Relationships between land use and water quality obtained for the evaluation of genotoxic effects in plant bioindicators

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ABSTRACT

Anthropic activities as well as land use and occupation are closely linked to water and soil quality. An alternative to assess their influence on water quality in water bodies is through biomonitoring using *Allium cepa* as a bioindicator organism. This technique allows the detection of cytogenetic abnormalities in *Allium cepa* root meristematic cells after contact with analyzed water samples. Thus, we evaluated the genotoxic potential of water samples from the Rio das Antas (Antas River) along the urban perimeter of the city of Irati, Paraná, Brazil. With the aid of GeoEye Satellite high-resolution fused orbital images, we searched for possible relationships between the use and occupation of land around this river. Biomonitoring was performed at three different sampling points. Statistical equality between the negative control and Point 1 and between Points 2 and 3 was obtained using Fisher's test and a Principal Coordinate Analysis (PCoA). The former presented a low frequency of abnormalities chromosomes and the latter presented larger averages. In addition, Point 1 had strong influence of tree vegetation. Points 2 and 3, demonstrated a strong influence of urbanized area, with significant degradation of permanent preservation areas (áreas de preservação permanente – (APP). The results showed remarkable anthropogenic interference to the ecosystem. Furthermore, this indicates importance of an APP watercourses functioning to preserve the quality of the water resources.

Keywords: alternative method of water quality analysis, chromosomal abnormalities, remote sensing.

Relações pontuais entre o uso e ocupação da terra e a qualidade da água obtida pela avaliação dos efeitos genotóxicos em bioindicadores vegetais

RESUMO

As atividades antrópicas bem como o uso e ocupação da terra estão intimamente ligadas à qualidade da água e do solo. Uma alternativa para avaliar suas influências na qualidade da água em corpos hídricos é através do biomonitoramento utilizando *Allium cepa* como organismo bioindicador. Esta técnica permite detectar anormalidades citogenéticas nas células meristemáticas radiculares de *Allium cepa* após o contato com a amostra de água analisada.
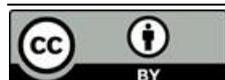
Assim, realizou-se a avaliação do potencial genotóxico de águas do Rio da Antas no perímetro urbano do município de Irati-PR e com o auxílio de imagens orbitais fusionadas de alta resolução do satélite *Geoeye*, buscou-se possíveis relações com o uso e ocupação da terra do entorno deste rio. O biomonitoramento foi realizado em três pontos amostrais distintos. Integrando-se os dados, obteve-se, com o teste de Fisher e mediante uma análise de coordenadas principais (PCoA), uma igualdade estatística entre o controle negativo e o ponto 1 e entre o ponto 2 e 3, sendo que os primeiros apresentaram baixa frequência de anormalidades cromossômicas e os últimos, médias maiores. Além disso, o ponto 1 apresentou forte influência de vegetação arbórea e os pontos 2 e 3, forte influência de área urbanizada, com expressiva degradação das áreas de preservação permanente (APPs). Os resultados confirmaram a notável interferência antrópica ao ecossistema, bem como, a importância da APP na preservação e qualidade do recurso hídrico.

Palavras-chave: anormalidades cromossômicas, metodologia alternativa de análise da qualidade da água, sensoriamento remoto.

1. INTRODUCTION

The urbanization process of many Brazilian cities occurred along riverbanks and brought detrimental consequences for bodies of water, the quality of the air, soil, fauna and flora of the region (Fendrich and Oliynik, 2002).

Anthropogenic activities such as land use and occupation are factors that interfere with water and soil quality. Another factor of significant relevance is the advancement of technologies and products in the industrial and agricultural sectors, which have helped to bring about the introduction of new chemical agents in the environment, thereby impacting the ecosystem equilibrium (FUNASA, 2002). The destruction of the riparian forests contributes to the silting of water bodies. As a result, this caused increases of turbidity, erosion, disequilibrium of the flood regime and the involvement of wild fauna (Oliveira-Filho *et al.*, 1994).

Environmental quality monitoring can be performed through conventional physico-chemical and microbiological assessments combined with specific assessments to investigate changes in the cellular metabolism of bioindicators. Moreover, they can applied to evaluate differences in some genetic structures due to the presence of substances with varying degrees of toxicity. In addition to representing a low costs, according to Grant (1994), plant species such as *Allium cepa* and *Tradescantia ssp.* are effective in environmental monitoring to assess the mutagenic potential of air, water and soil pollutants. Because of the similarities in the morphology of the chromosomes of eukaryotic organisms (plants and animals), they may also respond in an action mode of mutagenic agents (Constantin and Owens, 1982).

Experiments using *Allium cepa* as a test organism reveal and classify parameters such as cytotoxicity with mitotic index, mutagenicity with frequency of micronuclei, and genotoxicity with the frequency of chromosomal abnormalities during meristematic cell division of roots exposed to the analyzed samples (Fiskesjö, 1993). Thus, the genotoxic action of possible pollutants in the samples can be determined (Leme and Morales, 2009). The genotoxicity test using *Allium cepa* was validated by the United Nations Environment Program and the International Chemical Safety Program (Grover and Satwinderjeet, 1999).

It is a fact that the use and occupation of the land interfere with the quality of a water body, and this interference does not occur only in a localized way, but reaches the whole region that surrounds it (Oliveira *et al.*, 2012; Costa, *et al.*, 2015). Thus, proximity analyses are valuable to detect the use and occupation of the land around the sampling points and to assist in the evaluation of water quality.

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The use of remote sensing as a tool for analysis and control of environmental issues has become essential. The evolution in the technological area increasingly favors the development of numerous terrestrial-environmental monitoring satellites allowing for the collection of qualitative and quantitative data on a global, regional or local scale, as well as determining the degree of environmental degradation among other measurements (Mascarenhas *et al.*, 2009).

The purpose of this study was to evaluate the influence of urban and peri-urban soil use and occupation through the monitoring of the Rio das Antas' (Antas River) water quality and its possible genotoxic effects at predefined points. By means of high-resolution orbital images the classification of land use and cover was carried out by comparing them with the results obtained in the cytogenetic evaluations of the bioindicator vegetation.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in the urban area of the municipality of Irati, Paraná, Brazil. (Figure 1) located 150.34 km from the state capital, Curitiba (IPARDES, 2017). The municipality was founded in 1907, and since then it has experienced increases in population along with economic and industrial growth. These factors interfere directly and indirectly with the preservation of the water quality of the municipality. It has a 999,517 km² extension, an estimated population of 60,425 inhabitants with 73.6% of households having access to adequate sanitary sewage (IBGE, 2017). The rivers that surround the municipality are the Rio dos Patos, Rio Caratuva, Rio das Antas, Rio Preto, Rio Riozinho, Rio Cachoeira, Rio Ponte Alta, Río Quente and Rio Taquari, with their courses being directed to the Tibagi, Iguaçu and Ivaí river basins. These tributaries all belong to the Paraná River Basin (Andrade and Felchak, 2009).
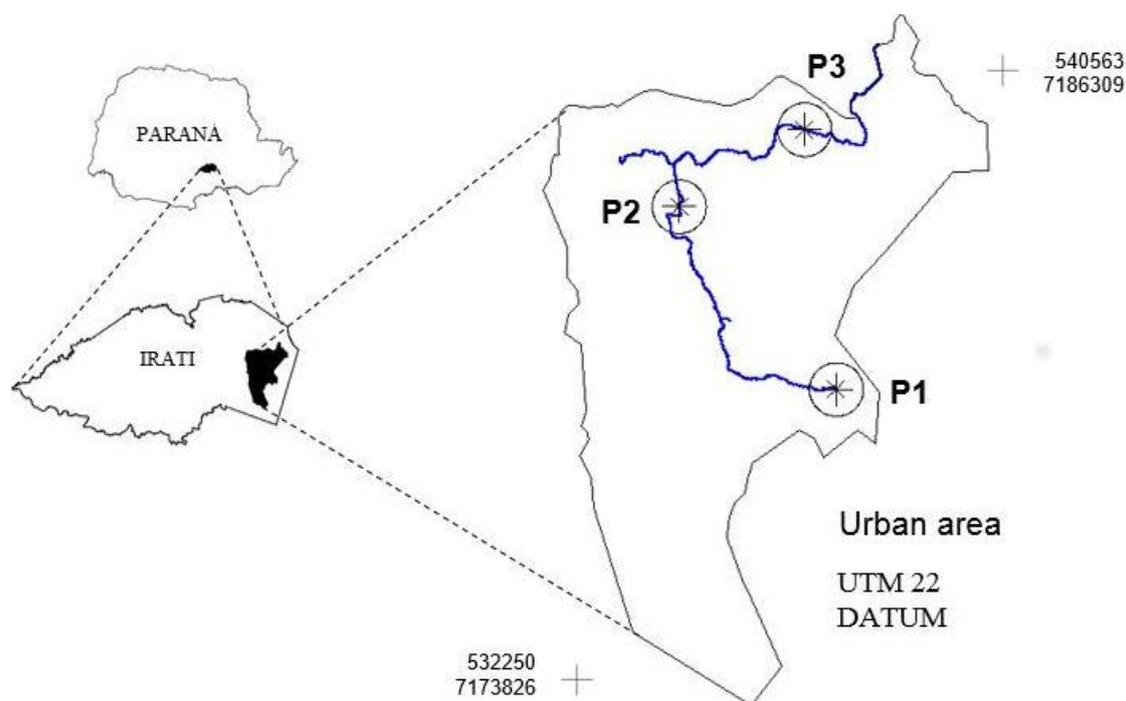

Figure 1. Urban area of the municipality of Irati, Paraná showing the sampling points P1, P2 and P3.
Source: research data.

The Rio das Antas is considered one of the main water bodies of the municipality of Irati. Its origin is in the Serra do Nhapindazal (Nhapindazal mountain range), an area of urban expansion of the municipality. Additionally, its greater extension crosses the urbanized area,

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crosses several neighborhoods, receiving several tributaries before leaving this perimeter when passing under the bridge in BR 277, between the coordinates 25º28'02" South and 50º39'04" West. It flows into the river Imbituvão, belonging to the Tibagi River Basin (Andrade and Felchak, 2009) and according to the SUREHMA Ordinance nº003/91 it is classified as a second order river (SUREHMA, 1991).

Through orbital images, an accurate visual analysis of the surroundings of the Antas River along its urban route was carried out at three different collection points (P1, P2 and P3) in relation to the use and occupation of the land in its surroundings. Since Point 1 is located near the springs; Point 2 and Point 3 are located in areas of urban consolidation.

2.2. Mapping of study areas

With the aid of multispectral orthoimagery fused with spatial resolution of 0.50 m, from the GeoEye satellite from 2013, obtained from the Municipality of Irati, the mapping of the study area was carried out, and for each collection point, a buffer zone, that is, a distance band that surrounds each of these points, with a radius of 500 m, covering a total area of 79 ha (encompassing the proximity around each point).

After the buffers were created, they were mapped. Classes were defined for the use and occupation of the land with the greatest influence in the analyzed areas. Then, through cognitive interpretation and utilizing the technique of vectorization on canvas, polygons were created and associated with the defined classes.

2.3. Assays with *Allium cepa*

Three water collections (campaigns) were carried out, in the months of September and December of 2016 and March of 2017, at the Antas River at predefined collection points. Bioassays with *Allium cepa* (onion) bulbs were placed in separate aquariums. Three assays were performed by collecting, one for each sample and leaving a fourth assay for positive control; following the protocols for plant cell mitosis (Jordão, 1998) along with adaptations of the assay as validated by the United Nations Environment Program (UNEP) as a standard efficient for the monitoring of genotoxicity and mutagenicity (Kumari *et al.*, 2009).

The bioassays were placed randomly by distributing twenty onion bulbs into four aquariums (five bulbs each) containing distilled water (negative control) and left for a period of 96 hours for root growth. Then, five roots of each bulb were randomly collected in each aquarium to perform cytogenetic analyses. The distilled water was replaced by water samples from each collection point of the river. And in the aquarium room, the distilled water was replaced by potassium dichromate solution 1.4 mg.L^{-1} (positive control).

After 48 hours, the same root harvesting procedure was performed and the roots collected were fixed in ethanol and acetic acid solution (3: 1 v / v) and stored in 70% alcohol under refrigerated conditions until they were analyzed by light microscopy. For the cytogenetic analyses, the roots were hydrolyzed in 1N HCl solution at 60ºC for 10 minutes and the cytological slides were made by a crushing technique and were stained with 1% carmin propionic.

Three cytological slides were prepared for the mitotic analyses. Each slide containing one root per bulb, being evaluated 500 cells per phase of mitosis totalizing 2000 cells in a total of 15 slides per treatment. Cells with the most representative abnormalities were photographed using the Olympus CX31 Optical Microscope / SC30 camera.

2.4. Statistical analyses

In order to determine whether there were statistical differences in the percentage of abnormalities (dependent variable) as a function of the 3 study points and the controls (factor), a one-way ANOVA analysis was performed after checking for gaussianity assumptions

(Shapiro-Wilk Normality test) and homogeneity of variances (Bartlett). Peer-to-peer comparisons were also performed by using Fisher's test.

The relationships between the percentage of chromosomal abnormalities and the classes of land use and occupation were investigated through a Principal Coordinate Analysis (PCoA), The analyses were carried out with the aid of software R (specifically, the vegan and MASS packages). For all statistical procedures, values of p <0.05 were considered significant.

3. RESULTS AND DISCUSSION

After completing the mapping of the study areas, eight classes were defined for land use and occupation, which through cognitive interpretation were more evident. They are:

• Urbanized areas: according to Magalhães *et al.* (2013): "[...]the result of anthropic occupation over the territory; are areas that have continuous occupancy of buildings, or that have suffered anthropic impact for urban occupation in some way ";
• Reforestation: Area used for the cultivation of species of commercial interest;
• Tree vegetation: can be defined as a group of species constituted by large trees, present mainly in forests and woodlands.
• Underground vegetation: mainly grasses;
• Bodies of water: rivers, lakes and lagoons;
• Exposed soil: soil devoid of any vegetation;
• Capoeira: area of secondary vegetation (Linhares *et al.*, 2003);
• Agricultural cultivation: occupied area by any stage of agricultural culture.

The classification of the image allowed the calculation of the areas equivalent to each class previously described. The results of area and respective occupation by class can be verified in Figure 2. Point 1 area near the springs of Rio das Antas showed about 77% of green areas, tree vegetation (48.21%), undergrowth (9.84%), reforestation (10.69%) and capoeira (8.07%). Along the way the population density increases and in Points 2 and 3 the predominant class was the urbanized area with 84.06% and 56.84% respectively.

Cytogenetic analyses in meristematic cells of Allium cepa roots showed some type of chromosomal abnormality in all phases of mitosis, which demonstrates the genotoxic potential of water samples at all collection points. Chromosome abnormalities visualized as chromosomal bridges and adhesions, inconclusive chromosomes in the equatorial plate of the cell, chromosomal fragments, early rise of chromosomes or retardant chromosomes culminated in the formation of micronuclei (Figure 3). The micronuclei are formed by cytoplasmic portions of chromatin, have a round or oval shape, are located near the nucleus and are formed by lysis in the DNA molecule during mitotic division, days or weeks after this undergoes mutagenic agent interference (Stich and Rosin, 1983; Stich et al., 1984). The other chromosomal alterations, according to Vidakovié-Cifrek et al. (2002) can be visualized at any stage of cell division as evidence of mutagenic effects arising from agents that cause DNA breaks (clastogenic agents) or that induce abnormal chromosomal segregation or aneuploidy (aneugenic agents), classified by the type of alteration it produces.

Genotoxic effects of different chemical agents have been described in the literature as being responsible for varied chromosomal alterations in different exposed organisms, among them man (Natarajan, 2002). According to Grant (1994), the evaluation of the genotoxicity caused by environmental pollutants in tests using Allium cepa as a bioindicator has been shown to be very efficient, as well as for the quality evaluation of waters affected by anthropic influences (Fiskesjö, 1993; Fatima and Ahmad, 2006).
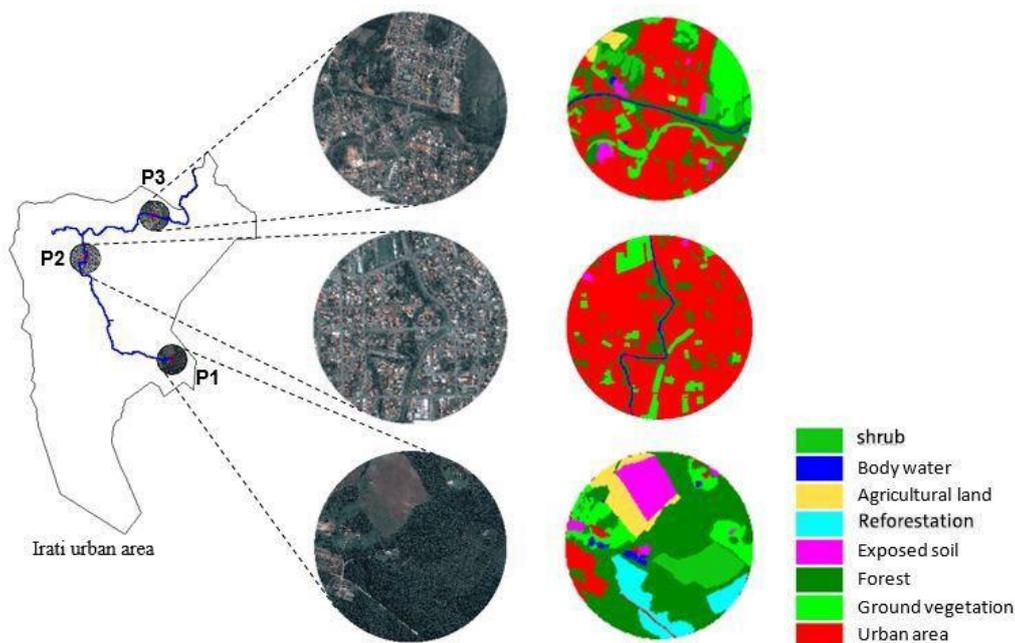
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Figure 2. Classification of the use and occupation of the land to the surroundings of the sampling points.
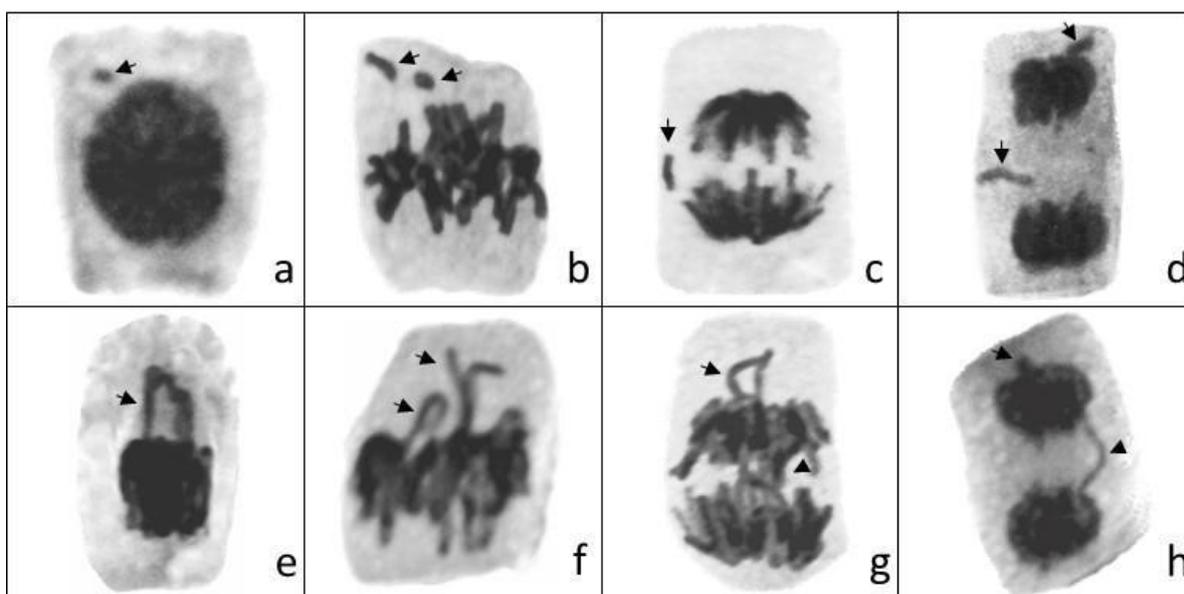**Source:** research data.

Figure 3. Major chromosomal abnormalities found in *Allium cepa* cells. a) Prophase with micronucleus (arrow); b) Metaphase with non-conjoined chromosome fragments (arrows); c) Anaphase with chromosome fragment (arrow); d) Telophase with chromosomes not assembled to the nucleus (arrows); e) Prophase with stickiness chromosomes (arrow); f) Metaphase with early ascending chromosomes (arrows); g) Anaphase with early ascending chromosomes (arrow) and stickiness chromosomes (arrowhead); h) Non-conjoined chromosome telophase (arrow) and chromosome bridge (arrowhead).
Source: research data.

In relation to the cytogenetic analyses in the three sample points and controls, three statistically distinct groups (F = 33,10; p <0,01) were identified, the first one referring to Points 2 and 3 (statistically equal averages) by Point 1 and the negative control and finally a third

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group formed by the positive control, (Table 1, Figure 4). These results are consistent with what is expected since Point 1, near the source (origin) of the study river, is characterized as a region with higher vegetation index and less urbanized when compared to Points 2 and 3, which are characterized as regions with less vegetation, more urbanized and consequently, more eutrophic. Moraes and Jordão (2002) report that plants collected in eutrophic rivers present a higher index of chromosomal alterations when compared to plants that develop in uncontaminated waters. Results similar to those found in this work are described by Oliveira *et al.* (2011; 2012), and Taniwaki *et al.* (2013).

Table 1. Number of cells analyzed and percentage of abnormalities in controls and by collection at each sampling point.

	Collection 1	Collection 2	Collection 3	NTCA	(%) TCA
	(%) CAA	(%) CAA	(%) CAA		
CN	-	-	-	6000	12.90c
CP	-	-	-	6000	24.80a
Point 1	2000 – 13.25	2000 – 13.50	2000 – 12.15	6000	12.97c
Point 2	2000 – 22.70	2000 – 19.45	2000 – 16.95	6000	19.70b
Point 3	2000 – 18.55	2000 – 19.35	2000 – 16.30	6000	18.07b

CAA= Number of Abnormal Cells Analyzed; NTCA= Total Number of Cells Analyzed; MCA= Average Cells Assayed; CN= Negative Control; CP= Positive Control. Averages followed by the same letter do not differ statistically from each other, by the Fisher test at 5% significance. Source: research data.
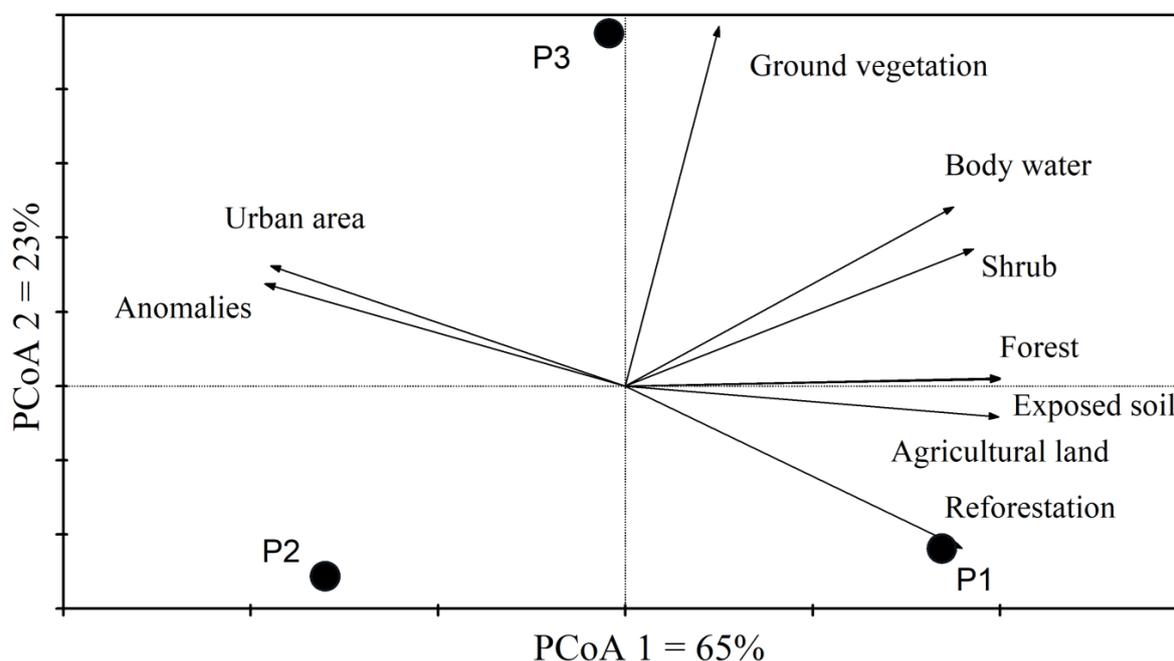

Figure 4. Principal Coordinates Analysis (PCoA).
Source: research data.

In relation to the results already presented, it is possible to discriminate the statistically significant interference of land use and occupation on water quality via genotoxic effect. The Principal Coordinates Analysis (PCoA) (Figure 4), showed that Point 1 is heavily influenced by tree vegetation, especially by areas of natural regeneration. Points 2 and 3, are strongly influenced by the urbanized area in part due to the percentage of chromosomal abnormalities in *Allium cepa* test.

The sampling Points 2 and 3 were the ones that presented the worst results referring to the water quality of the Antas River as evidenced by the presence of chromosomal abnormalities in the *A. cepa* toxicity test. Therefore, it is possible to say that the use and occupation of the land directly influence the quality of these waters, being that the anthropic occupation near the water course negatively impacts its quality.

According to a study by Andrade and Felchak (2009), the Antas River presents several problems resulting from disorderly occupation, such as the absence of riparian forest due to the occupation (accumulation) of the valley bottom, silting due to the creek-free banks and the recti linearization (straightening) process of some stretches of the river, accumulation of organic matter due to lack of sanitation and disposal of industrial chemical wastes not treated correctly. The main problem is the clandestine (possibly illegal) disposal of sewage and the extravasation of it in areas where the sewage network intersects with the rainwater network, and the pluvial network with the sewage network.

The riparian forests are areas around bodies of water, established as APPs. According to Law No. 12,651 / 2012, which established the New Forest Code, APP is: "[...]protected area, covered or not by native vegetation, with the environmental function of preserving water resources, landscape, geological stability and biodiversity, facilitate the genetic flow of fauna and flora, protect the soil and ensure the well-being of human populations." Also, in accordance with this law, it is considered as APP, in rivers with a width of less than 10m, a marginal range of 30m, as is the case in Antas River. The law also provides for the urban land regularization of specific interest of areas that are occupying APP and for environmental regularization purposes that a non-buildable strip with a minimum width of 15m on each side must be maintained. Nonetheless, it should be pointed out that in most of the areas analyzed in this study, it was not widely observed in Points 2 and 3, located in areas of urban consolidation.

Works such as Marmontel and Rodrigues (2015), Cândido *et al.* (2015) and Silva *et al.* (2016), have already verified that anthropic actions, together with the devastation of the ciliary forest, negatively influence the water quality of the water bodies. Furthermore, in places where there is greater preservation of the vegetation the quality water is also better.

4. CONCLUSIONS

The results were obtained in the bioassays using *Allium cepa* as test organisms. Combined with orbital satellite imagery, statistical analyses were effective as alternative validation tools to monitor the toxic effects of possible pollutants present in water bodies with compromised water quality. Accompanied by physical-chemical analyses to prove their results, these can become even more significant.

The Antas River (Rio das Antas) is experiencing significant degradation of PPAs, especially in urban consolidation areas. The results indicated that water quality is intrinsically related to land use and occupation. The sampling area near the sources of the Rio das Antas (P1) has a significant portion of arboreal vegetation, a fact that contributes to a better quality of the water of the locale (region). However, the remaining sample areas (P2 and P3), suggest a greater deterioration in water quality as evidenced by the greater presence of chromosomal abnormalities in the *Allium cepa* test. These areas suffer from the detrimental impact on the anthropic occupation and a consequent lack of PPAs; a fact that supports the observed results.

One can then verify the remarkable interference that man can bring to the ecosystem. Also, the importance of PPAs for a watercourse, can assist in greater preservation and better quality of the water resources.

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