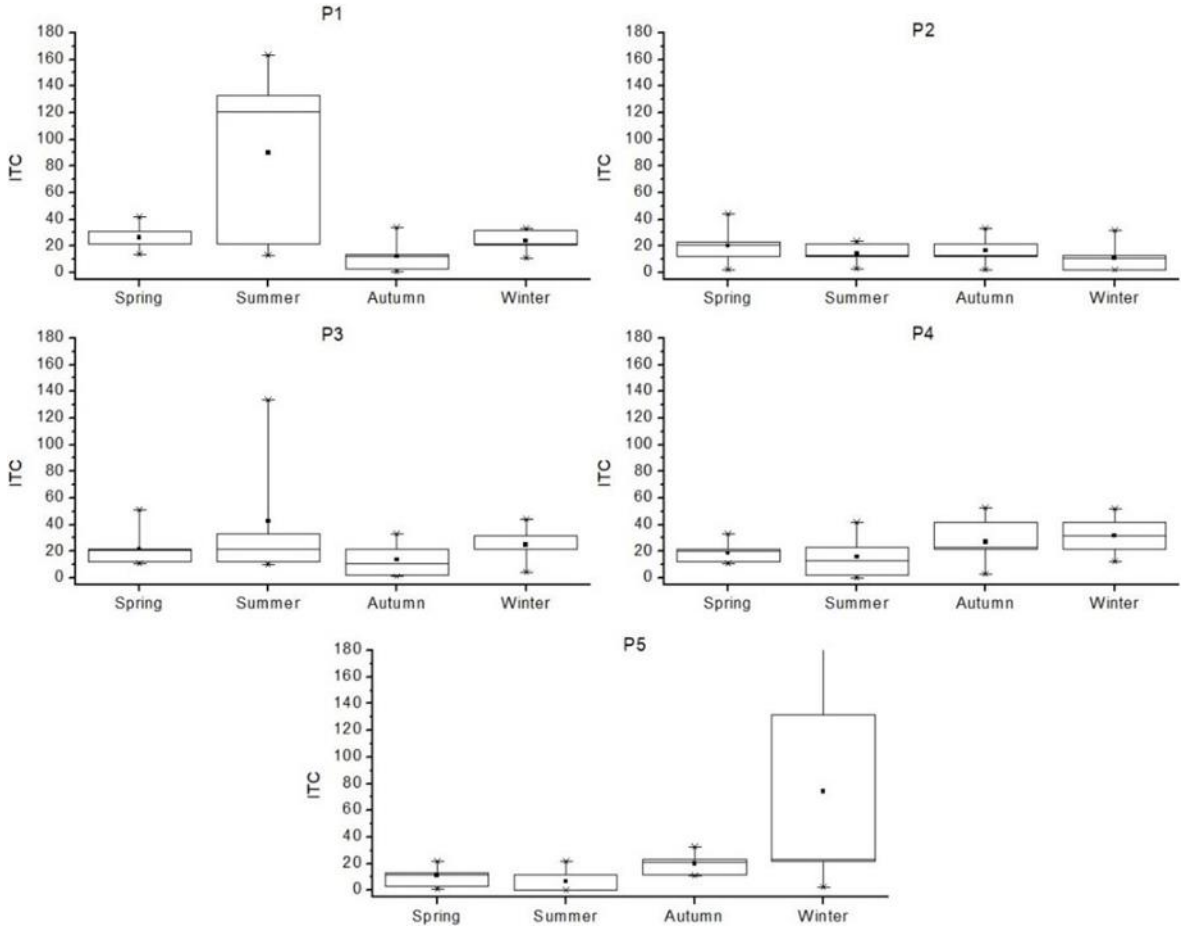


**Figure 5.** HCI observed in fish gills at the five points. Parameters: 0-10 = normal organ function; 11-20 = mild changes; 21-50 = moderate changes; 51-100 = severe injuries.

Figure 6 shows the histological characteristics of a normal gill. The damage most frequently observed in gills were Stage I: elevation of the epithelium (Figure 7A), proliferation of chloride cells (Figure 7B); Stage II: partial lamella fusion (Figure 7C) and complete lamellar fusion (Figure 7D).

The HCI of the livers showed moderate to severe lesions. Moderate grade lesions were observed in the summer season at points P2 and P4. The other seasons presented severe levels of HCI, indicating that the liver tissue functioning of the analyzed fish is compromised (Figure 8).

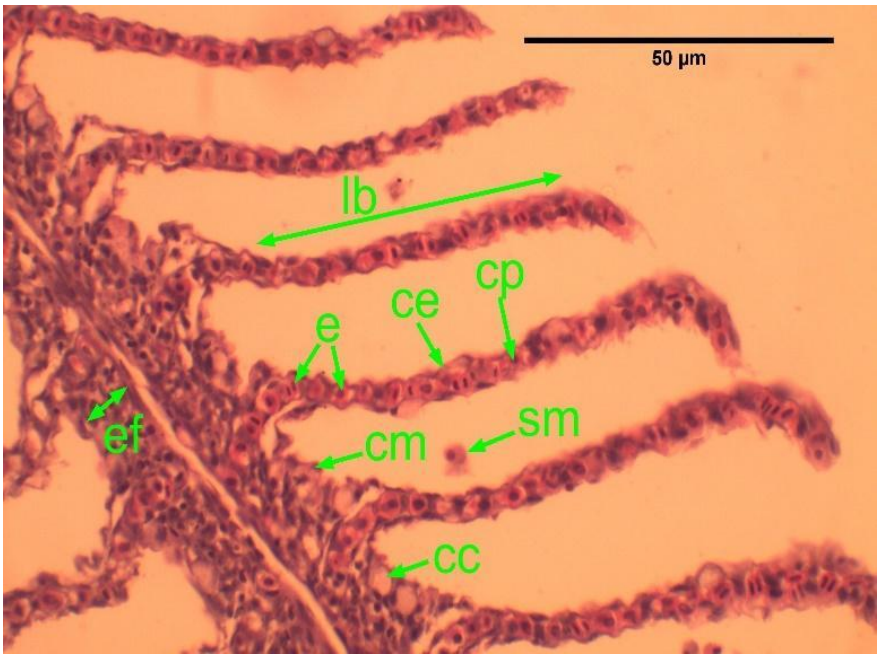


**Figure 6.** Normal gill (lb - gill lamellae; ef - filamentary epithelium; e - erythrocyte; ce - epithelial cell; cp - pillar cell; cm - mucous cell; sm - mucus secretion; cc - chloride cell).

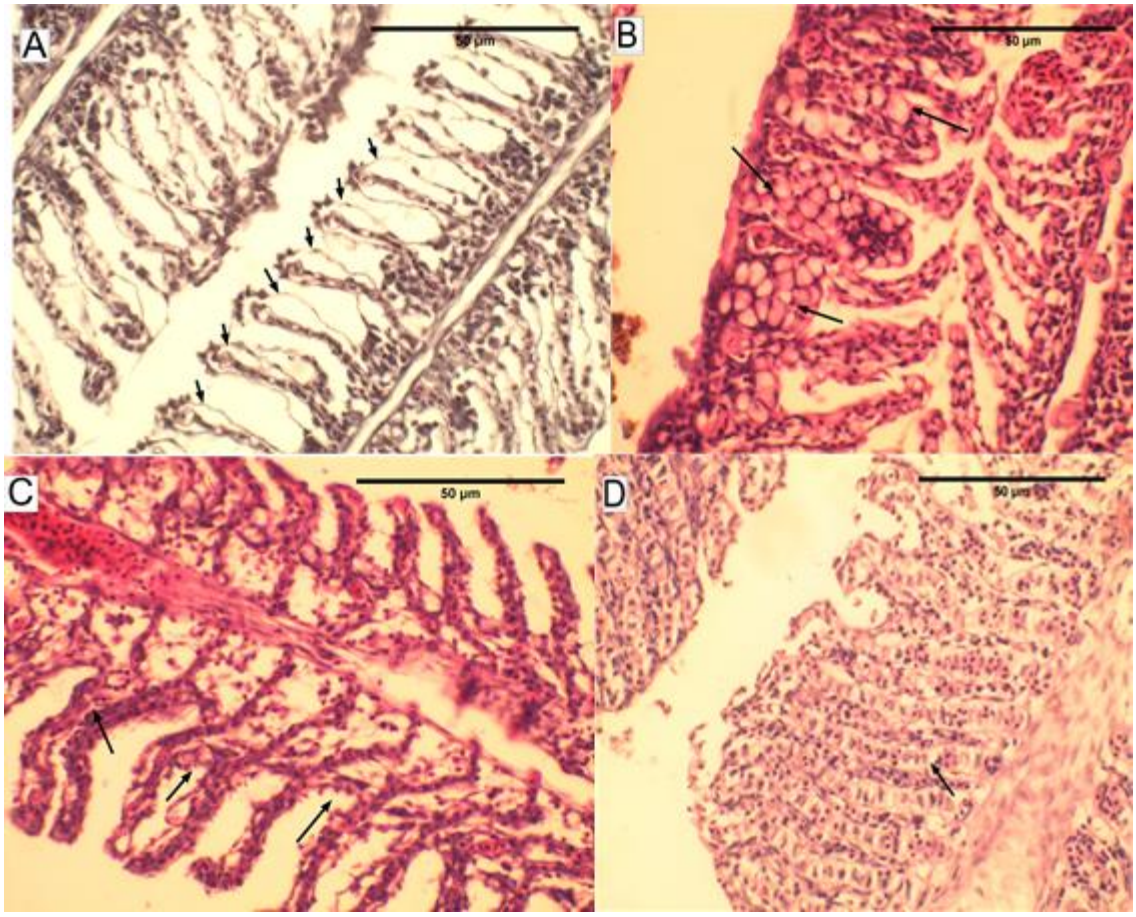


**Figure 7.** Histological changes observed in gills of fish from Ijuí River. Epithelial elevation - A; cc proliferation - B; partial fusion - C; complete fusion – D.

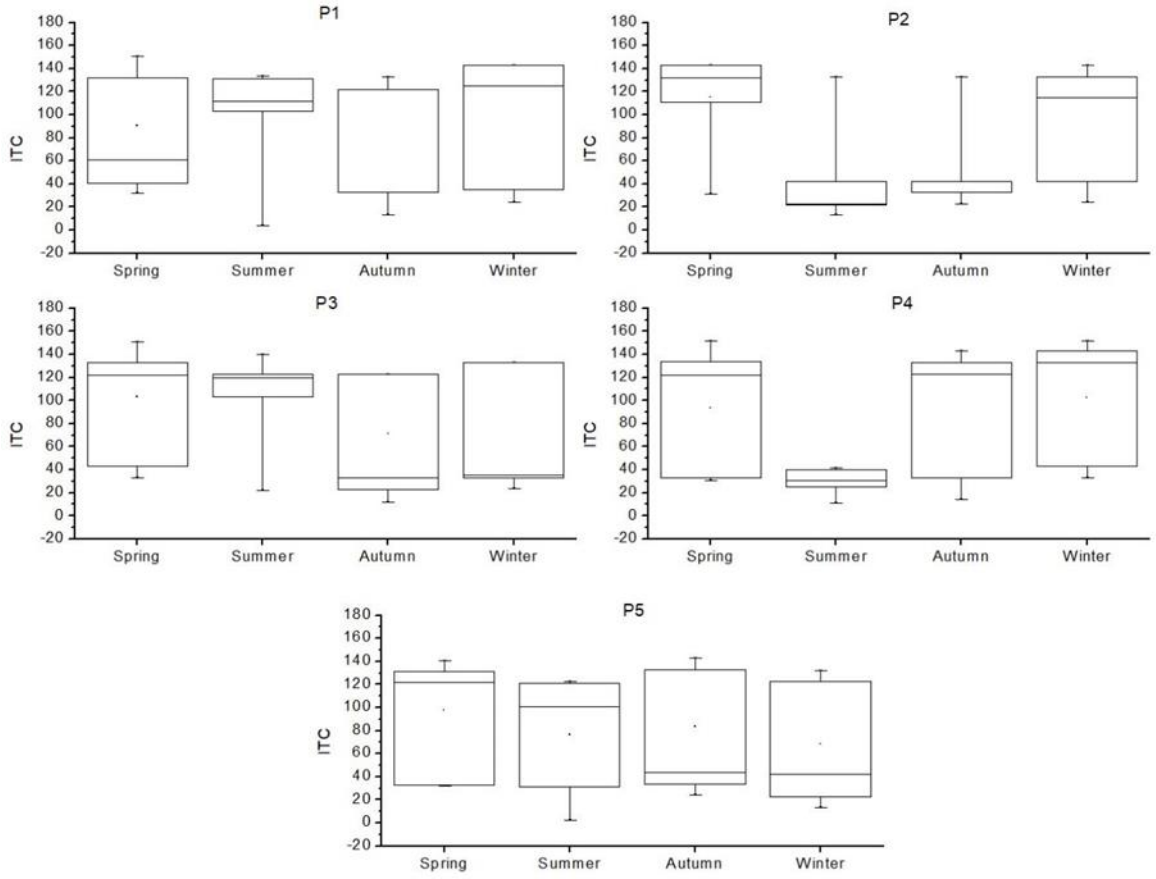


**Figure 8.** HCI observed in fish livers at the five points. Parameters: 0-10 = normal organ function; 11-20 = mild changes; 21-50 = moderate changes; 51-100 = severe injuries.

Figure 9 shows the normal histological characteristics of liver tissue, with the structure of the cords and the arrangement of hepatocytes, vessels and hepatopancreas. In livers, the most observed damages were nuclear vacuolization, cytoplasmic degeneration, nuclear degeneration, cytoplasmic vacuolization, biliary stagnation and hyperemia, which comprise Stage II, and the focal necrosis damage, of Stage III (Figure 10).

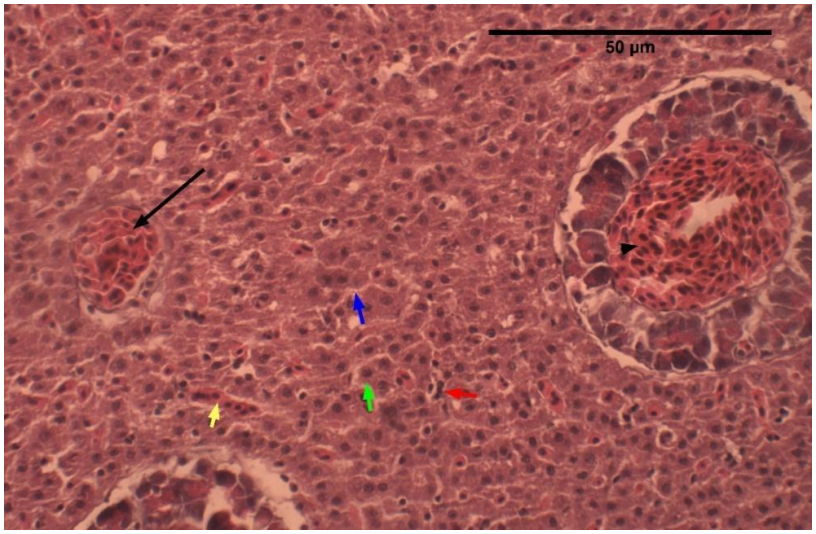


**Figure 9.** Normal liver - hepatocyte - green arrow; sinusoid capillary - blue arrow; kupffer cell - red arrow; erythrocyte - yellow arrow; vein - head of the arrow; hepatic artery - black arrow.

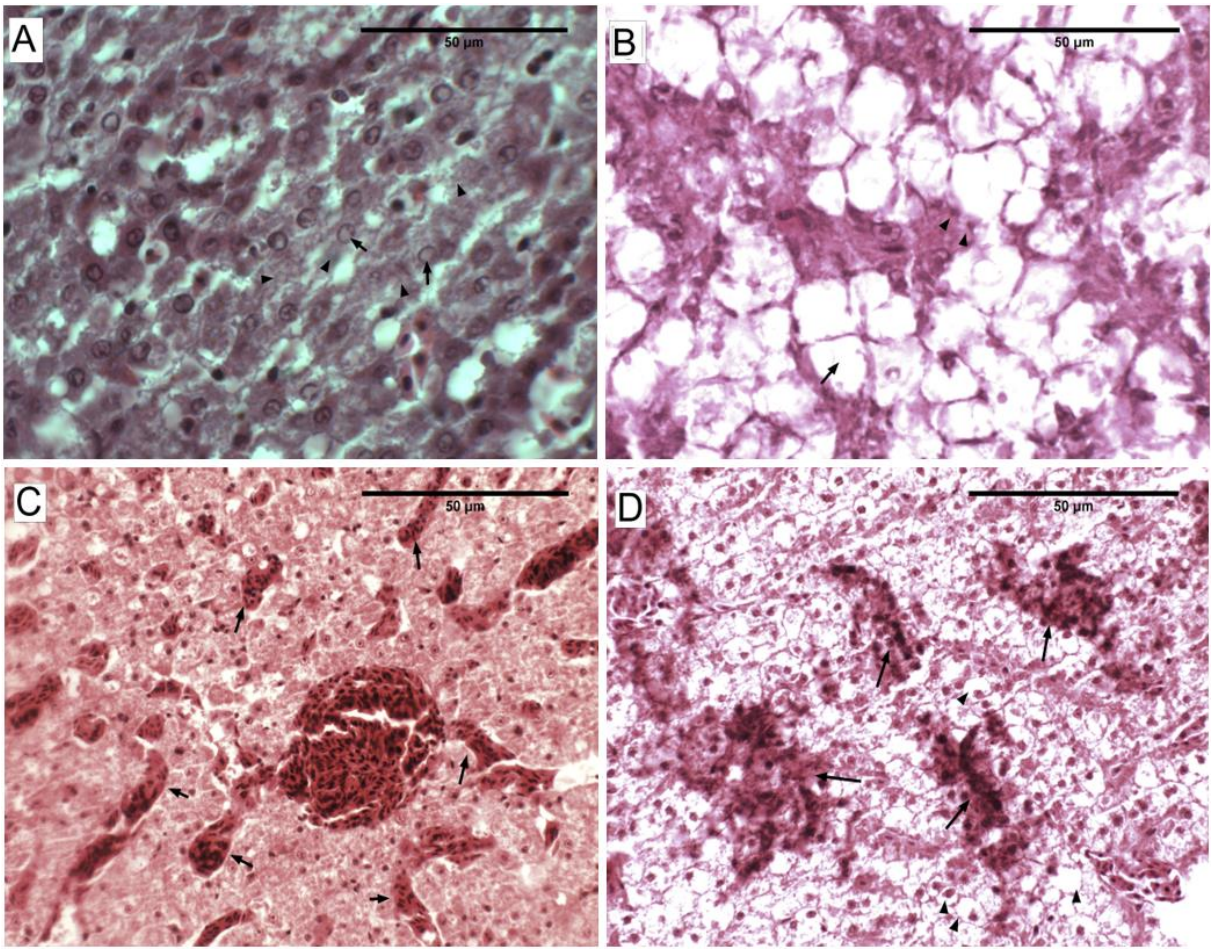


**Figure 10.** Histological changes observed in the livers of fish from the Ijuí River (A) nuclear vacuolization - arrow and cytoplasmic degeneration - arrowhead; (B) nuclear degeneration - arrowhead and cytoplasmic vacuolization - arrow; (C) hyperemia - arrow; (D) focal necrosis - arrow and cytoplasmic vacuolization - arrowhead.

At points P1, P3 and P4, more than 50% of the fish had moderate severity in the gills (Figure 11-A), which means its functionality was partially impaired. As for livers, severe alterations predominated, exceeding 50% of the analyzed fish at points P1, P3 and P5 (Figure 11-B).

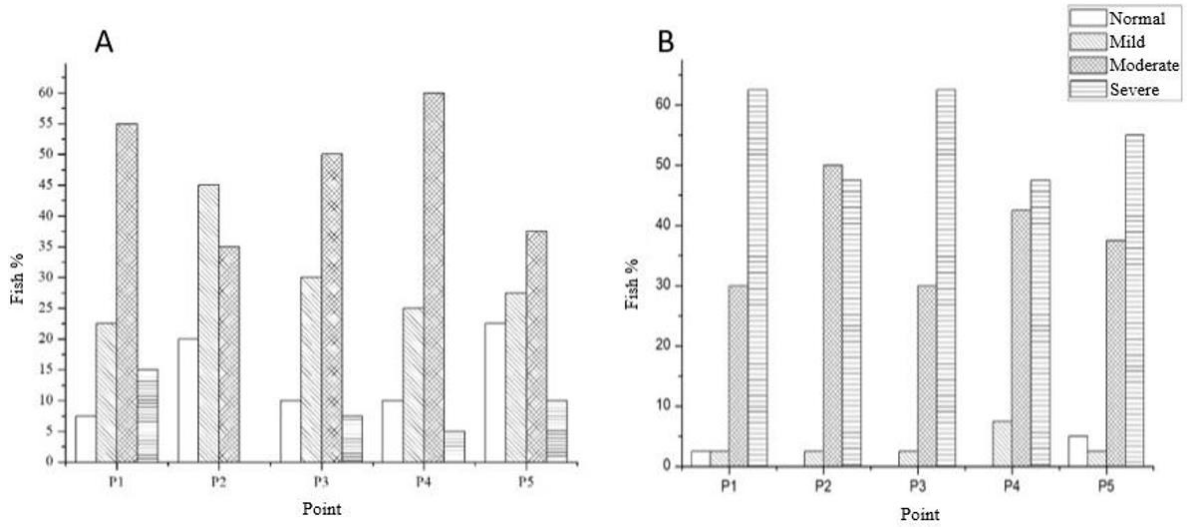


**Figure 11.** Percentage of fish and severities observed by point (A - gills and B - liver).

Regarding the histological analysis of the gills, the alterations found in the fish from the Ijuí River showed that, at Points P1, P2, P3 and P4, the highest HCI averages are found (Figure 5), with a predominance of moderate changes. At points P1, P3 and P4, more than 50% of fish show moderate changes, showing that the anthropization stage found at these points, can play a role in inducing these alterations, either by the presence of xenobiotics or by altering the environment, changing in the diet or promoting the presence of parasites and/or inductors of epigenetic effects (Simonato *et al.*, 2006).

The alterations caused in the branchial tissue are an attempt of the organism to defend itself in response to the presence of pollutants (Garcia-Santos *et al.*, 2006). This includes epithelial elevation, hyperplasia, partial and complete lamellar fusion (Figure 6), which were identified in fish from all points of this study. These alterations reduce the vulnerable surface area of the gills, thus increasing the diffusion barrier to pollutants; however, this barrier also hinders gas exchange and the hydromineral balance (Erkmen and Kolankaya, 2000).

The lesions identified in the gills are not specific from a single contaminant, but from a complex variety of stressing substances. Studies have shown that in high concentrations, cadmium, nickel and copper metals (Garcia-Santos *et al.*, 2006; Pane *et al.*, 2004; Arellano *et al.*,1999), acephate-based agrochemicals, diphenoconazole, sulfluramid, glyphosate, diflubenzuron (Virgens *et al.*, 2015; Shiogiri *et al.*, 2012; Maduenho, 2008) and diesel fuel (Simonato *et al.*, 2006) cause the same changes as the ones found in the present study.

When it comes to the liver, the data show that in all fish collected at all points, there are elevated HCI averages in the liver (Figure 4), configuring the changes of severe and moderate degree. More than 45% of the fish analyzed at all points showed severe injuries, indicating the occurrence of factors that are affecting the functioning of this organ.

The hepatic lesions most frequently documented in the literature are: nuclear vacuolization, cytoplasmic degeneration, nuclear degeneration, biliary stagnation and hyperemia, which comprise Stage II, and the focal necrosis damage, Stage III. Such changes are commonly reported in studies of monitoring aquatic environments or in substances analysed in laboratory (Meletti *et al.*, 2003; Rocha *et al.*, 2010; Santos Filho *et al.*, 2014; Pinheiro *et al.*, 2017) and are the same as the ones found in the samples assessed by this study.

Alterations such as nuclear and cellular degeneration may indicate dysfunctions induced by a toxic agent, since metabolically active areas of the liver are reduced, leading to a possible general reduction of the functions performed by this organ (Hinton and Laurén, 1990; Hinton *et al.*, 1992) Hyperemia, in turn, may indicate an adaptation process that leads to increased blood flow in the hepatic tissue, facilitating the transport of macrophages to the damaged regions of the tissue and improving the oxygenation of these areas or, additionally, it may indicate an auxiliary mechanism of detoxification (Simonato *et al.*, 2006).

In the analyzed samples, necroses evidence the presence of xenobiotics in the study sites, since it is the only Stage III lesion, and it was observed in 60% of the analyzed fish. This type of injury is considered irreversible, causing partial or total loss of the organ function (Simonato *et al*., 2006). These substances may be present in the environment. influencing the quality of the available food, of both animal and vegetable origin, that impacts the fish's liver physiology and metabolism.

The analysis of cells with MN and AN, combined with a histological evaluation of gills and liver, reveals a worrying situation regarding the conservation status of the Ijuí River in the sampled points. So far, human actions have not resulted in fish mortality; however, the impact on the physiology of the studied organs is evident and indicates the presence of pollutants derived from anthropization.

## 4. CONCLUSION

This research shows that fish found in anthropized waters present damage in tissues that have an important metabolic function for homeostasis, inducing significant alterations in the gills and liver, as well as important patterns of nuclear alterations in peripheral erythrocytes, which indicate contact with exogenous elements that interfere in cellular processes to the point of inducing repair mechanisms and the emergence of nuclear AN, minimizing the presence of MN.

These data are consistent with the environmental situation of the analyzed points, which present a degraded riparian forest, agricultural areas near the river banks, as well as the release of urban and industrial effluents directly into the river or into its tributaries.

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