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Genetic Biology

Fluvial water toxicology related to its land use and land cover

Relações entre toxicologia de águas fluviais e uso e ocupação da terra

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ABSTRACT

Due to uncontrolled population growth and intensified anthropic activities, numerous environmental impacts occur in water bodies, such as the toxicity of their waters. This study aimed to evaluate the water toxicity of the Antas River through ecotoxicological bioassays using neonates of the microcrustacean *Daphnia magna* and to relate it to land use and land cover in areas near the river. Fourteen sampling points were selected along the entire assessed stretch of the river, and water samples were collected and subjected to acute toxicity analyses using neonates. Land use and land cover mapping of the Antas River Watershed was generated, and an analysis was conducted within approximately a 400-meter radius from each sampling point. The occupation rates for each evaluated area were quantified and subsequently correlated with the obtained toxicity data. The results revealed a high significance level (99%), indicating that land use and land cover in the vicinity of water bodies directly influence water toxicity, impacting it in urban areas or preserving it in native forest areas, depending on the land use, as assessed through acute assays using neonates of the microcrustacean *Daphnia magna*.

Keywords: Water quality; Biomonitoring; Remote sensing

RESUMO

Decorrente do avanço populacional desordenado e da intensificação das atividades antrópicas, diversos são os impactos ambientais resultantes nos corpos hídricos, como por exemplo a toxicidade das suas águas. O presente estudo teve como objetivo avaliar a toxicidade da água do Rio das Antas por meio de bioensaios ecotoxicológicos utilizando neonatos do microcrustáceo *Daphnia magna* e relacionar com o uso e ocupação da terra nas áreas próximas ao rio. Foram adotados 14 pontos de amostragem durante todo o percurso do rio avaliado e posteriormente realizadas coletas de amostras de água, em seguida submetidas a análises de toxicidade aguda por meio da utilização dos neonatos. Foi gerado o mapeamento do uso e ocupação da terra da Bacia Hidrográfica do Rio das Antas e em seguida realizada uma análise de aproximadamente 400 metros de raio a partir de cada ponto de amostragem. Foram quantificadas as taxas de ocupação referentes a cada área avaliada e posteriormente correlacionado esses valores com os dados de toxicidade obtidos. Diante dos resultados, conclui-se com elevada significância (99%) que o uso e ocupação da terra nas áreas ao redor dos corpos hídricos influencia diretamente na toxicidade da água de forma a impactá-lo (áreas urbanas) ou preservá-lo (áreas de floresta nativa) a depender do uso quando avaliada por meio de ensaios agudos utilizando de neonatos do microcrustáceo *Daphnia magna*.

Palavras-chave: Qualidade da água; Biomonitoramento; Sensoriamento remoto

1 INTRODUCTION

Due to rapid urban growth and development in recent decades, various anthropogenic activities have resulted in extensive removal of riparian vegetation, discharge of untreated sewage, and numerous other environmental impacts. The situation is no different for the Mixed Ombrophilous Forest, also known as the Araucaria Forest, which is a typology of the Atlantic Forest. Only 0.8% of its natural remnants are in an advanced succession stage, fragmented among the three plateaus of the state of Paraná (Ribeiro et al., 2009). These impacts have caused a significant ecological imbalance, primarily due to the deterioration of water quality in water bodies, posing a major threat to the aquatic ecosystems present in these areas (Larson et al., 2019; Mendoza et al., 2017). Therefore, the few remaining natural forest remnants located in the most urbanized regions are heavily and frequently transformed in order to “benefit” society. However, they become one of the main factors contributing to the high water degradation in these regions (Uriarte et al., 2011; Su et al., 2016).

Such situations are commonly found in municipalities considered small-sized, which are experiencing rapid economic growth and development, and have urban water bodies with low flow rates. In addition to urbanization, it is important to highlight that other land uses directly or indirectly impact the quality and toxicity of water bodies, such as agricultural activities and improperly managed pastures. An example of this is the municipality of Irati, located in the state of Paraná, in the southern region of Brazil, which has the Antas River as its main water body, and the vegetation type of the ecosystem belonging to the city is called the Mixed Ombrophilous Forest

(Andrade; Felchak, 2009). However, its vegetation is increasingly being cleared due to urbanization, which directly affects the water characteristics of the main river in the watershed. According to Von Sperling (2005), vegetation serves as a barrier to protect water in these areas, preventing changes in its quality and toxicity.

The main environmental impacts on water bodies resulting from the absence of Permanent Preservation Areas (PPAs) are due to an increase in solid content, leading to high levels of color and turbidity, which reduce the euphotic zone. Additionally, the lack of PPAs contributes to sedimentation in water bodies, leading to bed siltation, and increased concentrations of nutrients (nitrogen and phosphorus), organic matter, and pathogenic microorganisms resulting from the discharge of untreated domestic sewage (Oliveira-Filho et al., 2007). Therefore, it is essential to conduct ecotoxicological biomonitoring in these regions due to the diverse impacts to which these environments are subjected.

When considering the harmful effects caused by chemical substances derived from water pollution on living organisms, these organisms exhibit responses according to the reactions arising from disturbances in the environment, leading to direct or indirect effects (Knie & Lopes, 2004). In some types of organisms, the impact is readily observed, making them biological sensors due to their sensitivity to environmental changes, resulting in acute or chronic effects, such as mortality and/or physiological changes (Zagato & Bertoletti, 2006). Among the microorganisms used for detecting the toxicity of the aquatic environment, microcrustaceans stand out, with Daphnia magna being an example. This species of Daphnia is considered one of the most widely used in ecotoxicological analyses due to its rapid growth and high productivity, as well as short life cycles and significant sensitivity to environmental changes in the presence of contaminants (Martins, Teles, Vasconcelos, 2007). However, the use of these microorganisms as indicators of toxicity in fluvial waters is scarce, with few studies related to this topic.

Due to the consequences of human encroachment on Permanent Preservation Areas, the application of Geographic Information Systems (GIS) techniques can be used as extremely important tools in monitoring land use and land cover changes more easily and efficiently, with the aim of curbing pollution and contamination of urban watercourses. However, several studies have been conducted to establish relationships that demonstrate the deterioration in water quality due to the intensification of anthropogenic activities related to land use in a watershed or sub-watershed (Melo et al., 2020; Dala-Corte et al., 2020; Hunt et al., 2017; Castro et al., 2018).

In this context, an analysis of the toxicological characterization of environmentally impacted water bodies becomes of utmost importance, considering that the water from these bodies may be subsequently collected and treated for population supply. Therefore, the present study aimed to assess the water toxicity of the Antas River through ecotoxicological bioassays using neonates of the microcrustacean Daphnia magna, in relation to land use and land cover in the areas adjacent to the river.

2 METHODS

2.1 Description of the study area

The study area of this research is known as the Antas River Watershed, located in the municipalities of Irati, Imbituva, and Fernandes Pinheiro in the state of Paraná, in the southeastern region of the state. The Antes River Watershed is part of the set of sub-watersheds that constitute the Tibagi River Watershed. The region has an average temperature ranging from 11.0°C in the month of June (cooler periods) to 24.2°C in January (warmer periods), with average precipitation around 4 mm in the driest months and reaching approximately 175 mm in the rainiest months (IAPAR, 2018). The study area is located in a phytogeographical region considered to be Mixed Ombrophilous Forest, specifically in the collection region of the sampling points, where it is characterized as Alluvial Ombrophilous Forest due to the presence of the water body and its respective floodplain areas.

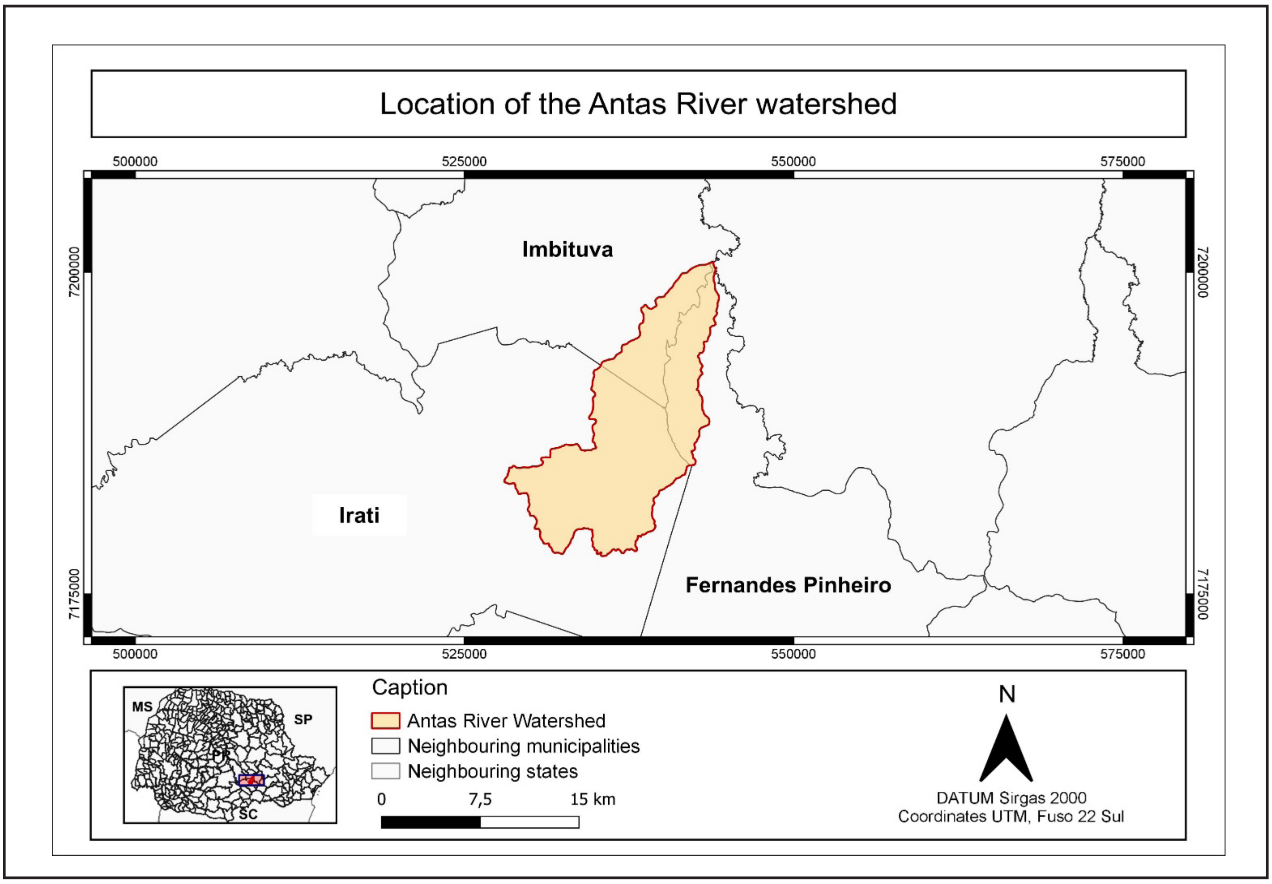
The study watershed is primarily fed by the Antas River, which stretches for approximately 80 km. According to Resolution CONAMA 357/2005, which classifies water bodies in Brazilian territory, the Antas River is categorized as Class 2, indicating considerably good water quality. The majority of the Antas River flows through the municipality of Irati, originating in the Nhapindazal neighborhood's mountainous region, which is predominantly composed of agricultural areas. The river then runs through the urban area of the municipality, where it serves as the main water body. Eventually, it merges with the Imbituvão River at its mouth, characterized by significant environmental preservation due to the presence of the Irati National Forest (Andrade & Felchak, 2009).

2.2 Adoption of sampling points and sample collection

For the selection of sampling points for water collection in the Antas River, several factors were taken into account, such as the location of industries, wastewater treatment plants (WWTP), residential areas, agricultural cultivation areas, pastures, reforestation areas, and native forests adjacent to the water body. In addition to selecting points based on the aforementioned land uses, the availability of entry into private properties, such as residences, country houses, companies, and farms, as well as ease of access to the water body, was also considered.

Fourteen sampling points were adopted from the source of the river (Point 1) to the mouth (Point 14). This number was chosen based on the positioning of the aforementioned land uses, taking into account that points in close proximity would not exhibit significant differences in toxicity tests due to the natural dynamics of the river. Another factor considered in selecting this number of samples is that the collections should be performed on the same day, and the laboratory analyses should be conducted within a maximum of 24 hours thereafter. This allowed for the comparison of the obtained data and minimized external interferences on the samples. Therefore, due to the time constraints and difficulties in accessing the water body, it would be unfeasible to increase the number of samples or travel longer distances. The geographic location of the Antas River Watershed is shown in Figure 1.

Figure 1 – Geographic location of the Antas River Watershed



Source: Authors (2023)

For sample collection, the condition of no rainfall in the Antas River Watershed area for at least 7 consecutive days was adopted to avoid interference with water quality. The samples were collected from three different surface sections of the river: the two sides and the center. Subsequently, they were homogenized to create composite samples.

2.3 Analysis of water toxicity in the Antas River

After being collected at their respective sampling points, water samples from the Antas River were subjected to analysis within a maximum of 24 hours. To assess the acute toxicity of the collected samples, analyses were conducted using neonates of the microcrustacean Daphnia magna. These organisms were chosen due to their high sensitivity to environmental pollution.

For the assays, adapted guidelines from NBR 12.713 dated 01/2022 were followed. Two neonates of *Daphnia magna*, aged between 2 and 26 hours, were used. The crustaceans were placed in beakers containing 50 ml of sample from each evaluated point and were kept for a period of 48 hours. The analyses were performed in triplicate, and no dilutions of the samples were carried out since they were collected directly from the water body. Samples were considered to have a toxic effect if they showed statistically significant differences compared to the negative control.

The analyses were conducted meticulously, observing the sluggishness, immobilization, death, or changes in the behavior of the crustaceans after 24 and 48 hours of the assay. To ensure the accuracy and reliability of the data, in addition to the analyses of the collected samples from the evaluated points, positive control analyses were also performed. In the positive control, the medium containing the Daphnia had a known level of toxicity, affecting all the neonates. Likewise, a negative control was included, where no interference was present, and the medium consisted of distilled water.

2.4 Evaluation of land use and land cover in the Antas River Watershed

To assess land use and land cover in the Antas River Watershed, the first step involved delimitating the watershed using the water divides (points of higher altitudes) and its main tributary. This was done using the QGIS software version 3.12.3 and the vector tool. The vectorization process was based on the geographical map of the region available in the cartographic database of the Instituto Terras, Cartografia e Geologia do Paraná (ITCG), provided by the Instituto Água e Terra (IAT). The chosen map (number 2839-4) has a scale of 1:50,000, meaning that each centimeter on the map represents 50,000 centimeters on the ground, and it was scanned at a resolution of 300 dpi. The selection of this specific map took into account its coverage area, ensuring that it encompassed the study area. Both created vector layers used the Cartographic Reference System (CRS) with EPSG:

31982 – SIRGAS 2000 / UTM zone 22S.

The data used for creating the land use and land cover map were collected by downloading images available on the digital platform of the Instituto Nacional de Pesquisas Espaciais (INPE). The satellite used for image acquisition was the CBERS4AWPM, which has a resolution of 8 meters. A search was conducted in the databases for a period of one year (from January 2022 to January 2023) within the study area region. Once the most representative images were identified, they were downloaded, including the blue, red, green, and infrared bands.

After obtaining the images of the study area with the aforementioned bands, georeferencing and clipping were performed using the QGIS software version 3.12.3. The images were clipped based on the desired area (Antas River Watershed) using the "raster extraction by mask layer" function in batch mode. The resulting product from this extraction had the same CRS as the other projects related to this research, which is EPSG: 31982 – SIRGAS 2000 / UTM zone 22S. After the clipping process, the raster layers were merged, resulting in a new composite layer as the final product.

Using the "Semi-Automatic Classification" plugin in the aforementioned software, specifically in the "SCP dock" function, supervised classification of land use and land cover was performed. This classification involved the use of 50 samples for each land use category, which were divided into native forest, reforestation, pasture, bare soil, urban areas, and agriculture. The sample collection process involved drawing free geometric shapes over the watershed area in regions where the predetermined land uses were present. After collecting the samples and assigning colors to each land use category, the "band processing – classification" function was used with the "maximum likelihood" algorithm. This algorithm aims to maximize the similarity between the sample points to generate the supervised classification.

Following the land use mapping, a buffer was created around each adopted sampling point with a radius of 400 meters, and the area within each buffer was clipped. The criteria for determining the buffer radius were based on the scale of land uses present in the watershed, considering that changes in water quality within 500 meters can impact the aquatic community (Yirigui et al., 2019; Frieden et al., 2014). After generating the buffers with their respective land use and land cover classes, the "r.report" processing tool was used to calculate the area occupied by each land use within the evaluated region at each point, as well as the respective percentages relative to the total area of the buffer.

2.5 Statistical analysis of the data

In order to test the hypothesis that land use and land cover in the vicinity of the sampling units (14 points) significantly influence the toxicity of surface waters, the land use data (continuous quantitative – %) and toxicity data (qualitative: Toxic and Non-toxic) were subjected to a multivariate technique called cluster analysis. The main objective of cluster analysis is to group objects based on their characteristics (Hair et al., 2005). In this study, the partitioning clustering method with the k-means algorithm was used, which is based on centroids.

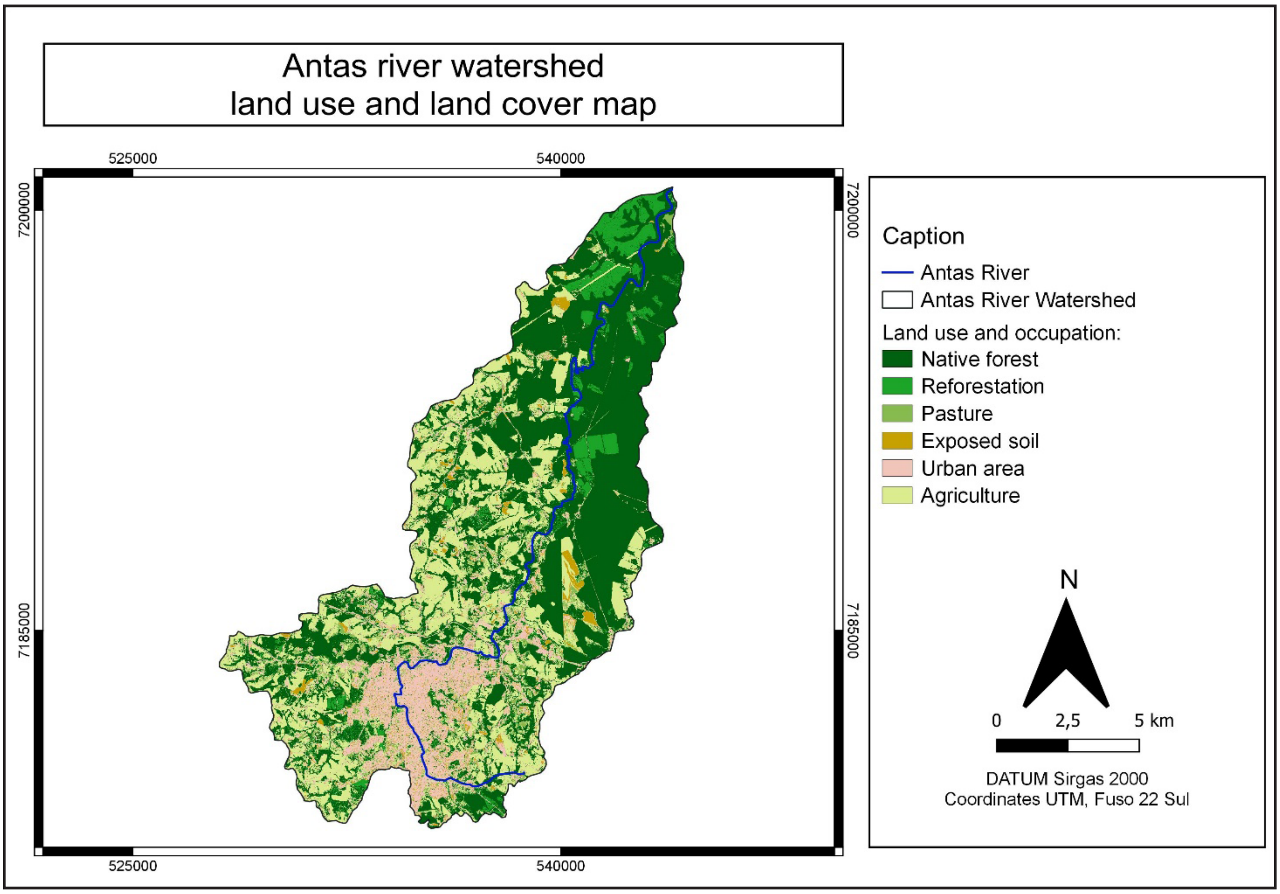
To determine whether the formed clusters correspond significantly to the non-toxic and toxic points, the obtained groups were associated with toxicity (Toxic and Non-toxic) and contrasted using the t-test. The significance level for all analyses was set at 5%. The assumptions of normality were assessed using the Anderson-Darling test, and the homogeneity of variances was tested using the Fligner-Killeen test. The results are expressed by the p-value (a value <0.05 indicates statistical significance). The analyses were conducted using RStudio software, version 4.0.2, with the packages MASS, cluster, and ggplot2 (RStudio, 2020).

3 RESULTS AND DISCUSSION

3.1 Evaluation of land use and land cover

Based on the land use and land cover mapping of the Antas River Watershed, the distribution of land uses was obtained, as shown in Figure 2.

Figure 2 – Land use and land cover mapping in the Antas River Watershed

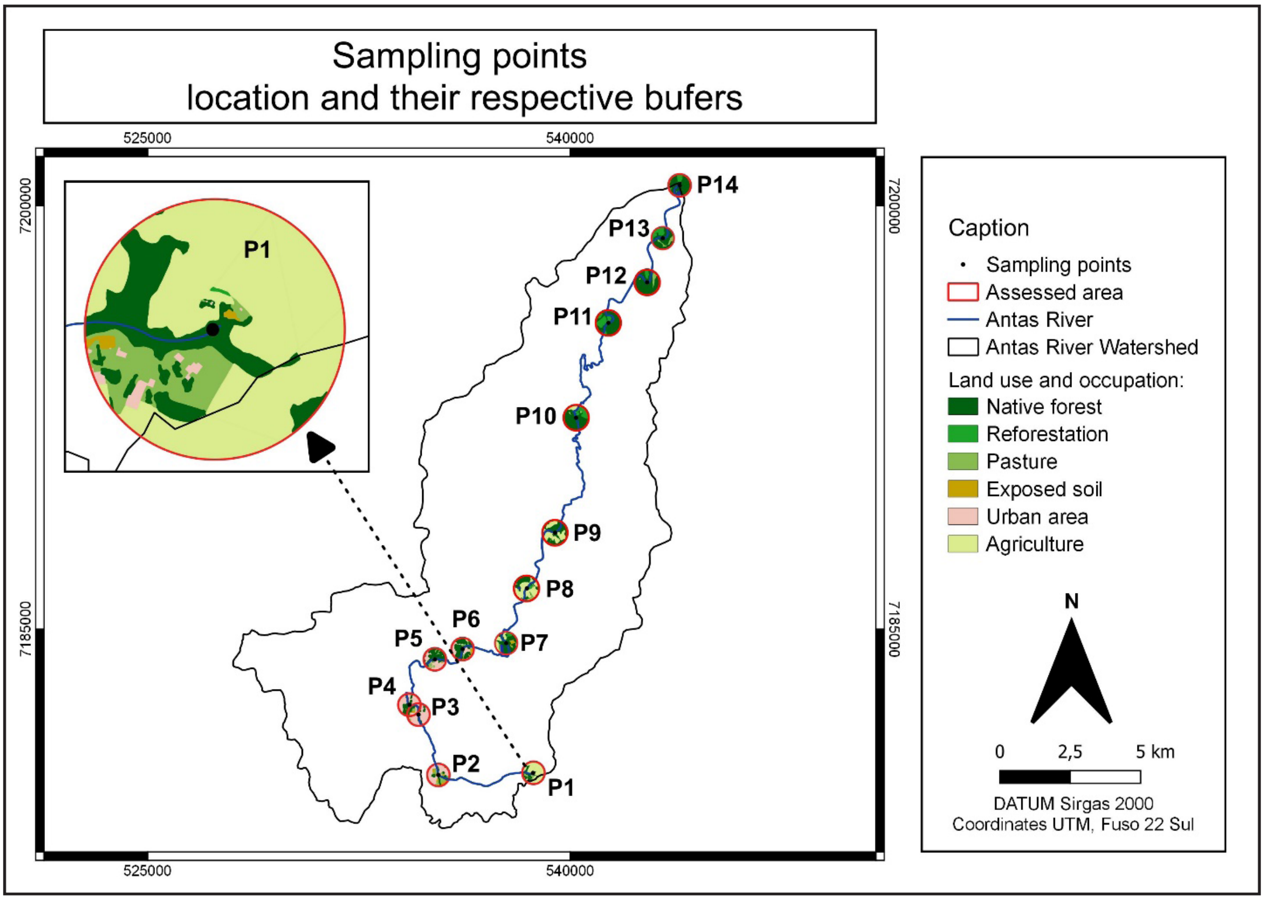


Source: Authors (2023)

In the studied Antas River Watershed, the predominant land use among those assessed is native forest, accounting for approximately 46.31% of the total area. This is followed by agricultural areas, urban areas, reforestation, pasture, and bare soil, which make up 25.67%, 13.42%, 6.48%, 5.42%, and 2.67%, respectively. The total area of the watershed is 167.82 km². These percentages are significant considering the findings of Dariva (2021), who assessed land use and land cover in the same watershed and reported a composition of 46% native forest, 33% for agriculture and bare soil combined, 11% for reforestation areas, and approximately 9% for urban areas. Some variance in the obtained percentages can be observed, which can be attributed to different GIS tools used and the ongoing development and urbanization in the municipality.

After obtaining the land use mapping of the watershed, the buffers were clipped based on their respective sampling points, as shown in Figure 3.

Figure 3 – Location of sampling points in the Antas River and their respective buffers



Source: Authors (2023)

Subsequently, calculations were performed to determine the areas corresponding to each land use within the buffers for the evaluated sampling points. The results are presented in Table 1.

Based on the presented data, a significant percentage of urbanized areas are observed in sampling points 2, 3, 4, 5, and 6, where this land use category comprises over 40% of the evaluated areas. Point 2 stands out in particular, with approximately 80.90% of its territory classified as urban land use. These points are located within the urban areas of the municipality of Irati, where few remaining forested areas are found. This indicates intense anthropogenic expansion and limited presence of reforestation areas, pastures, bare soil, and agriculture in these regions.

Upon analyzing points 1 (source), 8, and 9, a considerable presence of agriculture is observed in these regions. This can be explained by their locations, which are in the outer areas of the urban center. It is worth noting that point 7 is characterized by a wide range of land uses, but its main focus is the presence of a wastewater treatment plant (WWTP), which discharges its treated effluents into the evaluated river.

Table 1 – Percentage of each land use and land cover category assessed in their respective sampling point buffers

	Percentage of land use and land cover (%)					
Sampling point	**Native Forest**	**Reforestation**	**Pasture**	**Exposed soil**	**Agriculture**	**Urban area**
P1	20.02	0.17	11.18	0.65	66.14	1.82
P2	6.59	0	9.39	3.11	0	80.90
P3	15.81	0	4.51	2.25	0	77.42
P4	24.57	0	4.84	3.82	0	66.74
P5	32.93	0	10.38	1.03	0	55.63
P6	46.83	0	6.44	3.19	0.66	42.85
P7	52.14	5.07	8.08	13.65	9.25	5.64
P8	42.22	2.03	11.13	0.32	39.63	2.60
P9	71.50	0	1.27	0.96	23.67	2.57
P10	76.84	13.75	5.66	0	3.73	0
P11	80.12	19.87	0	0	0	0
P12	82.28	3.67	14.03	0	0	0
P13	62.87	18.08	19.04	0	0	0
P14	82.99	10.43	6.56	0	0	0

Source: Authors

In contrast to the other points, points 10, 11, 12, 13, and 14 (mouth) are located in an area of intense preservation within the Irati National Forest (FLONA). This explains the high percentages of native forest in these regions. The Irati National Forest is considered one of the main forest remnants with the presence of Araucaria trees in the region. It is located in proximity to the Araucarias Biological Reserve and the Fernandes Pinheiro Ecological Station (Grise et al., 2009; Brasil, 2000). However, these protected areas face various challenges, primarily related to land occupation. They should remain non-anthropized and free from environmental degradation (Manarim, 2008).

3.2 Toxicological assessment of samples based on land use and land cover

The sampling points were divided into toxic and non-toxic categories ($F_{14;30} = 7.57$, $p < 0.01$) according to the mean test. Points 2, 3, 5, 6, and 7 were statistically similar to the positive control (potassium dichromate – *Daphnia* immobilized or dead), while the remaining points (1, 4, 8, 9, 10, 11, 12, 13, and 14) differed from the control (Table 2).

Table 2 – Mean test applied to the sampling points based on toxicity data

Sampling Points	**P1**	**P14**	**P13**	**P4**	**P12**	**P11**	**P10**	**P9**	**P8**	**P6**	**P5**	**P3**	**P2**	**P7**	**CT**
immobilized (%)	0.66	0,66	0,66	0,66	0,66	0,66	0,66	0,66	1	2	2	2	2	2	3
Groups	c	c	c	c	c	c	c	c	c	ab	ab	ab	ab	ab	a

Source: Authors

Based on the data obtained regarding the percentages of each land use category at the sampling points, along with the results of toxicity analyses using the neonates, statistical treatment of the data was conducted. Through cluster analysis, two sets of data (clusters) were determined, broadly represented by the points classified as non-toxic (cluster 1) and toxic (cluster 2) according to the Daphnia toxicity test, as shown in Table 3.

However, it is noteworthy that the sampling points located in regions with higher percentages of urbanized areas (points 2, 3, 5, 6, and 7) are classified as toxic according to the statistical clustering based on the toxicity analyses. An exception is point 4, which, despite being situated in an urban area, has a significant amount of native forest. According to Metzger et al. (2019), this highlights the importance of protecting water quality, especially in urban rivers, emphasizing the relevance and significance of permanent preservation areas.

When evaluating points 10, 11, 12, 13, and 14, which have the highest percentages of preserved native forest areas, no toxicity is detected in the samples. This configuration significantly contributes to the preservation of terrestrial and aquatic ecosystems, facilitating their maintenance (Grise et al., 2009). Therefore, several studies have been conducted in Atlantic Forest areas as well (Gerhard; Verdade, 2016; Terra et al., 2015), reinforcing the importance of forest cover throughout the watershed, especially in riparian forests.

Table 3 – Components of the clusters generated in the cluster analysis based on the data matrix of land use and land cover variables and toxicity of 14 sampling units located along the Antas River in Irati, Paraná

Sampling Points	Cluster	Toxicity
P1	1	Non-toxic
P2	2	Toxic
P3	2	Toxic
P4	1	Non-toxic
P5	2	Toxic
P6	2	Toxic
P7	2	Toxic
P8	1	Non-toxic
P9	1	Non-toxic
P10	1	Non-toxic
P11	1	Non-toxic
P12	1	Non-toxic
P13	1	Non-toxic
P14	1	Non-toxic

Source: Authors

The land use and land cover also played a significant role in the formation of the identified clusters. In cluster one (Non-toxic), areas with higher forest and reforestation coverage, as well as lower percentages of bare soil and urban areas, predominated significantly. It is important to note that pasture did not show a significant influence on the formation of the clusters and, therefore, did not impact the toxicity of the analyzed water (Table 4).

Upon analyzing Table 4, a high level of significance ($p < 0.01$) is observed regarding the impact of urban areas and native forest areas on the toxicity of the sampled points, but in opposite ways. Urban areas contribute to toxicity, while preserved environments exhibit lower toxicity levels. However, when examining the t-test values, the influence of urban areas on toxicity is more pronounced (5.54), indicating that anthropogenic activities have a more intense impact on degradation compared to the protection provided by native forest areas. Therefore, the influence of land use and land cover on water quality is even more significant due to the absence of vegetation in permanent preservation areas (Dala-Corte et al., 2020).

Table 4 – Means and t-test and Chi-square values for the different clusters identified in the data matrix of land use and land cover and toxicity of the Antas River in Irati, Paraná

Variables	Cluster 1	Cluster 2	t-test or Chi-square test	p
Toxicity*	Não tóxico	Tóxico	10.37	p < 0.01
Forest (%)	64.85841	29.81575	3,13	p < 0.01
Reforestation (%)	8,505	0.845865	2.24	p = 0.04
Pasture (%)	8.613279	7.280022	0.48	p = 0.64
Exposed soil (%)	0.242277	4.512597	2.66	p = 0.64
Urban area (%)	0.8764	54.86871	5.54	p < 0.01

* Chi-square test

The toxicity observed in some sampling points of the Antas River in this study is a result of water quality degradation. This degradation is caused by various issues related to uncontrolled land occupation in the watershed, the absence of permanent preservation areas, riverbed siltation, dredging processes, reduction in river meandering, and the presence of sewage and solid waste in its course, as supported by Andrade and Felchak (2009). The deterioration of water quality due to anthropogenic activities and vegetation loss is also supported by other authors (Silva et al., 2016; Cândido et al., 2015).

Sieklicki et al. (2018), in their study evaluating the genotoxic effects of water from the Antas River in relation to land use and land cover using genetic assays with plant bioindicators (*Allium cepa*), concluded that there is a strong influence of urbanized areas on chromosomal abnormalities in plants due to the degradation of permanent preservation areas. This corroborates the results presented in this study, which used a different type of assay, the microcrustaceans (*Daphnia magna*).

4 CONCLUSIONS

Based on the presented evidence, it can be concluded with high significance (99%) that land use and land cover in the areas surrounding water bodies directly influence the toxicity of the water, either impacting or preserving it depending on the specific land use. This conclusion is drawn based on the assessment using neonates of the microcrustacean *Daphnia magna*. Specifically, urban areas followed by native forest areas have the greatest impact on the toxicity of the Antas River water, as confirmed by the t-test results for the generated clusters.

Thus, a significant harmful anthropogenic interference is evident in the evaluated aquatic ecosystem, highlighting the crucial role of riparian buffer zones (PPAs) in the conservation and preservation of watercourse quality.

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