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Kastoria and Mikri Prespa Lakes: The Impact of Anthropogenic Activities on the Differentiation in the Genotoxic and Toxic Profile of the Surface Water

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Abstract: Urban–industrial and agricultural waste can add significant amounts of pollutants to surface water. Therefore, the surface water from Mikri Prespa and Kastoria lakes was assessed for its toxic, genotoxic, and cytotoxic effects. Water samples were collected during the spring of two different years (S1 and S2) in order to investigate the anthropogenic effects on both lakes. Physicochemical parameters were identified, while significant elements were determined via ICP-MS/MS. The in vitro cytokinesis-block micronucleus (CBMN) assay in cultured human lymphocytes and the *Aliivibrio fischeri* bioassay were applied to evaluate the genotoxic–cytotoxic and toxic effects, respectively. In the CBMN assay, significant differences were observed in genotoxic and cytotoxic indices between the different time periods. Decreased indices in S2 could be correlated with a potential diminution in the negative human effect on the environment along the lakes. In the *Aliivibrio fischeri* bioassay, no significant difference in the toxicity of the surface water samples from Mikri Prespa was observed, whereas significant differences were identified in the samples from Lake Kastoria. The differences in toxic, genotoxic, and cytotoxic effects reveal the impact and the influence of anthropogenic activities in the areas of land around the lakes in relation to their surface water quality.

Keywords: surface water; lakes; toxicity; genotoxicity; cytotoxicity; micronuclei; anthropogenic activities; physicochemical parameters; elements

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1. Introduction

Lakes constitute significant freshwater resources and unique ecosystems. They have been utilized by humans for irrigation purposes, fishing, agricultural applications, and recreational activities, amongst others. Moreover, various lakes are considered as habitats for many species of great importance [1]. A fundamental issue concerning the contribution of urban and agriculture land to an environmentally sustainable "green" growth model is whether land use (LU) and its management practices can achieve the preservation and protection of our environment for the succeeding generations [2]. However, it is well known that human intervention in freshwater ecosystems via various activities, including agricultural practices such as irrigation and fertilization management, pesticide use, and urban–industrial and agricultural wastes, can significantly affect the quality of the habitat, the species' well-being, and the water chemistry [3,4]. Moreover, cyanobacteria, which often constitute the fundamental phototrophic component in lakes under specific conditions, can form cyanobacterial blooms which subsequently negatively affect their ecological state. Reduction of productivity at higher trophic levels, depletion of oxygen leading to the death of aquatic species, and diminished aesthetic value are among the most significant

negative effects generated by cyanobacteria [5]. In addition to this, secondary metabolites of cyanobacteria which have been found to be toxic could constitute a potential hazard to human and animal health through the use of lake water for drinking, recreation, and irrigation purposes [6]. Considering the rapid rate of global warming, leading inevitably to climate change, lake habitats are bound to be considerably affected. The combination of acute weather conditions and enhancement of nutrient run-offs is expected to magnify the problem of cyanobacterial blooms in freshwater ecosystems [7,8].

The present study focuses on two Greek lakes, i.e., Mikri Prespa and Lake Kastoria (Figure 1). Considering the fact that Mediterranean areas are categorized as climate change hot spots and that toxic cyanobacterial blooms occur frequently in most Greek lakes [9,10], it is crucial to investigate the quality of the surface water and its potential adverse effects concerning their cytotoxic and genotoxic impact.

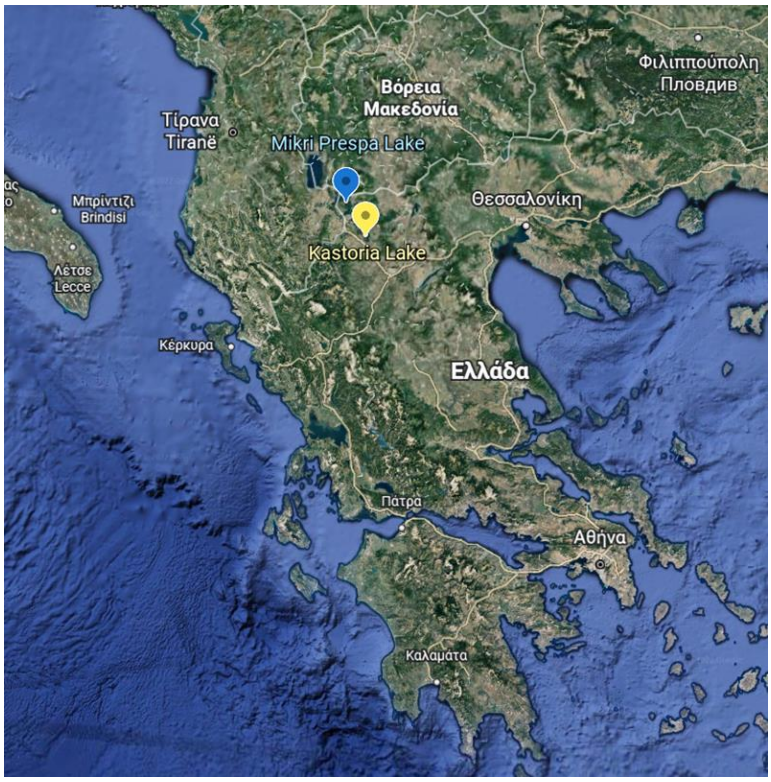

Figure 1. Geographical sites of Mikri Prespa and Kastoria lakes (maps by Google earth).

The Prespa lakes constitute two interlinked lakes: Megali Prespa (Great Prespa) which is shared by three countries (Greece, Albania, North Macedonia) and Mikri Prespa (Lesser Prespa) which is shared by Greece and Albania [11]. Mikri Prespa, which is located south of Megali Prespa at an altitude of 850 m, is considered to be one of the oldest lakes in Europe, with a surface area of 47.4 km^2, while its average and maximum depths are 4.1 and 8.4 m, respectively [12,13]. It is classified as a shallow, eutrophic lake and is a Ramsar Wetland of International Importance and a Special Protected Area (Directive 79/409/EEC) since it has a very high ecological value. In fact, it includes a large number of rare and endemic species of both flora and fauna [11,14,15]. Bean farming is the major agricultural activity within the area of Mikri Prespa [16]. Human interventions, including waste discharge and unsustainable agricultural and irrigation practices, could have a significant impact on the aquatic habitats of the lake [11,16].

Lake Kastoria or Orestiada is located in Western Macedonia, Greece, at an altitude of 630 m in the Kastoria prefecture [17]. It is considered to be a potentially ancient lake in the Balkan region [18] and is a swallow, polymictic, and eutrophic lake [10]. Its surface area is 27.9 km and it has an average depth of 4.4 and maximum depth of 9.1 m [17]. Given the

fact that the city of Kastoria is situated on the western part of the lake while agricultural activities are conducted in its catchment area, it is apparent that human interference affects the water quality and the status of the lake [19]. Lake Kastoria belongs to the Natura 2000 Special Protection Area GR1320003 and Site of Community Importance GR1320001 since it is also considered to be a hotspot for European biodiversity [20,21].

Considering the various contaminants that could possibly be present in lake water. originating from pesticides, sewage, and/or other urban sources, a holistic approach was followed for the assessment of surface water samples from lakes Kastoria and Mikri Prespa. Specifically, the physicochemical parameters of both lakes were determined as well as the elements concentrations via ICP-MS/MS. Afterwards, a cytokinesis-block micronucleus (CBMN) assay, a method known for its simplicity and reliability, was applied for the assessment of both the genotoxic and cytotoxic profile of surface water samples from the two lakes, via the evaluation of the micronuclei (MN) induction frequency and the calculation of the cytokinesis-block proliferation index (CBPI), respectively [22]. This assay has been applied extensively for the determination of the genotoxic and cytotoxic activity of a plethora of substances as well as freshwater samples [23–25]. Toxicity of the surface water was further evaluated via the Microtox test by the estimation of the percentage inhibition of the luminescence of the bacteria *Aliivibrio fischeri*.

2. Study Areas, Materials, and Methods

2.1. Study Areas

Surface water samples were collected from Lake Mikri Prespa (MP) (Figure 2), which is located south of Megali Prespa (surface area = 47.4 km^2, average depth = 4.1 m, and maximum depth = 8.4 m) [12,13], and Lake Kastoria (K) or Orestiada (Figure 3), which is in Western Macedonia, Greece (surface area = 27.9 km, average depth of 4.4 m, maximum depth = 9.1 m) [17].

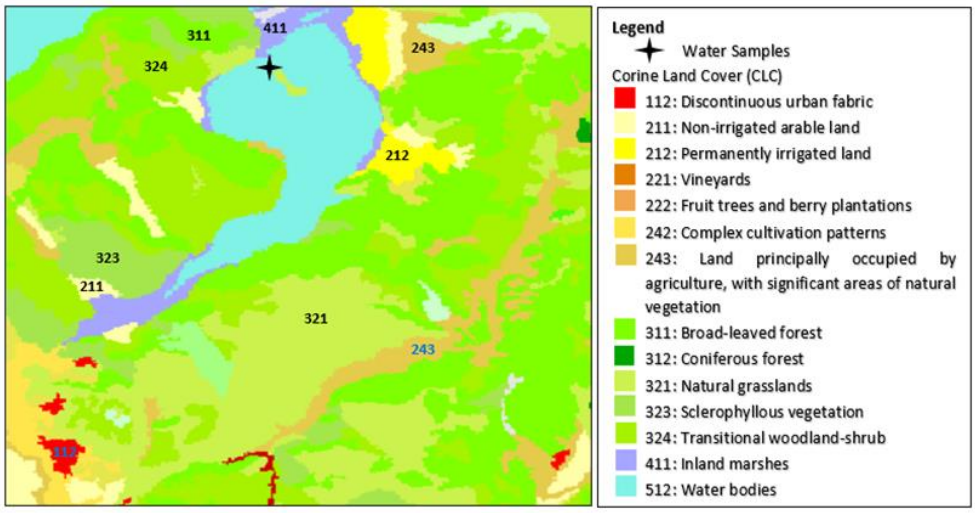

Figure 2. Map and sampling site (black mark) of Lake Mikri Prespa (**40°47′23.2″ N 21°04′35.6″ E**).

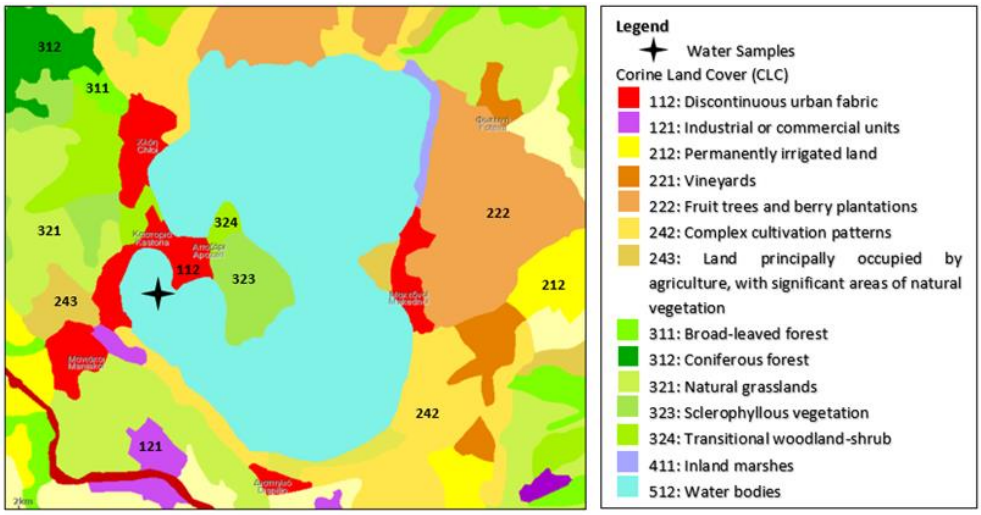

Figure 3. Map and sampling site (black mark) of Lake Kastoria (40°30′46.8″ N 21°15′49.8″ E).

2.2. Water Samples Collection

Water samples (0 to 0.5 m) were collected in spring (May) of two different years (S1 and S2). It has been shown that freshwater, i.e., rivers and lakes, have a higher pollutant load during the end of spring and beginning of summer. In fact, low concentrations of pollutants such as pesticides are usually observed during the winter months because of dilution effects due to high-rainfall events and the increased degradation of pesticides after their application [26]. Thus, the specific period was chosen in our case for the sampling of surface water. Moreover, specific sampling points (one for each lake) were carefully chosen, which were close to potential pollutant sources to identify the most pronounced effects.

The samples were collected in plastic (polyethylene) containers, at the north part near the island of Agios Achilleios from Lake Mikri Prespa (Figure 2) and near the western shore (south from Kastoria Peninsula) from the Lake Kastoria (Figure 3). The surface water samples were obtained by plunging an open 1 L polyethylene sample bottle just below the water surface. Surface water samples were transferred to the laboratory, filtered with a Whatman GD/X filter (0.25 mm and 0.2 μm pore size) for the removal of dissolved particles, and subsequently kept in sterile containers at −80 °C. For metals analysis, the samples were pre-treated with the addition of acid before storage. The analysis of the other parameters as well as the toxicity tests were immediately conducted on return to the laboratory.

2.3. Reagents

Ham's F-10 medium, foetal bovine serum (FBS), and phytohaemaglutinin (PHA) were commercially supplied from Gibco (UK), while cytochalasin-B (Cyt-B) was purchased from Sigma (St. Louis, MO, USA). All other chemicals and solvents were of the highest grade commercially available.

2.4. Physicochemical Analysis

A pH meter (Jenway 3310) and a conductivity meter (CDM 230, MeterLab, Radiometer Analytical) were used for the determination of the pH and conductivity, respectively. The StandardMethod 2540 D was used for the determination of total suspended solids (TSS) [27]. The detection and quantification of anions were performed by a Dionex ICS-1500 Ion chromatography system. For the elution, an IonPac AS9-HC column with an aqueous sodium carbonate (9 mM) solution (flow rate of 1 mL min^{-1}) was used. Ammonium ions were quantified photometrically using the method based on the indophenol blue formation [28].

2.5. ICP-MS/MS Analysis

Elements were determined in an acid matrix of 2.5% v/v HNO_3 and 0.5% v/v HCl using an Agilent 8900 Triple Quadrupole ICP-MS/MS (Agilent Technologies, Tokyo, Japan) with an Agilent SPS4 Autosampler and an Agilent Integrated Sample Introduction System (ISIS). ISO 17294-2 testing protocols [29,30] were applied for the establishment of the method used for their determination.

2.6. Genotoxic and Cytotoxic Effects on Human Lymphocyte Cultures

2.6.1. Ethic Statement

Approval by the Research Ethics Committee of the University of Patras (Ref. No: 19686/27-05-2015) was acquired for the CBMN assay and the use of human lymphocytes. Healthy males (<30 years old), who did not smoke and did not receive any radiation or drug treatment recently, were used as blood donors after written, informed consent.

2.6.2. Experimental Procedure

The standard protocol of the CBMN assay with minor modifications was applied [22]. Following the addition of the appropriate volumes of whole blood, Ham's F-10 medium, foetal bovine serum, and phytohaemagglutinin (0.5, 6.5, 1.5, and 0.3 mL, respectively) in culture flasks, 1, 2, and 5% of filtrated surface water samples were added 24 h after culture initiation. Cytochalasin-B was added at 44 h, at a final concentration of 6 μg mL^{-1}, in order to hinder dividing cells cytokinesis. Cultures were incubated for a total of 72 h at 37 °C and 5% CO_2 supply, followed by harvesting and collection of cells via centrifugation. Cells were treated with a hypotonic solution (Ham's medium and milli-Q water; ratio 3:1) and left for 3 min at room temperature. Subsequently, cells were fixated for 10 min (×3) with a methanol and acetic acid solution (ratio 5:1). Cell staining was conducted for 10 min with 7% Giemsa [31]. MN frequency was assessed via the scoring of 2000 binucleated (BN) cells with preserved cytoplasm in each concentration according to standard criteria [32,33]. Furthermore, 1000 cells with one ($M1$), two ($M2$), three, and/or four ($M3/4$) nuclei were counted for the evaluation of the CBPI for each concentration [34] according to the following equation:

$$CBPI = \frac{(M1 + 2 \times M2 + 3 \times M3/4)}{N}$$

where N is the total number of cells.

2.7. Aliivibrio Fischeri Toxicity Test (Microtox Assay)

Surface water samples' toxicity from both lakes was determined using the marine luminescence bacteria *Aliivibrio fischeri*. A Microtox Model 500 Toxicity Analyzer was utilized for the analysis and the 81.9% Basic Test of the Microtox program was selected. Recording of luminescence took place after 15 and 30 min of incubation at 15 °C. All samples were analysed in triplicate, while the results represent the % inhibition of *Aliivibrio fischeri* bioluminescence.

2.8. Statistical Analysis

CBMN results are expressed as the mean frequency ± standard error. A G-test for independence on 2 × 2 tables was used for MN data analysis, whereas the chi-square (χ^2) test was used to analyse CBPI data. The statistical decisions were based on a significance level of 0.05. The Statistical Package for Social Sciences (SPSS) for Windows, version 17.0, was used for data analysis.

3. Results

3.1. Physicochemical Analysis

The physicochemical analysis of the surface water samples from the two lakes is presented in Table 1. The mean values of three different measurements are provided. The

pH was neutral in the samples from lake MP in both time periods, while conductivity, TSS, and SO_4^{2-} were found at relatively low and/or normal levels and had similar values. PO_4^{3-}, NO_3^-, NO_2^-, F^-, Br^-, and NH_4^+ were not present in any of the samples of S1 and S2. The concentration of Cl^- was higher during S2.

Table 1. Physicochemical analysis of the collected samples during different time periods (S1 and S2) from Mikri Prespa (MP) and Kastoria (K) lakes.

Site		pH	[1] Cond	[2] TSS	[2] PO_4^{3-}	[2] SO_4^{2-}	[2] Cl^-	[2] NO_3^-	[2] NO_2^-	[2] F^-	[2] Br^-	[2] NH_4^+
MP	S1	7.14 ± 0.20	55.82 ± 3.10	1.18 ± 0.45	0.00 ± 0.00	1.12 ± 0.21	5.42 ± 0.26	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	S2	7.22 ± 0.12	47.63 ± 2.05	1.24 ± 0.12	0.00 ± 0.00	0.64 ± 0.32	10.34 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
K	S1	5.74 ± 0.12	193.27 ± 1.27	5.72± 0.18	15.34 ± 0.56	10.37 ± 0.39	31.67 ± 0.44	20.32 ± 0.37	12.67 ± 0.66	10.56 ± 0.72	0.25 ± 084	2.47 ± 0.26
	S2	7.01 ± 0.18	42.64 ± 2.01	1.05± 0.64	0.00 ± 0.00	2.24 ± 0.27	20.43 ± 0.38	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.03

[1] $\mu S\ cm^{-1}$. [2] $mg\ L^{-1}$.

As far as lake K is concerned, the samples had a slightly acidic pH value, i.e., 5.74, during S1, whereas the pH of the samples from S2 was neutral. Conductivity was significantly greater during S1 in comparison with S2 with the values being 193.27 ± 1.27 and 42.64 ± 2.01 $\mu S\ cm^{-1}$, respectively. TSS values were relatively low in both cases. In the case of PO_4^{3-}, NO_3^-, NO_2^-, NH_4^+, and F^-, high values were measured in samples during S1, while none were present during S2. SO_4^{2-} and Cl^- were found in samples from both time periods, but they were also higher during S1. Br^- ions were found in samples of S1 at a concentration of 0.25 ± 0.84 $mg\ L^{-1}$ but were not present in S2.

Comparing the parameters from both lakes, it is clear that values of the different physicochemical parameters are higher in the surface water samples from K lake.

3.2. Element Concentration via ICP-MS/MS

The elements identified in surface water samples from the two lakes are presented in Table 2. According to the results, magnesium and calcium were found at the highest concentrations in both MP and K lakes, with the values being higher during S1. A similar pattern was observed for silica and potassium, while phosphorus had similar concentrations for MP surface water in both sampling periods and slightly lower values were recorded for K samples in S2. Copper was not present in either lake in S1 sampling, while it was found at low concentrations during S2. Finally, zinc had small values in MP in S1 and S2, whereas it was only found in K lake during S1.

Table 2. Concentration of elements measured in samples collected during different time periods (S1 and S2) from Mikri Prespa (MP) and Kastoria (K) lakes.

	MP S1	MP S2	K S1	K S2
Mg [1]	14,981.69	9749.36	15,903.18	9922.45
Al [1]	-	-	-	-
Si [1]	4187.58	2380.99	4835.98	4199.23
K [1]	1598.09	1254.79	3495.23	1961.03
Ca [1]	10,696.50	5518.65	6405.31	6003.50
P [2]	6.47	6.47	7.11	6.35
Cr [1]	-	-	-	-
Mn [1]	-	-	-	-
Fe [1]	-	-	-	-
Co [1]	-	-	-	-
Ni [1]	-	-	-	-

Table 2. *Cont.*

	MP S1	MP S2	K S1	K S2
Cu [1]	-	3.51	-	5.44
Zn [1]	3.62	8.90	-	7.58
As [1]	-	-	-	-
Se [1]	-	-	-	-
Mo [1]	-	-	-	-
Ag [1]	-	-	-	-
Cd [1]	-	-	-	-
Sn [1]	-	-	-	-
Hg [1]	-	-	-	-
Tl [1]	-	-	-	-
Pb [1]	-	-	-	-

[1] μg L^{-1}. [2] mg L^{-1}.

3.3. Genotoxic and Cytotoxic Effects Assessment

According to the results (Figures 4 and 5), the samples from the surface water of MP lake increased the induction of MN, compared with the control cultures, which was not, however, statistically significant. On the other hand, all samples collected from K lake during S1 demonstrated a significant increase in MN frequencies, while those collected in the S2 did not exert genotoxic effects. Regarding the cytotoxic potential of the samples, statistically significant diminution of the CBPI was detected for the two highest doses of surface water from MP lake, i.e., 2 and 5% (v/v), and the highest dose of surface water from K lake (5% v/v) collected in S1, in relation to the negative control. However, none of the samples of either lake collected in the S2 sampling were cytotoxic.

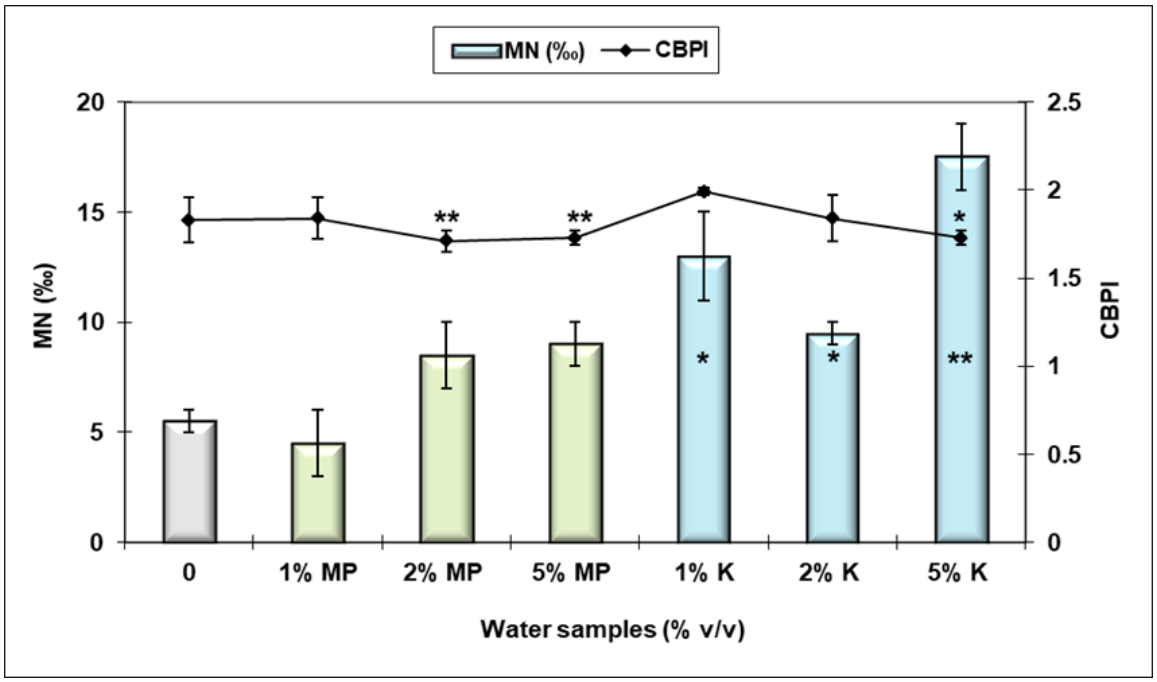

Figure 4. Frequencies of MN (histograms) and CBPI values (solid black line) in cultured human lymphocytes treated with different concentrations of surface water (1, 2, and 5%, v/v) from Mikri Prespa (MP) and Kastoria (K) lakes (S1). MN, micronuclei; CBPI, cytokinesis-block proliferation index; mean MN frequencies, expressed as number of MN (‰) ± standard error per 2.000 binucleated cells per experimental point; mean CBPI values, expressed as number of CBPI ± standard error per 1.000 binucleated cells per experimental point [G-test for MN; χ^2 for CBPI]. The significant difference from control is indicated by the asterisks in each case (* $p < 0.01$; ** $p < 0.001$).

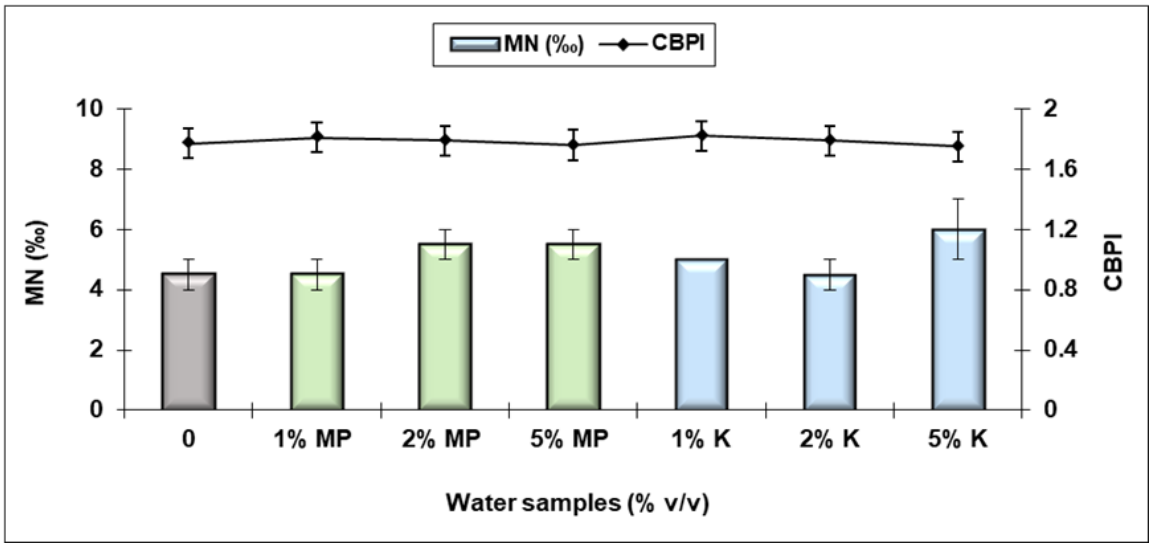

Figure 5. Frequencies of MN (histograms) and CBPI values (solid black line) in cultured human lymphocytes treated with different concentrations of surface water (1, 2, and 5%, *v*/*v*) from Mikri Prespa (MP) and Kastoria (K) lakes (S2). MN, micronuclei; CBPI, cytokinesis-block proliferation index; mean MN frequencies, expressed as number of MN (‰) ± standard error per 2.000 binucleated cells per experimental point; mean CBPI values, expressed as number of CBPI ± standard error per 1.000 binucleated cells per experimental point [G-test for MN; χ^2 for CBPI].

3.4. Ecological Assessment

Based on the results of the Microtox test, the percentage inhibitory effect of water samples from MP lake was relatively low for both time periods, i.e., 11% for S1 and 14% for S2 (Figure 6). On the other hand, the inhibition percentages exhibited by the samples from K lake for both S1 and S2 sampling periods were significantly higher. Most notably, 78% inhibition of the bacteria luminescence was recorded for S1 water samples, whereas the percentage for S2 was 23%.

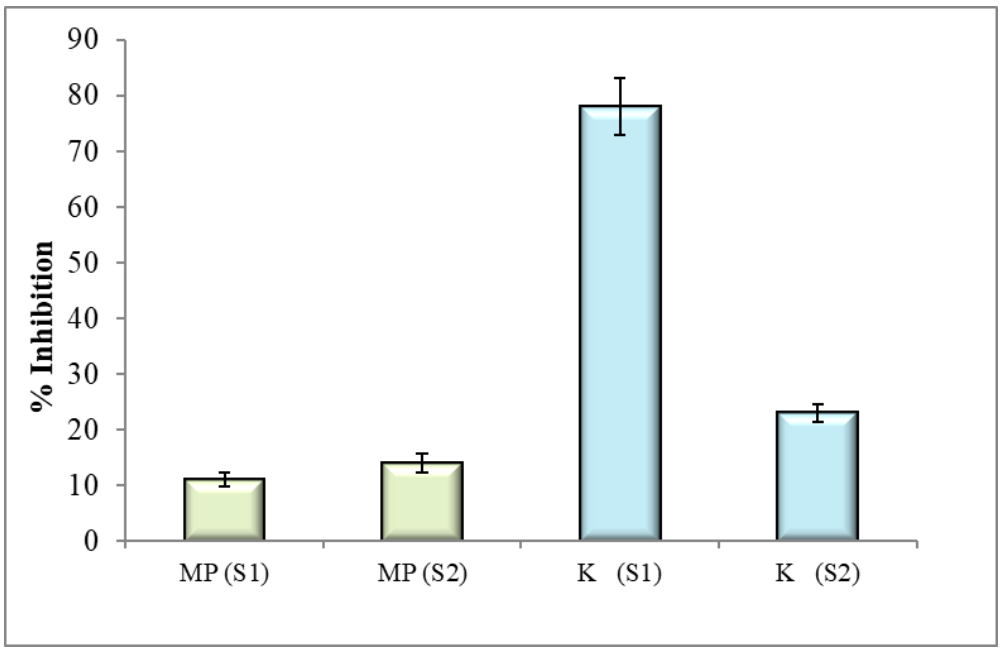

Figure 6. Percentage inhibition of *Aliivibrio fischeri* after exposure in water samples collected during different time periods (S1 and S2) from Mikri Prespa (MP) and Kastoria (K) lakes.

4. Discussion

In the present study, a concise analysis was performed in surface water samples from two Greek lakes, i.e., Mikri Prespa and Kastoria, both known for their high biodiversity and ecological value. Specifically, a physicochemical analysis was conducted entailing essential physicochemical parameters as well as a determination of elements via ICP-MS/MS, followed by the assessment of their potential genotoxic, cytotoxic, and toxic potential via a CBMN assay and Microtox test, respectively.

According to the results from the physicochemical analysis, the samples from Lake Mikri Prespa showed normal or low, even negligible, levels of the tested parameters in both time periods (S1 and S2). A similar pattern was noticed for the samples of Lake Kastoria collected in S2. On the other hand, samples from S1 had conductivity values > 100 μS cm^{-1}, which suggest impairment of the aquatic environment. Moreover, PO_4^{3-}, NO_3^-, NO_2^-, NH_4^+, SO_4^{2-}, Cl^-, and F^- demonstrated high values which could be attributed to domestic, industrial, and agricultural waste, including organic and inorganic pesticides and fertilizers [26]. It could be classified as a point source of pollution due to the mixed land use (industrial/urban/agricultural) in the study area. As shown in Figure 2, industrial or commercial units are located near the sampling point as well as discontinuous urban fabric and agricultural land use. The above observation is reinforced by the fact that in a short sampling period with a one-year difference, the values of the analysed physicochemical characteristics were different between the first and second samples.

Regarding the structure of the agricultural sector of the study area, cereals, beans, apples, and maize occupy approximately 50%, 6%, 5%, and 3%, respectively, of the total cultivated area in the prefecture of Kastoria. Usually, sowing and the subsequent application of agrochemicals to spring crops such as beans, maize, etc., take place in the period from 15 April to 30 May depending on the weather conditions. In addition, during the same period, agrochemicals are applied to tree crops such as apple trees, peach trees, etc., while winter cereals are subjected to basic fertilization in autumn and the remaining nitrogen is added in spring. According to the nutrient requirements in kilograms per hectare for cereals, roughly 120–160 Kg ha^{-1} of nitrogen (N), 30–50 Kg ha^{-1} of phosphorus (P_2O_5), and 30–50 Kg ha^{-1} of potassium (K_2O) are added. However, smaller amounts (Kg ha^{-1}) of nitrogen fertilizers are applied to bean cultivation (20–50 N, 40–70 P_2O_5, 50–60 K_2O) and greater in maize (200–300 N Kg ha^{-1}, 60–120 P_2O_5 Kg ha^{-1}, 40–80 K_2O Kg ha^{-1}) compared with cereals. Furthermore, in apple trees, approximately 80–150 Kg ha^{-1} of nitrogen (N), 50–100 Kg ha^{-1} of phosphorus (P_2O_5), and 150–250 Kg ha^{-1} of potassium (K_2O) are added. All the above cropping practices under conditions of heavy rainfall seem to have affected the variation, mainly of nitrate concentrations, in spring between the sampling years and, in general, the pollutant load of Lake Kastoria. Usually, in the agricultural land, the arbitrary application of agrochemicals such as N-P-K fertilizers and pesticides, when followed by heavy rainfall, usually leads to a run-off event, especially on sloping land such as the study area [35]. Lassaletta et al. reported that although the use of synthetic N fertilizers has increased enormously, only 47% of the reactive nitrogen applied to crops worldwide is converted into harvested products, compared with 68% in the early 1960s [36]. In addition, there is often a lack of drainage infrastructure and connection to biological purification units in a discontinuous urban fabric. As a result, house sewage ends up directly in water receivers [37]. Finally, industries without or with poorly maintained biological wastewater treatment are the most common point sources of pollution in water receivers such as lakes.

Regarding the genotoxicity and cytotoxicity of the samples from S1, Mikri Prespa surface water samples induced cytotoxic activity at the two highest concentrations, while lacking any genotoxic potential. All concentrations from Lake Kastoria were genotoxic and the highest dose, i.e., 5% v/v, was cytotoxic. The aforementioned negative effects may not be solely due to the agricultural and urban discharges but could also be related to other environmental stresses including increased temperature due to global warming, which is predicted to increase proliferation of cyanobacterial blooms and eutrophication [7]. In fact, it has been previously mentioned that the Mediterranean climate favours the spreading

of cyanobacterial blooms, especially in eutrophic freshwaters, during extended periods of time. Thus, toxic cyanobacterial blooms have been found in most of the lakes in Greece. Microcystins, which are produced from cyanobacteria, could pose a significant health hazard to wildlife, livestock, and humans [38]. It is noteworthy to mention that cyanobacteria including *Microcystis aeruginosa* and *Microcystis wesenbergii* have been previously found in Lake Mikri Prespa, especially during the warmer periods of the year [39]. Bean farming constitutes the main agricultural activity in the proximity of Mikri Prespa lake, which, coupled with the frequent use of fertilizers, caused its progressive eutrophication [16]. In addition, populations of waterbirds have been found to enhance algal production through their excrements, which are rich in N and P [39]. Furthermore, samples from Lake Kastoria possessed the highest variety of cyanotoxins, according to Christophoridis et al. [40], among the Greek lakes tested, including saxitoxins, which are known as the cyanotoxins with the most acute toxicity [41]. Human intoxications have been previously recorded due to saxitoxins, while the exposure can take place via inhalation, contaminated food, and contaminated drinking water [42,43]. Agriculture, livestock farming, and human activities increase the annual quantities of fertilizers and pesticides to the soil, which finally end up in the lake as well [44]. Similarly, a significantly high level of % inhibition in the bioluminescence of *Aliivibrio fischeri* was noted for the samples from Lake Kastoria, especially during S1.

The differences observed between the samples collected during S1 and S2 time periods could be due to different weather conditions either enhancing or hindering the proliferation of cyanobacteria blooms, respectively. In addition to that, more intensive agricultural and urban activities during the first time period might have taken place leading to the observed results. As far as the variations between the two lakes are concerned, considering the fact that Lake Kastoria is an urban lake, having approximately 17,000 inhabitants on its shoreline and 35,000 in the whole catchment, it is apparent that the specific lake is significantly affected by anthropogenic activities [20]. Furthermore, before the establishment of a sewage treatment plant in 1990, sewage was disposed of in the lake without proper handling. Even though the water quality has since improved, pesticides are used, presently, on a large scale affecting the soil and enhancing the eutrophication and the degradation of the lake [44].

5. Conclusions

Freshwater pollution constitutes a serious issue for public health as well as various aquatic organisms, affecting the general status of the aquatic ecosystem. In the present study, two Greek lakes of high ecological importance were investigated, i.e., lakes Mikri Prespa and Kastoria. After physicochemical analysis and determination of elements of surface water samples from both lakes during S1 and S2 sampling periods, their genotoxic, cytotoxic, and toxic potential were assessed in vitro, showing the differences between the two time periods as well as the lakes. The land use and the intensity of the anthropogenic impact, depending on the population density and various agricultural and urban activities, could lead to the observed differentiation. Furthermore, the increased MN frequencies recorded in this research represent the first evidence of the presence of genotoxic compounds in the studied lakes.

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