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Evaluating the Trace Element Concentration in Sediments and Assessing Their Genotoxicity in Ichthyofauna of a Coastal Lagoon in Southeastern Brazil

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Abstract: Lacustrine ecosystems are constantly affected by industrial and domestic effluents, which are considered to be the main sources of trace elements in the environment. The physicochemical characteristics of trace elements undergo modifications that can cause reversible genotoxic damage to ichthyofauna. This study aimed to assess the environmental quality of a lagoon (Mãe-Bá) that receives industrial effluents from one of the largest iron ore companies in the world, located in southeastern Brazil. The physicochemical parameters of the lagoon water were analyzed monthly, the trace element levels in the sediment were quantified, and the risk of genotoxic damage to fish was quantified using a micronucleus test and comet assay. We verified the poor environmental quality of the lagoon, and strong anthropic action was evident, with particularly high levels of Cr and Ni and genotoxic damage being observed in fish. It is not possible to state a relationship between the increase in Cr and Ni with the mining company since we found high concentrations of these elements in a reference lagoon (Nova Guarapari) with no connection to the mining company. Even if the bioavailability of the trace elements in the water resource is low or if their concentration is below the permitted limit, their presence can cause genotoxic damage. These findings can enable us to assist in planning suitable remediation strategies to decrease the genotoxic effects observed in these sensitive eco-systems. A multidisciplinary approach is needed in studies involving ecotoxicology to develop conservation strategies for both the biotic and abiotic environments.

Keywords: comet assay; genotoxicity; micronucleus test; trace elements

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Citation: Camargo Filho, C.B.; dos Santos, A.R.; Ferrari, J.L.; Preto, B.d.L.; Kunz, S.H.; Senhorelo, A.P.; Burak, D.L.; Rossi Junior, J.L. Evaluating the Trace Element Concentration in Sediments and Assessing Their Genotoxicity in Ichthyofauna of a Coastal Lagoon in Southeastern Brazil. *Diversity* **2022**, *14*, 151. https://doi.org/10.3390/d14020151

Academic Editor: Michael Wink

Received: 31 January 2022
Accepted: 18 February 2022
Published: 20 February 2022

1. Introduction

Trace elements released from industrial and domestic effluents can affect the physical, chemical, and biological characteristics of the water and sediment of lake ecosystems [1]. In addition, strong winds [2] and varying degrees of pollution [3] and rainfall [4–6] can influence sediment characteristics.

The physicochemical characteristics of water, rainfall, and wind can cause unpredictable toxicity in natural environments [7]. For example, temperature has been indicated to be an environmental disturbance in several previous studies [8–10].

Domestic effluents containing excessive amounts of xenobiotics and pathogens can cause toxicity in fish and in the subsequent consumers of fish in the food chain through bioaccumulation and biomagnification [11,12]. Furthermore, several studies have reported

that sediments comprise a large repository of carbon and trace elements [13–19]. Anthropic factors are primarily responsible for the deposition of these pollutants in sediments. However, most studies have analyzed trace elements separately, and their combinations have often been neglected [20].

Fish can lose their ability to respond to changes in their natural environments due to the presence of trace elements, even at very low concentrations [21]. Research has indicated that fish may be potential bioindicators of environmental variability, and the comet assay and micronucleus (MN) test have been used to assess the genotoxicity of water pollutants in fish [22]. These two methods are mainly used to detect DNA damage resulting from the xenobiotic contamination of organisms [23].

The simple, sensitive, and rapid comet assay allows for the detection of DNA damage in individual cells [24,25] and the assessment of the effects of genotoxic agents from domestic and industrial effluents on organisms that have been exposed to contaminants [26]. For example, DNA replication in fish erythrocytes can not only be affected by untreated wastewater from industries and urban areas [22], but also by stressors such as excess organic matter and microbiological agents [27] and extreme climatic factors such as rainfall [2,27,28] and wind [27]. Furthermore, the assay is useful for assessing short-term genotoxicity [29], and although it does not detect genomic mutations, it can identify lesions that can be corrected [24]. Moreover, if the mutated DNA is not corrected, biological reactions can be affected, primarily at the cellular level, which, in turn, can affect the organism as a whole by interfering with its growth process and reducing its survivability during the embryonic, larval, or adult stages [30].

The MN test identifies entire chromosomes and chromosomal fragments that are generated during cell division due to the absence of a centromere, which is the result of damage during the cytokinesis stage [31] or chromosomes that are lost during cell division [24,32]. Thus, these replicated chromosomes are morphologically identical to but smaller than those that were originally in the nucleus [33]. The presence of MN is not only a sign of genetic damage, but also of carcinogenesis [34], because tumors originate from mutations, which are the result of replication errors or defects in the DNA repair mechanisms that are caused by radiation, chemical products, etc., that can lead to structural changes in the DNA [35].

Interactions with genotoxic compounds, such as heavy metals, can also cause cell lesions and tumor formation [36]. For decades, the effects of pollutants on DNA integrity have been studied in aquatic animals, particularly in fish [37]. Furthermore, pollutants can cause hereditary defects and can have teratogenic effects on germ cells, causing changes in the reproductive behavior and growth of aquatic organisms [30], thereby reducing their populations [38]; fish are therefore considered bioindicators of environmental quality.

Studies quantifying the concentrations of trace elements in water resources are easily found in the literature [1,13–16,19,39–41]. However, such studies have not determined the real damage to the aquatic community since the bioavailability of these elements is influenced by environmental factors such as water and sediment. Additionally, studies involving the quantification of trace elements and their genotoxicity in free-living environments are scarce. For example, we only found one genotoxic study on lagoons [2] and four studies on rivers [22,27,28,36], but in these examples, the genotoxicity was not correlated with trace elements in the water. A previous study on the Mãe-Bá Lagoon observed that the mining process contributed to the reduced bioavailability of trace elements in the analyzed biota [13], but whether this result was caused by another factor impacting that site was not established. We hypothesized that in water resources, even in areas where there is a low bioavailability of trace elements, such characteristics can cause other types of damage to aquatic fauna, such as genetic damage to the biota. Such damage can be identified with simple methods such as the micronucleus test and comet assay. Therefore, we aimed to investigate the environmental quality of a coastal lagoon through genotoxicity tests of its inhabitant free-living fish. In addition to domestic effluents, this lagoon receives industrial effluents from one of the largest iron ore companies in the world.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Mãe-Bá Lagoon, the second largest lagoon in the state of Espírito Santo. It is situated between the municipalities of Guarapari and Anchieta (latitude 20°45′ S, longitude 40°34′ W). The lagoon has been under tremendous anthropic pressure since the 1970s, initially with the closure of its connection to the sea, and later with the installation of an iron ore beneficiation plant, which releases treated industrial effluents. The effluent is released from the northern dam, which was built to separate one of the arms of the lagoon and was subsequently used to receive water from the iron ore pipeline. The establishment of the iron ore plant increased the presence of local communities around the lagoon, which was used for artisanal fishing, captive fish breeding, and recreation, and it also receives diluted and untreated domestic wastewater.

The entire area of the Mãe-Bá Lagoon (4.67 km^2) can be divided into three regions: the southern region (1.38 km^2), which is located close to the iron ore plant and connected to the northern dam from which industrial effluents are released; the central regions (1.51 km^2), which is located relatively close to the iron ore plant and the Mãe-Bá community, which releases its sewage directly into the lagoon without proper treatment by the sewerage company despite the existence of a sewage collection system; and the northern region (1.78 km^2), which is located across from a mining company and close to the local Porto Grande and Condados communities (Figure 1).

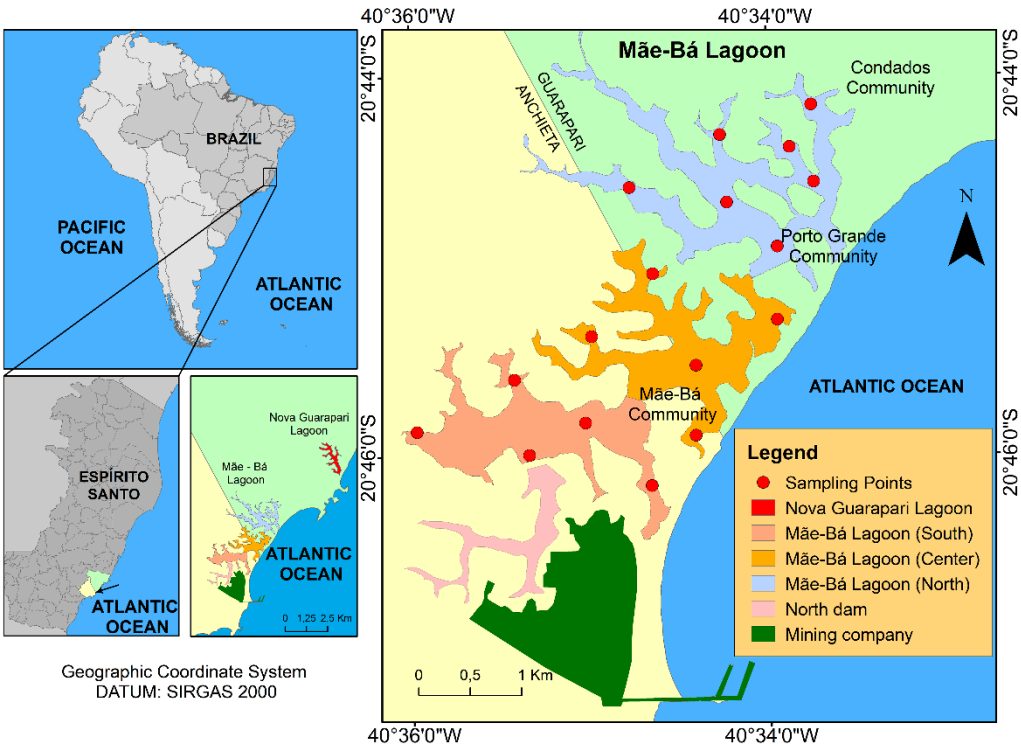

Figure 1. Location of Mãe-Bá Lagoon in Espírito Santo state, Brazil.

Nova Guarapari Lagoon, which is located 4 km from the Mãe-Bá Lagoon, was used as a reference site to determine whether the concentrations of trace elements in the Mãe-Bá Lagoon were affected by the mining company. We want to emphasize that the analysis of the trace element concentrations was carried out for the two lagoons and that the water quality analysis, MN tests, and comet assay were only carried out for Mãe-Bá.

2.2. Water Quality Analysis

The water quality of 204 samples collected from 17 sampling points in the Mãe-Bá Lagoon from January to December 2015 was analyzed (Figure 1).

The biochemical oxygen demand (BOD), total phosphorus (TP), orthophosphate (OP), total nitrogen (TN), hardness (DH), and total alkalinity (ALK) of the water samples were determined. Samples were collected in 1 L polyethylene bottles at a water depth of 10 cm, and the bottles were kept in a thermal box with ice for 3 h during transit from the study site to the Laboratory of Aquatic Ecology and Plankton Production at the Federal Institute of Espírito Santo, Alegre Campus. The preparation, collection, and storage of the samples for the BOD, OP, DH, and ALK analyses were conducted following the Standard Methods for the Examination of Water and Wastewater guidelines (Apha, 2005) [42]. TN and TP concentrations were determined according to the experimental recommendations by Valderrama (1981) [43].

The temperature (TEMP), percentage of dissolved oxygen (ODP), dissolved oxygen (DO), conductivity (COND), and potential of hydrogen (pH) were measured on site using a multiparameter probe YSI Professional Plus immediately after sample collection, and transparency (TRA) was measured with a Secchi disk.

2.3. Analysis of Trace Elements in Sediment

Sediment samples (N = 114) were collected during the summer, autumn, winter, and spring in 2015 from the three Mãe-Bá Lagoon regions and from the reference lagoon (Nova Guarapari).

Samples were collected using a Peterson stainless steel dredge; subsequently, the 50 g samples were stored in polyethylene bottles and refrigerated at 4 ± 2 °C. Sample collection and storage followed the Standard Methods for the Examination of Water and Wastewater guidelines (Apha, 1998) [44].

2.3.1. Determination of Sediment Organic Matter (OM) (Loss on Ignition Calcination Method)

The organic matter in the sediments was determined by differences in weight (Davies, 1974) [45].

2.3.2. Preparation of Sediment Samples to Determine Trace Elements

The sediment samples were oven-dried with air circulation at 60 °C until a constant weight was achieved, and they were then macerated and homogenized using a different porcelain mortar and pestle for each sample to prevent cross-contamination. The equipment was cleaned and decontaminated before use (Apha, 1998) [44].

After maceration, the sediment was sieved manually using a <63 µm mesh sieve made of 100% nylon PA66; subsequently, 500 mg sediment samples were stored in cryopreservation tubes at −4 °C for further analysis.

2.3.3. Sample Preparation

The concentrations of chromium (Cr), nickel (Ni), cadmium (Cd), lead (Pb), copper (Cu), manganese (Mn), cobalt (Co), iron (Fe), potassium (K), calcium (Ca), magnesium (Mg), and zinc (Zn) were determined using the 3051A method (USEPA, 2007) [46] by transferring approximately 500 mg (precision of 0.0001 g) of each sediment sample to a digestion flask in a microwave oven.

Subsequently, 9.0 mL of nitric acid (Merck® 65% PA) and 3.0 mL of hydrochloric acid (Merck ® 65% PA) were added. The solution was allowed to stand for 15 min with the lid open in a chapel with an exhaust gas system. The flasks were then sealed and kept in the microwave oven for 40 min at approximately 172 °C using 650–1000 W of power, and an approximate pressure of 130 psi.

After the reaction period, the flasks were allowed to cool and were later opened in the chapel with the gas exhaust system. The flask contents were transferred to sterile polypropylene conical tubes, which were filled to the 25 mL mark with Milli-Q® ultrapure water and stored under refrigeration until further atomic absorption analysis. Two blank samples were prepared for each series of 10 samples.

2.3.4. Determination of Trace Elements and Quality Control

The levels of the trace elements were determined by ICP-OES (Optima 3300 DV, PerkinElmer) with high-purity argon (>99.995%); an amount of 1000 mg L^{-1} of multi-elemental standard solution (Sigma-Aldrich©) was used to build the calibration curve. The limit of detection (LOD) and practical limit of quantification (PLOQ) were calculated using blanks prepared during the digestion procedure. The PLOQ and LOD values were calculated using the slope of the calibration curve [47]. A dilution factor was used to determine the PLOQ. The analysis was quality controlled using a certified ERM-CC141 sample—loam soil (trace elements)—and 2 samples were digested for each series of 10 samples. The recovery rate that was calculated for each metal varied between 86 and 119% (Table 1), which is considered satisfactory for the 3051A method [48] under the operating conditions for LOD and PLOQ. The recovery rates are shown in Table 1.

Table 1. Wavelength, limit of detection (LOD), quantification (PLOQ), and percent recovery from certified reference material of sediment (ERM-CC141—loam soil) for Cr, Ni, Cd, Pb, Co, and Zn.

Element	Wavelengths nm	LOD µL L^{-1}	PLOQ mg kg^{-1}	Recovery %
Cr	267.71	2.34	0.47	119
Ni	231.60	6.62	0.92	89
Cd	214.44	0.64	0.13	86
Pb	220.35	0.68	0.18	102
Co	228.61	2.02	0.20	101
Zn	213.85	3.24	0.65	88
Mn	259.37	0.32	0.06	102

Notes: Limit of detection (LOD): 3 σ $(tg\ \alpha)^{-1}$. Practical limit of quantification (PLOQ) = LOD × dilution factor (FD). % Recovery = (recovered value/certified value) × 100.

2.4. Genotoxicity Analysis

2.4.1. Fish Collection

The genotoxic effects of anthropic actions on the Mãe-Bá Lagoon were assessed in three fish species (all omnivorous): *Astyanax bimaculatus*, *Geophagus brasiliensis*, and *Oreochromis niloticus* (Table 2). These fish were found at all of the collection points in the lagoon (Figure 1). In total, 113 fish were collected and analyzed. Fish collection was authorized by SISBIO (no. 40278-4 and 40278-5) and the Ethics Committee on the Use of Animals (No. 304/2013) for six points in the southern, central, and northern regions of the Mãe-Bá Lagoon.

Table 2. Number of fish species sampled at each site of Mãe-Bá Lagoon.

Sites/Species	*A. bimaculatus*	*G. brasiliensis*	*O. niloticus*
Southern	27	5	9
Central	27	3	7
Northern	22	9	4

Specimen collection took place between January and September 2015 for 48 h at each sampling point; the nets were inspected every morning. Only live fish were collected, packaged, and transported in plastic bags (filled with water from the collection site and medicinal oxygen) to the on-site research area.

2.4.2. Sample Preparation

The collected fish were kept in the sample bags for a maximum of 2 h and were then transferred to an aquarium containing 4 L of water. For sedation, 200 mg L^{-1} of eugenol was added to 20 mL of alcoholic solution [49].

After sedation, 0.5 mL of blood was extracted from each fish by intracardiac puncture using a 3 mL syringe containing 0.2 mL of EDTA anticoagulant, and the blood samples were stored in 2 mL cryopreservation tubes and preserved on ice for the MN test and comet assay. Finally, the animals were euthanized following the protocols of the National Council for the Control of Animal Experimentation (Conselho Nacional de Controle de Experimentação Animal (CONCEA)) and Resolution 1000/2012 of the Federal Council of Veterinary Medicine (Conselho Federal de Medicina Veterinária (CFMV)).

2.4.3. Micronucleus Test

Micronuclei were identified and classified according to Grisolia (2002) and Grisolia and Cordeiro (2000). For this, a few drops of a collected blood sample were transferred onto a glass slide to prepare two blood smears. The smears were left to dry, and the slides were then subjected to a 30 min bath of methanol PA (100%) for fixation, stained with 5% Giemsa solution for 40 min, washed with distilled water, and dried. Subsequently, the samples were observed under an optical microscope at 400× magnification with 1000 blood cells (erythrocytes) counted in each slide to quantify the MN in the cells [50,51].

The MN test was used to determine the MN frequency in the three fish species.

2.4.4. Comet Assay

The comet assay was conducted twice using an alkaline method (pH > 13) and silver nitrate as a stain. From each slide, 100 cells were counted and classified into four classes of DNA damage: I, no damage; II, minor damage; III, intermediate damage; and IV, autolysis (Tice et al., 2000) [52]. In total, 22,600 cells were analyzed.

The damage classes among the species, regions, and months were compared via a damage index (DI), which was determined in Grisolia et al. [53]:

$$DI = \frac{N1 + 2N2 + 3N3 + 4N4}{S/100}$$

where *DI* is the damage index for the DNA; *N1*, *N2*, *N3*, and *N4* are the number of cells in damage classes 1, 2, 3, and 4 respectively; and *S* is the number of nucleoids analyzed.

2.5. Statistical Analyses

Water quality and the concentrations of the trace elements in the sediment samples were statistically analyzed (SAS software, University Edition) using the sampling plots (southern, central, and northern regions of Mãe-Bá Lagoon) as the main plots. Data were analyzed by repeated measures, and in the case of significant interactions, means were sliced by time. Means were compared using Tukey's test at $p < 0.10$. In this analysis, an additional location was included as a control group. The Nova Guarapari Lagoon is a lagoon that does not receive waste from mine ore activity and was used as a control for the trace elements.

To verify the interaction between fish species (*A. bimaculatus*, *G. brasiliensis*, *O. niloticus*), regions (southern, central, northern), and dates (January, February, March, April, May, June, July, August, September) in the micronucleus test, DNA damage index, and damage classes (I, II, II, IV) assessed by the comet assay, the Kruskal–Wallis test was used. For the MN test, 2000 cells per fish were used, and for the comet assay, 200 cells per fish were analyzed.

3. Results

3.1. Water Quality

The physicochemical parameters of water in the Mãe-Bá Lagoon are shown in Figure 2.

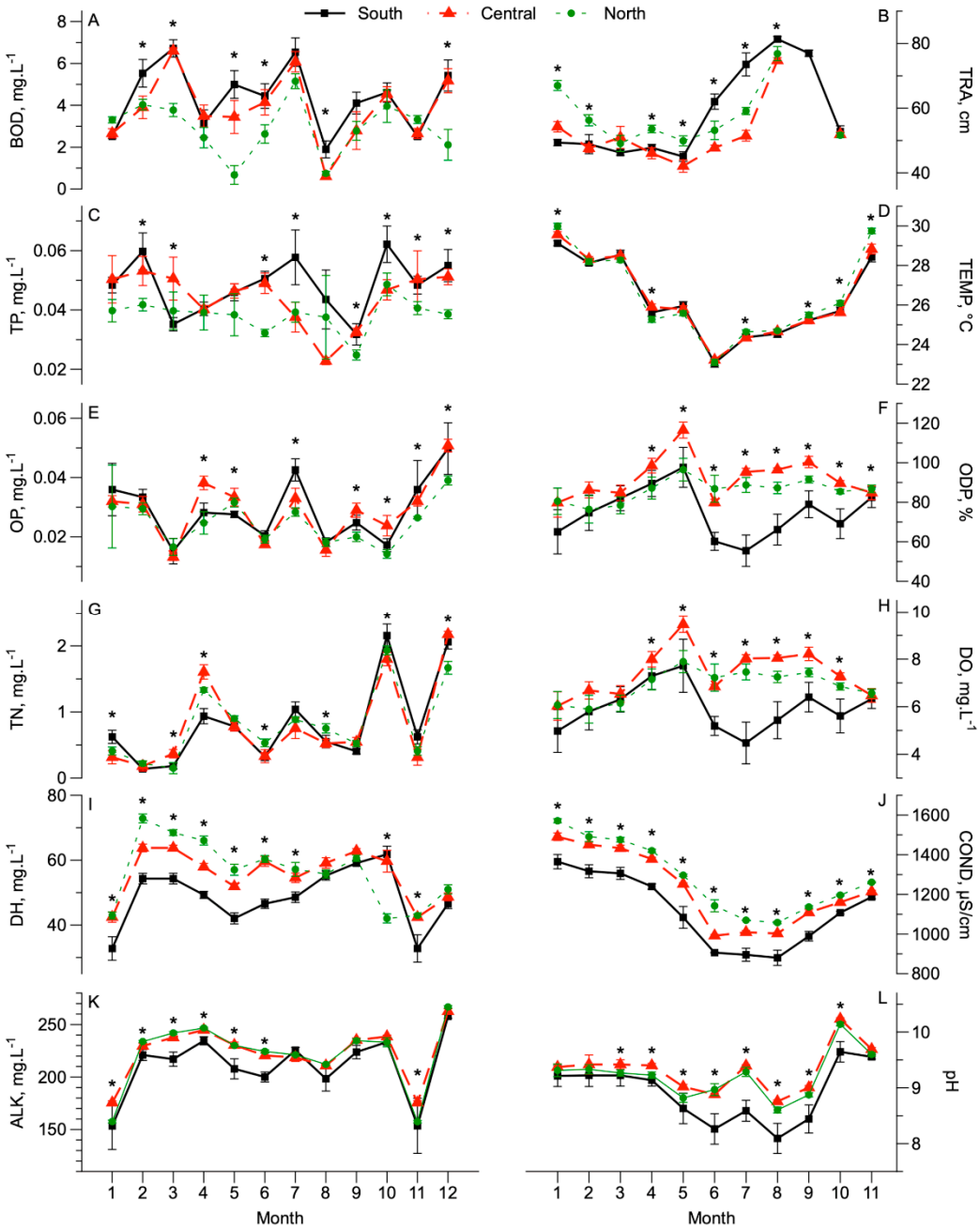

Figure 2. Physicochemical parameters of water in southern, central, and northern regions of Mãe-Bá Lagoon in (1) January, (2) February, (3) March, (4) April, (5) May, (6) June, (7) July, (8) August, (9) September, (10) October, (11) November, and (12) December. * Tukey's test showed statistically significant differences between regions during each month ($p < 0.1$).

Although the annual results were similar among the three regions, Tukey's test showed statistically significant differences between the regions for each month ($p < 0.1$).

3.2. Concentrations of Trace Elements in Sediment

The trace element concentrations in the sediment collected from the Mãe-Bá and Nova Guarapari Lagoons were similar, with no significant differences being observed according to Tukey's test ($p < 0.1$); however, differences in the Pb, Mn, Co, K, and OM in the three regions of the two lagoons were observed (Table 3).

Table 3. Mean and standard deviation of trace element concentrations (mg kg^{-1} ± SD of dry weight) in the southern, central, and northern regions of the Mãe-Bá and Nova Guarapari (reference) Lagoons and the percentage of organic matter (OM) in the sediment.

Trace Element	*p*-Value	Southern Region (N = 30)	Central Region (N = 30)	Northern Region (N = 30)	Nova Guarapari Lagoon (N = 24)	CONAMA No. 454		MacDonald et al. (2000) [54] [3]
						(Level 1) [1]	(Level 2) [2]	
Cr	0.260	59.62 ± 15.517	13.52 ± 24.611	64.95 ± 13.679	69.51 ± 15.260	37.3	90.00	111.00
Ni	0.347	29.65 ± 10.482	3.89 ± 16.625	31.27 ± 9.240	39.57 ± 10.308	18.00	35.90	48.60
Cd	0.284	0.02	0.02	0.00	0.01	0.60	3.50	4.98
Pb	0.006	2.76ab ± 0.340	1.14c ± 0.539	2.37b ± 0.300	3.36a ± 0.334	35.00	91.30	128.00
Cu	0.047	3.81 ± 0.294	3.06 ± 0.466	4.50 ± 0.259	4.10 ± 0.289	35.7	197.00	149.00
Mn	0.038	38.84b ± 4.600	60.55a ± 7.296	36.45b ± 4.055	39.19b ± 4.523	-	-	-
Co	0.007	0.75b ± 0.108	0.97a ± 0.172	1.25a ± 0.095	0.88b ± 0.107	-	-	-
Fe	0.001	13945 ± 1080	22303 ± 1080	18990 ± 1080	21168 ± 1080	-	-	-
K	0.062	358.15a ± 34.910	181.47b ± 55.370	286.95a ± 30.774	306.96a ± 34.330	-	-	-
Ca	0.200	623.77 ± 70.755	861.06 ± 112.220	590.57 ± 62.375	676.79 ± 69.582	-	-	-
Mg	0.141	686.67 ± 77.134	358.48 ± 122.340	532.08 ± 67.998	551.49 ± 75.855	-	-	-
Zn	0.412	9.08 ± 0.865	9.71 ± 1.373	9.20 ± 0.763	10.89 ± 0.851	123.00	315.00	459.00
OM	0.001	14.98c ± 2.090	59.16a ± 3.315	20.54b ± 1.842	19.63bc± 2.055	-	-	-

[1] Level 1, threshold below which there is less likelihood of adverse effects on biota. [2] Level 2, threshold above which there is a greater probability of adverse effects on biota. [3] Numerical sediment quality guidelines (SQGs) for freshwater ecosystems. Different letters between columns in same row indicate significant differences ($p < 0.1$) as determined by Tukey's test.

The Pb concentrations in the Nova Guarapari and in the southern region of the Mãe-Bá were high, differing from the other regions, which exhibited low Pb concentrations.

The OM content in the central region of the Mãe-Bá Lagoon showed the greatest differences among the study locations because of domestic effluents containing a high proportion of organic matter released by the sewerage company. The Mn presented a similar behavior to the OM, with the central region obtaining a higher value and being statistically different from the other regions. Higher Co concentrations were observed in the central and northern regions of the Mãe-Bá Lagoon.

3.3. Genotoxicity

According to the Kruskal–Wallis test, there were no statistical differences ($p < 0.05$) observed between the fish species and regions that were observed during genotoxicity testing (micronucleus test), and similar findings were determined for the damage index and DNA damage classes during the comet assay (Tables 4 and 5). Even so, the highest cell damage frequency was observed in the damage classes II and IV in the three fish species and the three regions that were studied.

According to the Kruskal–Wallis test, there were no significant differences based on the micronucleus test ($p < 0.05$) during the studied months. However, when comparing the results between the months via the comet assay, we observed a statistical difference ($p < 0.05$) using the Kruskal–Wallis test; in May, August, and September, we found the highest percentage of class IV damage, which consequently increased the damage index in those months. January, March, and April were the months with the lowest damage index values (Table 6).

Table 4. Micronucleus test and DNA damage index assessed by comet assay for all studied species (mean ± SD).

Species/Test	Micronucleus Test (‰)	Damage Index	Class I (%)	Class II (%)	Class III (%)	Class IV (%)	N
A. bimaculatus	0.64 ± 1.028	151.78 ± 42.305	7.28 ± 15.573	30.94 ± 33.133	12.64 ± 15.249	49.12 ± 39.226	76
G. brasiliensis	0.18 ± 0.393	139.91 ± 44.263	10.21 ± 24.246	38.09 ± 36.294	13.38 ± 18.626	38.32 ± 36.029	17
O. niloticus	0.40 ± 0.400	135.49 ± 46.510	10.10 ± 21.222	44.25 ± 38.987	12.22 ± 17.153	33.92 ± 38.323	20
p-Value *	0.179	0.372	0.781	0.428	0.733	0.217	

* Kruskal–Wallis test.

Table 5. Micronucleus test and DNA damage index assessed by comet assay from three monitored regions of the Mãe-Bá Lagoon (mean ± SD).

Region/Test	Micronucleus Test (‰)	Damage Index	Class I (%)	Class II (%)	Class III (%)	Class IV (%)	N
South	0.63 ± 1.000	139.55 ± 46.311	12.55 ± 23.711	34.52 ± 35.135	15.17 ± 16.979	38.00 ± 36.800	41
Center	0.50 ± 0.816	149.71 ± 46.009	7.99 ± 17.638	34.20 ± 37.204	8.11 ± 13.624	49.68 ± 41.838	37
North	0.43 ± 0.850	153.21 ± 36.795	3.40 ± 5.650	34.37 ± 32.615	14.59 ± 16.472	47.63 ± 37.702	35
p-Value *	0.398	0.355	0.137	0.841	0.079	0.336	

* Kruskal–Wallis test.

Table 6. Micronucleus test and DNA damage index evaluated by comet assay during the months in which the Mãe-Bá Lagoon was monitored (mean ± SD).

Data/Test	Micronucleus Test (‰)	Damage Index	Class I (%)	Class II (%)	Class III (%)	Class IV (%)	N
1	0.34 ± 0.598	109.31a ± 39.871	30.03e ± 34.707	36.84cd ± 26.926	17.59bc ± 19.387	15.531ab ± 26.478	16
2	0.65 ± 0.964	160.21b ± 36.305	8.73bcd ± 17.224	18.26bc ± 15.658	16.85bc ± 14.509	56.147cd ± 30.408	17
3	0.62 ± 0.866	101.89a ± 14.719	6.94cde ± 8.173	85.62e ± 17.001	4.16a ± 11.704	3.281a ± 12.474	16
4	0.46 ± 1.305	118.15a ± 33.048	9.58de ± 12.989	64.87de ± 33.719	5.21a ± 10.953	20.333ab ± 29.562	12
5	0.85 ± 1.107	190.97cd ± 11.080	0.10a ± 0.316	0.80a ± 0.422	15.75abc ± 21.888	83.250de ± 21.959	10
6	0.45 ± 0.568	159.43bc ± 32.433	2.77abc ± 5.811	31.64bcd ± 25.830	9.54abc ± 8.493	56.045cd ± 30.848	11
7	0.92 ± 0.917	181.46bcd ± 16.331	0.75ab ± 1.837	7.67ab ± 12.148	19.50bc ± 17.487	72.083cde ± 23.094	6
8	0.04 ± 0.144	193.94d ± 7.358	0.54ab ± 0.498	3.37a ± 5.059	3.58ab ± 6.424	92.458 e ± 9.969	12
9	0.61 ± 1.102	155.63b ± 34.591	2.42ab ± 4.041	30.23bc ± 30.211	24.08c ± 17.124	44.038bc ±35.510	13
p-Value	0.162	<0.001	<0.001	<0.001	<0.001	<0.001	

Different letters in same column indicate significant differences ($p < 0.05$) according to the Kruskal–Wallis test. 1, January; 2, February; 3, March; 4, April; 5, May; 6, June; 7, July; 8, August; 9, September.

4. Discussion

4.1. Water Quality

The physicochemical parameters of the water samples varied significantly (Figure 2). CONAMA Resolution no. 357 from 17 March 2005, which provides environmental guidelines in Brazil, classifies waterbodies into three classes according to the water quality standards [55]. The BOD, TP, OP, and TN values in the Mãe-Bá Lagoon varied significantly in classes 1, 2, and 3, indicating the instability of environmental problems that have been aggravated by climatic factors.

In estuarine lagoons, strong winds easily disturb sediments [2]. In addition, primary sedimentation can influence pollution levels [3], consequently increasing the eutrophication of water bodies due to higher nutrient availability. However, the input can increase BOD and TP levels in shallow lagoons without a hypolimnion, which can lead to sudden changes in the BOD and TP (Figure 2) and other aquatic physicochemical parameters due to the sediment resuspension that is caused by the strong influence of the wind on coastal lagoons.

4.2. Concentrations of Trace Elements in Sediments

The increasing concentrations of trace elements in the study area are not specific to the Mãe-Bá Lagoon, as other lagoons have encountered similar problems due to identical causes. Since the last century, these occurrences have been a common global phenomenon because of intense anthropogenic processes [19,40,56–58]. Thus, the levels of most of the trace elements in this lagoon were similar to those reported in studies carried out around the world [1,13–15,19,41,59], reinforcing the notion that the contamination of water resources is a chronic global problem that is caused by both natural processes and anthropogenic activities, such as the release of industrial and domestic effluents, agricultural runoff, land-use, or atmospheric activities.

We did not find any statistical differences among the mean trace element concentrations (Cr, Ni, Cd, Cu, Fe, Ca, Mg, and Zn) in the southern, central, and northern regions of the Mãe-Bá Lagoon (Table 3). This was expected because the lagoon, despite its large size, is in fact a single water system. However, trace elements can exhibit minor concentration

variations due to the differences in their transfer as a result of several factors, such as water depth, sediment composition, water flow speed, and pH [58].

The Cd, Pb, Cu, Mn, Fe, and Zn concentrations in the Mãe-Bá Lagoon that were observed by Pereira et al. [13] were higher than those in the present study, with the exception of Cr and Ni, which were lower in some regions. Studies have indicated that cleaning products from anthropic sources, such as powdered soaps, have high levels of Cd, Zn, and Cu [60].

The Nova Guarapari (reference) Lagoon presented trace element concentrations similar to those of the Mãe-Bá Lagoon even though it has no direct contact with the mining company. Significant increases in the Cr and Ni concentrations were observed in the lagoon, indicating an association of its water quality with the domestic effluents released from urban areas lacking sewage treatment [61]. However, significant differences among the concentrations could not be determined.

The Mãe-Bá Lagoon is not only impacted by the release of untreated domestic sewage. Previous studies showed an increase in the Cr and Ni concentrations as well as those of other metals (Fe, Mn) as the result of the improvement of iron ore in the region. This is likely because these trace elements had higher concentrations in the places of origin (mining) compared to the concentrations at the final destination (Mãe-Bá Lagoon) [13]. The gradual reduction in the concentrations of trace elements in the iron ore beneficiation process is due to the treatment processes carried out by the mining company itself. This process begins at the mining sites and reaches the North Dam, where the industrial effluent is released into the Mãe-Bá Lagoon. Clearly, Co does not show a relationship with Mn and Fe, indicating that its source is not related to the geochemical cycle since these elements appear together in geogenic minerals bearing these elements. Among anthropogenic sources, the residual phase of the metallurgical industry stands out as a potential source of Co as well as of Ni and Cr [62]. Within the study region, the regions close to the mining company tend to have lower levels of Mn and Co, meaning that further studies into the probable anthropogenic sources of Co and Mn in the sediments of the Mãe-Ba Lagoon are required.

Pereira et al. (2008) mentioned reductions in the Fe, Mn, Cr, Cu, and Hg concentrations between the mining sites and the Mãe-Bá Lagoon [13]. However, they did not mention Ni, which explains the significant increase in its concentration in this study compared to in the previous study, as gradual deposition has occurred over the years, and Cr, despite showing a gradual reduction, showed the same trend as Ni. Gopal et al. (2017) associated high Pb and Zn concentrations with agricultural activities and untreated domestic solid waste and effluents. Conversely, trace elements such as Cr, Cu, Fe, and Mn are associated with natural soil erosion [15]. All of these results reinforce the idea that the potential sources of trace elements are of anthropic origin, causing environmental problems in water ecosystems. We would like to emphasize that all of these anthropic activities occur in the study area.

In addition to the iron ore industry and the surrounding communities, agriculture, as a form of land use and occupation, leads to soil exposure. Agricultural sources contribute to trace elements entering into the lagoon [59] due to the proximity of anthropic sources [39].

Lower concentrations of some elements, such as Fe, Mn, and Co, which are abundant in the Earth's crust, are found in the Mãe-Bá Lagoon [63]; these values are also lower compared to those determined in previous studies [13,14,41,58]. According to CONAMA Resolution no. 454 from November 1, 2012 [64], the concentrations of most trace elements in the Mãe-Bá Lagoon are within permissible limits, with the exception of Cr and Ni, which were above level 1 (above which the possibility of adverse effects on the biota is low) in the southern and northern regions but below the tolerable level 2 (above which the possibility of adverse effects is high). Thus, according to the legislation, there is a low probability of adverse effects, such as genotoxic damage to the biota, in the lagoon. When using the probable effect concentration (PEC) as a standard [54], the trace element concentration in the Mãe-Bá Lagoon is within acceptable standards (Table 3). However, although these results do not indicate any potential threat, understanding them is critical since they serve as a basis for future comparisons [41].

Conversely, high concentrations of some of the elements found in the Mãe-Bá and Nova Guarapari Lagoons, including Ca and Mg, can be advantageous because of their strong interactions with other elements. This verifies the findings of other researchers who have stated that ions such as Ca^{2+} and Mg^{2+} affect the absorption and toxicity of trace elements due to their competitive interactions [20]. Additionally, ions also affect the granulometry of these fractions, whereby finer ions decrease in bioavailability [39]. Furthermore, previous studies have shown that a high binder content, such as carbonates, further decreases the low bioavailability of trace elements in the Mãe-Bá Lagoon [13].

4.3. Genotoxicity

The trace element concentrations in the Mãe-Bá Lagoon, with the exception of Cr and Ni in some locations, were within acceptable ranges (Table 2). The Cr and Ni concentrations in the southern and northern regions were between level 1 and 2, showing a median probability of adverse effects on the biota, such as bioaccumulation, which can lead to increased concentrations of these elements at various trophic levels.

The MN test and comet assay results showed that fish in the Mãe-Bá Lagoon had experienced genotoxic damage. These results were similar to those of other studies that identified micronuclei in lotic environments adjacent to urban areas [22,24]. However, it was not possible to obtain a statistical relationship between the three studied species, as they have similar trophic levels, or between the three studied regions. A similar study also did not observe significant differences ($p > 0.05$) between species of the same trophic level or between the regions of the same lagoon [53].

Although the MN test and comet assay are excellent tools for analyzing genotoxicity in fish cells [22], care should be taken when presenting data, since natural environments have variables that cannot be controlled or isolated, such as trace element levels, physicochemical water parameters, and climatic factors, when compared to experimental conditions. This was observed in our study due to the statistically significant differences (Kruskal–Wallis test, $p < 0.05$) only being observed when the studied months were compared, and variations were observed in both the damage index and classes of damage, reflecting the differences in the climactic factors between months.

Climatic factors such as rainfall directly dilute pollutants, thereby decreasing the frequency of cellular changes [2]. In other cases, rainfall contributes to water dispersion and the bioavailability of contaminants [28], which alter the relationship between genetic damage and various organic matter and the microbiological parameters of urban discharge, which may also be affected by seasonal factors such as precipitation and wind [27]. Moreover, genotoxic damage in fish is also correlated with TP content [65], which was found to vary in the Mãe-Bá Lagoon along with the action of wind. Lagoons are favorable environments for cyanobacteria flowering, which is promoted by the phosphorus supply of the sediment, high temperatures, and alkaline conditions found in these environments [66].

Annual seasonal differences in the water temperature of the Mãe-Bá Lagoon may have influenced our results since variations in temperature and oxygen were previously shown to affect DNA mutations obtained from the erythrocytes of fish (*Cyprinus carpio*) and the hemolymph of mussels (*Dreissena polymoha*). The highest proportion of genetic damage to fish erythrocytes was observed in the winter, likely because cellular repair efficiency decreases as the temperature decreases [10]. Similar behavior was observed in native marine fish along the southwestern Mediterranean coast of Turkey; significant differences were observed in the frequency of large MN in the summer versus in the winter [9].

Environmental analysis results should be interpreted with caution due to the effect of variations in natural stressors, such as temperature and anthropic pollutants [8]. The comet test should also be interpreted with care, since it detects genotoxic effects in a broad sense, and a complementary test will always be necessary to determine the contamination level [67]. Nevertheless, past results indicate that DNA damage could lead to the induction of carcinogenesis [68]. Such DNA damage is associated with abnormal development, growth reductions, and influences the survival of embryos, larvae, and adult animals [30]

We should also emphasize that chemical analyses were performed to estimate the pollutant sources and the risk to the biota [28], as industrial and domestic effluents as well as natural processes cause pollution [1]. The joint action of specific and nonspecific toxic factors and their interaction can result in unpredictable toxicity in in situ situations, increasing or inhibiting their toxicity [7]. The Mãe-Bá Lagoon, which was studied in the present research, is an example of such an ecosystem under tremendous anthropic pressure due to industrial and domestic activities. Even if there is a low bioavailability of trace elements in the environment for aquatic biota, which is the case in the Mãe-Bá Lagoon [13], types of damage other than bioaccumulation can occur as well, such as genetic damage, which can harm the populations of certain species in the long term.

5. Conclusions

The results of this study indicate that the environmental quality of the Mãe-Bá Lagoon is poor and that this can be attributed to human activities as well as the natural environmental characteristics. The concentrations of the trace elements Cr and Ni in the lagoon sediment exceeded the permitted levels. The mining company was not identified as the primary source of these elements, as high concentrations were also observed in the lagoon used as a reference (Nova Guarapari). The fish from the Mãe-Bá Lagoon were found to have experienced genotoxic damage; however, it was not possible to identify the sources of this damage due to the presence of numerous possible polluting agents.

The low bioavailability of trace elements in a water resource does not indicate good environmental quality since there are other important forms of damage that should be analyzed in addition to the bioavailability of elements in the environment.

Natural environments comprise multiple factors that can cause genotoxicity in fish, even if these agents occur naturally, with or without anthropic actions. The latter, even if it occurs below the allowed limits, can cause genotoxic damage in fish.

There is a need for more studies on the environmental quality of the water in lacustrine ecosystems, as healthy and safe water resources are essential for the growth and development of aquatic life. However, a multidisciplinary approach is evidently needed in studies involving ecotoxicology in order to develop conservation strategies.

Author Contributions: Conceptualization, methodology, writing—original draft, writing—review and editing, C.B.C.F.; conceptualization and methodology, A.R.d.S.; software and validation, J.L.F.; writing—review and editing, B.d.L.P., S.H.K. and A.P.S.; Methodology, software, and validation, D.L.B.; writing—original draft, J.L.R.J. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to extend their thanks to the Society for the Support of Technological and Cultural Development (SADTEC) for financing the project, to the Federal Institute of Espírito Santo–Campus Alegre for the support and scholarship from Doctor/CAPES (Edital PRPPG 18/2014), and to the Graduate Program in Forest Sciences at the Federal University of Espírito Santo.

Institutional Review Board Statement: The study was conducted according to authorization of SISBIO no. 40278-4 and 40278-5 and according to the Ethics Committee on the Use of Animals (CEUA–UVV) No. 304/2013.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to thank the Instituto Chico Mendes de Conservação da Biodiversidade for its permission, the technicians from the chemistry laboratory, and the General Directorate of the Federal Institute of Espírito Santo–Campus Alegre.

Conflicts of Interest: The authors declare no conflict of interest. This manuscript has not been published or presented elsewhere in part or in its entirety and is not under consideration by another journal. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these.

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