Article

The Snowball Effect of Anthropogenic Alterations in a Karst Tropical Lake in Yucatán Peninsula, Environmental Variability and Risk Assessment

Sergio Cohuo[1]*, Antonia Moreno[1], Naydelin Escamilla[1], Alondra M. Pérez[1], Iván Santos-Itzá[1], Laura Macario-González[2]*, Carmen A. Villegas-Sánchez[1], Alejandro Medina-Quej[1]

[1] Tecnológico Nacional de México – I. T. Chetumal., Av. Insurgentes 330, Chetumal, 77013 Quintana Roo, Mexico
[2] Tecnológico Nacional de México – I. T. de la Zona Maya, Carretera Chetumal-Escárcega Km 21.5, Ejido Juan Sarabia, 77965 Quintana Roo, Mexico
* Correspondence: sergio.cd@chetumal.tecnm.mx (S.C.); laura.mg@zonamaya.tecnm.mx (L.M.)

Abstract: In the Yucatán Peninsula, anthropogenic activities such as urbanization and final disposal of solid and wastewaters critically impact aquatic systems. Here, we evaluated the anthropogenic-related environmental alteration of Lake La Sabana, located in the northern limit of one of the main cities of the Mexican Caribbean. We evaluated lake water quality using physical, chemical, and microbiological indicators, and heavy metals in surficial sediments and fish tissue to evaluate the potential environmental risk. Multivariate analyses revealed that environmental conditions in La Sabana are spatial and temporal heterogeneous. Medium to bad water quality was determined within different basins by the National Sanitation Foundation water quality index, related with the degree of anthropogenic influence at each zone. The center-south zones displayed critical microbiological values largely exceeding national standards. Heavy metals in sediments and fish tissue such as Zn and Hg were relatively low, but Hg concentration threatens the ecological environment. Incipient wastewater treatment and final disposal in La Sabana is the main responsible of changes in the trophic status and nutrients availability, which in turn may have promoted changes in the biological structure and aquatic plant invasions. Lake La Sabana can be considered a model of the potential sequential effects of the anthropogenic alterations in oligotrophic karst tropical aquatic systems in Yucatn Peninsula.

Keywords: heavy metals; Lake La Sabana; Chetumal; bathymetry; water quality index

1. Introduction

The Yucatán Peninsula in southern Mexico is a continental karstic platform composed by highly permeable limestone deposits [1–3]. Rainfall in this region infiltrates rapidly down to the water table, forming large reserves of underground water [3–5]. The Yucatan Peninsula aquifer is one of the most prominent of the world, as it extends more than 165,000 km^2 and displays a complex interaction with surrounding marine environments such as Gulf of Mexico and Caribbean Sea [1,6]. The aquifer is mostly unconfined, forming networks of karst conduits driving subterranean water discharge mainly northward and eastward of the Peninsula toward the Gulf of Mexico and Caribbean Sea [7–9]. Groundwater flow in the Yucatán Peninsula (horizontal and vertical) is responsible for a highly diverse aquatic systems development, such as cenotes (sinkholes), lakes, wetlands, and "aguadas" (permanent and ephemeral freshwater ponds) [1,10,11]. In the southeastern of the Yucatán Peninsula, groundwater flow rate is significant, forming the Bacalar hydrological system [3,12]. Bacalar system is composed of eight surficial or subterranean interconnected lakes (Bacalar, Mariscal, Chile Verde, Agua Salada, Guerrero, Xul-ha, Milagros and La Sabana), at least ten cenotes, wetlands and a small and permanent surface water outlet (Estero de Chac) that communicates the systems and flows about 20 km from

Lake Bacalar to the Hondo River and then to Chetumal Bay watershed (Caribbean Sea) [13,14]. Bacalar system is mostly oligotrophic, with well oxygenated and alkaline waters with high concentrations of calcium (Ca^{2+}), bicarbonates (HCO_3^-), magnesium (Mg^{2+}), sulfates (SO_4^{2-}), and chloride [12,15,16]. Waters are saturated with calcite and dolomite as consequence of limestone dissolution [3,17].

Bacalar hydrological system due to its landscape nature and water transparency has become an important touristic site in southern Mexico. During the last ten years, pristine conditions of the hydrological system has progressively changed, presumably as the consequence of increasing tourism activities, population growth, urbanization, land-use change, and cities with poorly regulated solid and wastewater management [18,19].

Lake La Sabana is located southern of the Bacalar hydrological system, parallel to the Chetumal bay (Caribbean coast) and currently along the northwest limits of the Chetumal city. Chetumal, is one of the main cities in the Mexican Caribbean with a population growth rate of about 2.9% in 2010 [20], which was higher than Mexican national average of 1.4%. In 2020, Chetumal city had a population of about 169, 000 inhabitants [21]. With the progressive urbanization, Lake La Sabana has been severely impacted by anthropogenic activity including the modification of littorals, alterations of natural surficial flows, the remotion of littoral native vegetation and changes in the trophic status [22]. Since 1999, "El Centenario" wastewater treatment plant was established next to La Sabana, discharging about 120 lt s^{-1} of treated waters to the lake [23]. Additionally, the open-air landfill of the city is located less than 5 km away from the lake. During 2017, the no-native and invasive aquatic plant *Pistia stratoites* established in the lake and had grown rapidly covering extensive areas of the lake surface, a novel condition in the region. Currently, the magnitude of environmental alteration and the relative environmental risk for biota is undetermined in La Sabana, thus precluding institutional and governmental actions towards restauration and conservation.

In this study, we comprehensively evaluated the anthropogenic-induced environmental alteration in Lake La Sabana by spatially analyzing 1) water quality using physical, chemical, and microbiological indicators; 2) heavy metals in sediments as evidence of leeching, and 3) presence of heavy metals in fish tissue as consequence of contaminant transfer to food webs. We estimate the current environmental risk of heavy metals in sediment and fish tissue and document how the increase of nutrient availability promotes sequential alterations in lake ecosystem structure and functionality. A bathymetric map of the Lake La Sabana and direction for management are provided.

2. Materials and Methods

2.1. Study site

Lake La Sabana is a freshwater body located along a geological fault zone, in southern Quintana Roo, Mexican federal state [24]. The lake has an elongated form with approximately 6,5 km length (considering the associated wetland) and less than 600 m width (Figure 1). La Sabana is currently, divided into four sub-basins, given the construction of three dams used to access from one side of the lake to the other, each basin with variable conditions in regard of color, transparency, and biota [25,26]. La Sabana belongs to the Bacalar hydrological system, and it is assumed a subterranean connection with it, as a surficial connection lack. The main basin (northern basin) is about 3 km long and < 600 m wide; southern basins correspond to wetlands and are more sensitive to dry seasons and can dry out sporadically. The climate in the region is tropical (A-climate "tropical/megathermal climate") and warm subhumid (Aw) according to the Köppen classification [27]. Mean annual temperature is 26°C and is related with the descending limb of a Hadley cell, centered at 20 °N [28]. Precipitation is the most fluctuating variable in the region, and it depends on the seasonal migration of the Intertropical Convergence Zone (ITCZ). The rainy season occurs in summer, associated with the northern position of the ITCZ in the American continent, whereas the dry season occurs during winter-spring. Mean annual precipitation in Yucatán Peninsula is 1600 mm but ranging monthly from 300-1800 mm [29,30].

Figure 1. Satellite map showing the geographical location of Lake La Sabana. Yellow dots represent sampling sites in the north (N), center (C), south (S) and wetland (W).

2.2. Water physical, chemical and microbiological parameters, and quality assessment

Lake La Sabana was divided into four zones: north, center, south, and wetland (Figure 1). At each zone three sampling sites were established, except for wetland in which six sampling sites were considered because of its extension. Seven environmental parameters were quantified at each site: temperature, conductivity, pH, and dissolved oxygen were measured *in situ* with a Hach HQ40D multiparametric probe; transparency was measured with a turbidity tube; 5-day Biochemical Oxygen Demand (BOD_5)) was measured with a portable dissolved oxygen and BOD meter HI98193 of Hana instruments. Total Suspended Solids (TSS) were quantified gravimetrically using a glass fiber filter of 2 μm. For nutrient content determination, 1.5 L of water was collected in plastic bottles, previously washed with distillated water. Collected samples were preserved in ice *in situ* and then transported to the Laboratorio de Química analítica from El Colegio de la Frontera Sur, Chetumal unit. Nitrites, nitrates, orthophosphates, and sulfates were measured with a Shimadzu UV-1700 spectrophotometer. Samples were measured in triplicate and for all runs two blank samples were used. Calibration curves reached an r^2=0.990-0.995. All variables were measured, near surface at 0.5 m depth, during February and August 2021, coinciding with the dry and rainy season, respectively.

For microbiological analysis three surficial water samples (at 0.5 m depth) were collected at each lake zone during May (coinciding with the dry season), and July 2021 (coinciding with the rainy season). We applied the Most Probable Number (MPN) technique to estimate total and fecal coliforms in La Sabana, as this is the technique suggested by Mexican national regulations. This technique is used to estimate microbial populations in waters and to determine the probability to contain disease-producing organisms making the water unsafe for consumption or recreational activities [31,32]. We used sterilized Whirlpack bags® and collected about 100-150 ml of sub-surficial waters (0.5 m depth). Samples were labeled and stored in ice and then transported to the Laboratory of microbiology of food and water of the Laboratorio Estatal de Salud Pública del Estado de Quintana Roo for analysis. Presumptive test for both total and fecal coliforms were first conducted to detect coliform presence in samples. We used the method proposed by Peeler et al. [33], which is based on lake water inoculation in a series of lactose rich medium at different concentrations. Given that all presumptive test for both total and fecal coliforms were positive, we conducted confirmatory tests. For total coliforms, from each tube with presence of gas, a loopful of the sample was streaked onto the selective medium Brilliant Green Bile Lactose Broth and incubated at 35 +/- 0.5°C for 24-48 hrs. Confirmatory tests for fecal coliforms were conducted inoculating subsamples from positive tubes in the selective medium EC Broth (*Escherichia coli* Broth). Determination of viable number of bacteria in the samples was based on the Bacteriological analytical manual (BAM) Appendix 2: Most Probable Number from Serial Dilutions of the U.S. Food and drug administration (FDA) [34]. For comparison purposes, we used the threshold values of the Mexican Standard NOM-210-SSA1-2014 for total coliforms, which established guidelines for water consumption and recreational activities. Fecal coliforms reference values were obtained from the Laboratorio Estatal de Salud Pública, Laboratorio de Patógenos. Reference values for both total and fecal coliforms are expressed in MPN 100 ml^{-1}.

The National Sanitation Foundation water quality index (NSF-WQI) was applied to obtain relative values of quality in La Sabana. Nine environmental parameters were used pH, dissolved oxygen, turbidity (derived from transparency tube values, expressed in nephelometric units), fecal coliform, BOD_5, orthophosphates, nitrates, temperature, and TSS. The NSF-WQI weighted average each parameter and its relative importance is summed to obtain values between 0 (zero) representing very bad quality and 100 representing excellent quality [35].

2.3. Heavy metals in sediments, fish tissue and ecological risk assessment

A total of eight sampling sites were selected for heavy metal determination in sediments. Two sampling sites were taken in the north (N1, N3), center (C1, C3), south (S1, S3), and wetland (W1, W2). Samples were collected during August-September 2020 with the aid of an Ekman dredge. Only the uppermost 3 cm of each grab were used for analysis. For heavy metal determination in fish tissue, a total of 15 individuals of the non-native fish *Oreochromis niloticus* were collected in La Sabana during November 2020 and January 2021 using an individual cast net. After the capture, individuals were kept in ice and immediately transported to the Laboratorio de Zoología of the Instituto Tecnológico de Chetumal and stored at −5°C. Total length and weight of the fishes were recorded. For heavy metal determination, fish liver was used because it effectively accumulates metals that cannot be metabolized or assimilated by the individuals [36]. Heavy metal concentration in liver can be comparable or even higher than other tissue such as muscle or gills [37]. For fishes of La Sabana, the whole liver was removed and stored in Eppendorf tubes with 70% ethanol until analysis.

For both, sediment, and fish liver, Pb, Cd, Zn, and Hg were measured, as they are the most frequent contaminants in sediments [38,39] and biological groups [40,41] in the region. The atomic absorption spectrophotometry technique was used. For sediment digestion, standard methods 3051A [42] and 3015A [43] were used. Fish liver acid digestion was performed as described in Buenfil-Rojas et al. [40]. Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) (Avanta PM– 105 GF3000, GBC) was used for Pb, Cd and Zn and Hydride Generation Atomic Absorption Spectrophotometry (HGAAS) (Avanta PM–HG 106 3000, GBC) for Hg. Quality control for heavy metals in sediments was performed as in Tun-Canto et al. [39]. Recuperation percentage was between 90-110% for all elements. Accuracy of the analysis of heavy metals in fish tissue was determined using certified reference material TORT-2 (lobster 72 107 hepatopancreas) from National Research Council of Canada (NRC-CNRC) and sample blanks. For reference material, Hg concentration was 0.27 ± 0.04 μg g^{-1} dry weight and our results were on average 0.25 ± 0.009 μg g^{-1} dry weight, with a recuperation percentage of 95 ± 3%.

Two approaches were used to evaluate the relative ecological risk of heavy metal in sediments. The total metal content, based on the Canadian Sediment Quality guidelines

(CSQG) for the protection of aquatic life developed by the Canadian Council of Ministers of the Environment, which establishes threshold values for ecotoxicological effects on aquatic biota [44]; and total content index, using the ecological risk factor (E*r*). This index is calculated using the following equations:

$$Er = T_r \text{ x } C_f \quad (1)$$

$$Cf = C_i/C_n^i \quad (2)$$

Where T_r is the toxic response factor values for each different metal, and C_f is the contamination factor, determined by the content of target metal in sediments (C_i), divided between the background value of metal in the study area or consensus reference values for a given substance (C_n^i) [45]. For our samplings, given the lack of heavy metals reference values in La Sabana and surrounding environments, consensus preindustrial reference values were taken: Pb=70, Cd=1, Zn=175, and Hg=0.25 [45]. Toxic response factors are as follows: Pb=5, Cd=30, Zn=1, and Hg=40 [45,46]. RI consists of five levels of environmental risk: RI < 40 suggest low risk; 40 ≥ RI < 80, moderate risk; 80 ≥ RI < 160 considerable risk; 160 ≥ RI < 320 high ecological risk; and RI > 320, very high ecological risk [43,47].

The relative contamination of heavy metal in fish liver of *O. niloticus* from La Sabana was assessed by comparison with national standards and with the metal pollution index (Pi), which is a mathematical method to assess and monitor contamination of individual or a set of heavy metals in aquatic ecosystems [48] . The Mexican Standard NOM-242-SSA1-2009, which determines sanitary specifications for fishing fresh products for human consumption was used for comparison. The Pi was calculated using the following equation [49]

$$Pi = Cn/GB \quad (3)$$

Where C*n* is the content of metal in sediment and GB is the geochemical background value. For this study, the geochemical background value of Hg was taken from the NOM-242-SSA1-2009, in fish samples 0.5 mg kg^{-1} wet weight. Pi values classify four pollution levels, Pi < 0.2 no significant pollution; 0.2 ≥ Pi < 0.6 minor pollution; 0.6 ≥ Pi < 1 moderate pollution; and Pi > 1, severe pollution. The Pearson correlation test was used to identify relation of fish length and weight with heavy metal content in liver.

2.4. Spatial and temporal environmental variability between lake zones

For statistical analysis, physical and microbiological data with different measured units were normalized. In order to determine if the samples can be assigned to statistically significant groups regardless of the known factor levels, a Hierarchical Cluster Analysis (CLUSTER) coupled with a Similarity Profile Test (SIMPROF) with 999 permutations and a significance level of 5% was performed based on Euclidean distances. To explore the multivariate composition of the samples, a non-metric Multidimensional Scaling (nMDS) was used. Significant groups identified by cluster and SIMPROF were overlaid as ellipses in nMDS plot.

A Test of Homogeneity of Dispersions (PERMDISP) and a two-way Permutational Multivariate Analysis of Variance (PERMANOVA) were used to detect significant differences in dispersion and location, respectively, among the four levels of the Lake zones factor (north, center, south, wetland) and the two levels of the Season factor (dry, rainy). These analyses were performed with 999 permutations based on the Euclidean distances.

The relation between the studied samples and environmental variables was evaluated by means of the ordination obtained in a Principal Components Analysis (PCA). All calculations were performed in the statistical software PRIMER v7.0.13 [50].

2.4. Bathymetry of Lake La Sabana

A rigid bottom inflatable boat of about 2.8 m long and 1.2 m beam, attached with a 6.5 hp outboard motor, was used as platform to determine the bathymetry of La Sabana. Given the elongated morphology of the lake, we established seven main longitudinal

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transects and three transversal transects in each of the basins of the lake. Additional transects were performed in the lake margins. All transects were equidistant, with about 60 m of distance between each other and a total of 726 depth measurements were taken from La Sabana. Resulting mesh had irregularly distributed sampling points. Latitude, longitude, and depth of sampling points were recorded with a Garmin (Lenexa, Kansas) ecosounder model Echomap Chirp 42CV. GPS had an accuracy of 1-3 m, and the transducer has a precision of 95%. A polygon of the coastline was delineated with satellite imagery, using the google satellite base map and then converted to a vectorial file in the open source QGIS software 3.22.1. The XYZ data (longitude, latitude, and depth) was visually inspected in an excel work sheet. Then, using the Surfer software (Golden Software Inc., Golden, CO, USA), we applied the kriging interpolation algorithm to obtain the bathymetric map and the isobaths to represent water depth.

3. Results

3.1. Coliform bacteria and NSF water quality index in La Sabana

Spatial and temporal patterns of distribution and concentration of coliform bacteria was variable in La Sabana. During the dry season, coliforms are almost homogenously distributed in the center-south of the lake, where they reach concentrations >16,000 MPN 100 ml^{-1} and > 1000 MPN 100ml^{-1} for total and fecal coliforms (Figure 2a, b), respectively. North was characterized by drastic reductions in microbial concentrations with an average of ~1000 and ~100 MPN 100 ml^{-1} for total and fecal coliforms (Figure 2a, b), respectively, whereas in wetlands values were variable with generally low values of ~1100 and ~120 MPN 100 ml^{-1}. During the rainy season, interpolated maps show that coliforms are almost restricted to the center of the lake (Figure 2c, d). Total coliform concentration significantly increased to > 50,000 MPN 100 ml^{-1} and fecal coliform concentration to >18,000 MPN 100 ml^{-1} compared with the dry season. Bacterial lower values were observed in the wetlands where values remain < 2500 and <260 MPN 100 ml^{-1} for total and fecal coliforms, respectively (Figure 2c, d). Compared with reference values of the Mexican Standard NOM-210-SSA1-2014, which indicates a threshold of <16,000 MPN 100 ml^{-1} for total and <450 MPN 100 ml^{-1} for fecal coliforms, for recreational use of waters, La Sabana largely exceeded the threshold in almost all sections, during the two climatic seasons. Wetlands are the exception as coliform values remained below the threshold, during both, dry and rainy seasons (Figure 2a-d).

The NSF WQI was relatively homogeneous spatial and temporal, with values fluctuating from 41 to 61 (Figure 2e, f and Table S1). The north showed values between 48 to 56, suggesting that water quality fluctuates between bad to medium conditions throughout the year. The center displayed mostly medium water quality conditions (NSF WQI= 53 average) during the dry season but dropped to bad conditions in the rainy season (NSF WQI= 48). The south displayed mostly bad condition throughout the climatic seasons with NSF WQI values ranging from 46-48. Wetlands demonstrated the higher values of the index ranging from 57 to 61, and thus suggesting medium water quality conditions through the year.

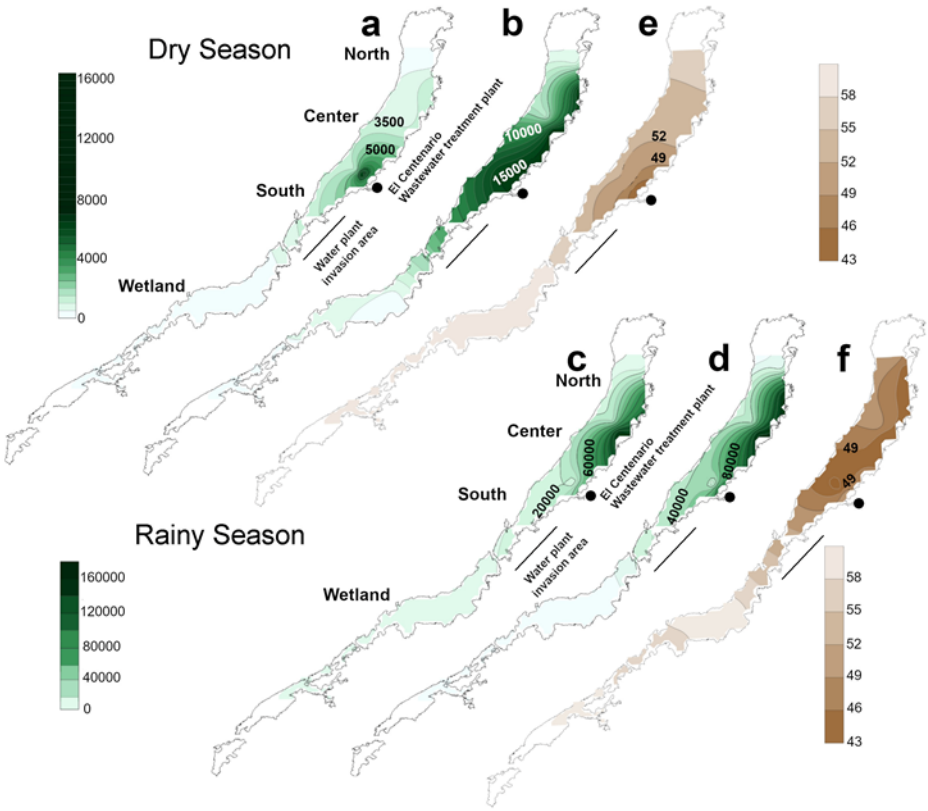

Figure 2. Interpolated map of fecal and total coliform content and water quality of Lake La Sabana during two climatic periods. Dry season: (a) fecal coliform content in surficial waters; (b) total coliform content in surficial waters; (e) National Sanitation foundation (NSF) water quality index. Rainy season: (c) fecal coliform content of surficial waters; (d) total coliform content of surficial waters; (f) NSF water quality index.

3.2. Heavy metals in surficial sediments and ecological risk index

The heavy metal analysis showed two available elements in surficial sediments of Lake La Sabana, Hg and Zn (Table 1), both detected in 100% of the samples. The Cd and Pb were below of the limit of detection (LOD= 0.125 mg g^{-1} and 0.12 mg g^{-1}, respectively) in all samples (Table 1). Therefore, Cd and Pb were excluded for further analyses. Concentration of Zn was relatively higher than Hg and displays higher variability among sites. Table 1 shows Hg and Zn concentrations (in mg kg^{-1} wet weight) per site, and basic statistics of total content in La Sabana. The CSQG revealed that Zn in most sites were below the threshold effect levels (TELs, Zn= 124 mg kg^{-1}), except for the sites C1 and S3, for which concentrations were above the probable effect level (PEL = 271 mg kg^{-1}), suggesting that adverse effects for aquatic biota may occur in those sites. For Hg, concentrations in all sites were between TELs (0.13 mg kg^{-1}) and PEL (0.7 mg kg^{-1}), and therefore falling within the range where adverse effects may occasionally occur [51]. The Er which are based in a score index, highlight that Zn concentrations were in low environmental risk, whereas for Hg, moderate environmental risk was detected.

Table 1. Heavy metals concentration and basic statistical metrics in surficial sediments in La Sabana and associated ecological risk factor.

Site	Heavy metals in surficial sediments (in mg kg^{-1})				Ecological risk factor (E*r*)	
	Pb	Cd	Zn	Hg	Zn	Hg
N1	<LOD	<LOD	73.2	0.25	0.41	41.29
N3	<LOD	<LOD	52.1	0.27	0.29	43.88
C1	<LOD	<LOD	590.1	0.44	3.3	70.99

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C3	<LOD	<LOD	40.4	0.12	0.23	19.55
S1	<LOD	<LOD	41.3	0.14	0.23	22.94
S3	<LOD	<LOD	664.5	0.30	3.79	49.24
W1	<LOD	<LOD	104.2	0.34	0.59	55.37
W2	<LOD	<LOD	42.3	0.31	0.49	49.6
Mean	-	-	202.1	0.27		
St dev	-	-	264	0.10		
Cv (%)	-	-	130.6	37.9		

3.3. Heavy metal in Oreochromis niloticus and metal pollution index

Total length of *O. niloticus* ranged from 10 to 26 cm, while weight ranged from 170 to 300 gr (Table 2). From the four nonessential heavy metals suspected to be available in lake waters and to be accumulated in fish liver, Zn, Cd and Pb were below of LOD (0.065, 0.125 and 0.12 mg g^{-1}) of the analyte, thus suggesting negligible concentrations in liver. Mercury (Hg), on the contrary, was detected in all specimens, with values ranging from 0.008 to 0.2 mg kg^{-1}. The Hg values were mostly below of the threshold of 1 mg kg^{-1} of Hg and 0.5 mg kg^{-1} of methylmercury, established by Mexican Standard NOM-242-SSA1-2009, which determines sanitary specifications for fishing fresh products for human consumption. Metal Pollution index, however, demonstrated that most individual falls into moderate pollution and in four individuals severe pollution was inferred. Pearson correlation test reveal negative correlation between fish length and Hg content in liver (r= -0.59, *p-value* 0.01 at 0.05 significance level) suggesting that smaller individuals have higher Hg concentrations, whereas statistical significance lack for fish weight and Hg concentration (r= -0.45, *p-value* 0.08 at 0.05 significance level).

Table 2. Size measurements and Hg concentration in *Oreochromis niloticus* from Lake La Sabana.

Weight (gr)	Total lenght (cm)	Hg concentration (mg kg^{-1})	Metal pollution index
300	26	0.0085	0.17
200	22	0.019	0.38
225	21	0.0247	0.494
300	25.5	0.0205	0.41
200	22.5	0.1289	2.578
190	20	0.0233	0.466
350	26	0.0254	0.508
200	23	0.0145	0.29
330	25	0.0278	0.556
210	25	0.0227	0.454
200	22	0.0129	0.258
180	19	0.1196	2.392
200	21	0.1362	2.724
190	19	0.0451	0.902
170	18	0.2073	4.146

3.4. Lake La Sabana bathymetry, spatial and temporal environmental variability

The bathymetric map revealed that La Sabana is a shallow lake that does not exceed 1.9 m depth during the dry season (Figure 3). The northern basin displays deeper waters, whereas in wetlands average depth is 1.1 m (Figure 3). During the rainy season, lake water levels can increase 40 cm, and then a water outlet is formed in the northernmost section, discharging waters northward toward the Chetumal bay (Caribbean Sea). Lake area is 1.48 km^2, perimeter is 18.81 km and length at middle of the lake is 6.43 km.

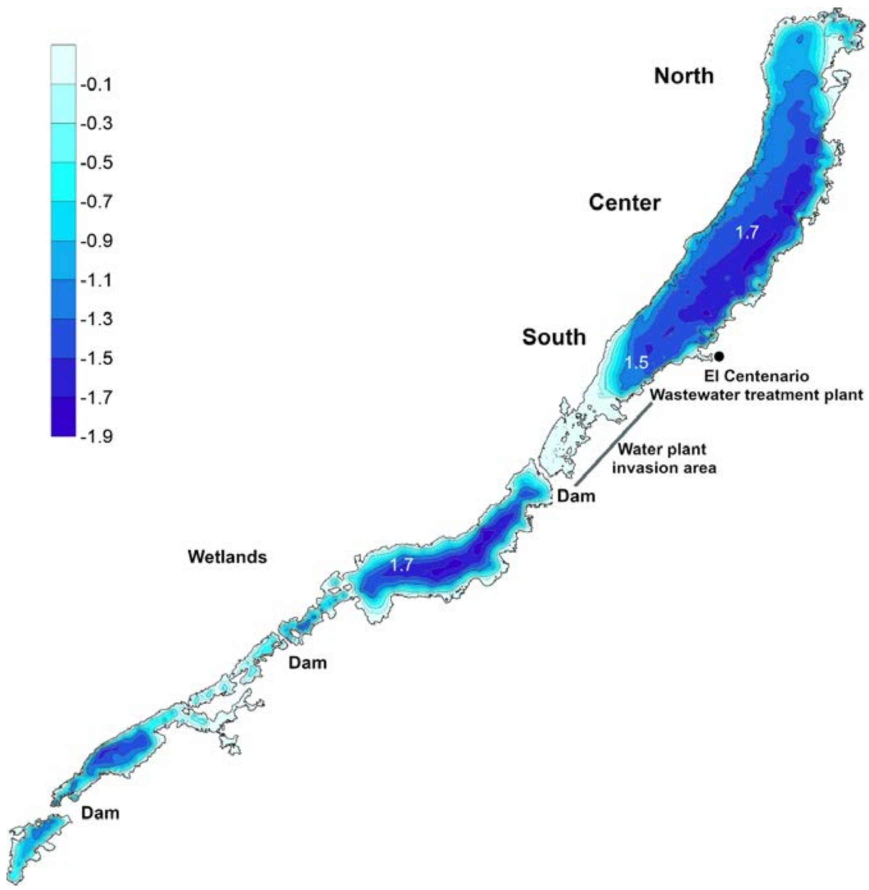

Figure 3. Bathymetric map of Lake La Sabana, isobaths show differences of 0.2 m depth.

The SIMPROF test projected on the nMDS ordination plot, with a good level of stress (0.09), clearly showed five significant groups (Figure 4). First group was constituted by samples from center zone of the lake in rainy season (Figure 4). The samples from wetland formed two well-defined and coherent groups, one corresponding with samples obtained in the rainy season and the other one comprising samples of the dry season (Figure 4). The remaining two groups included samples from north, south and central zone of the lake, differentiated by the climatic seasons (Figure 4).

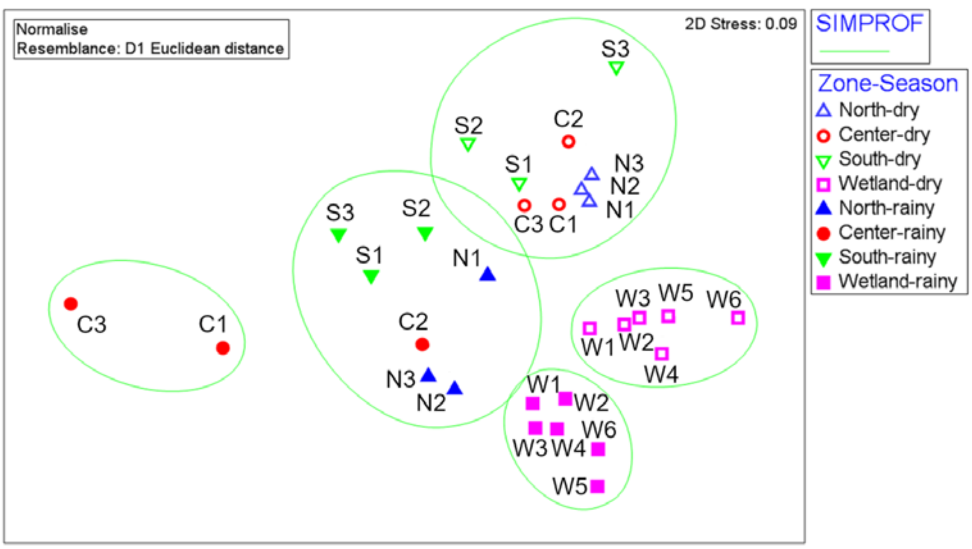

Figure 4. Non-metric Multidimensional Scaling (nMDS) projecting the Euclidean distances among the 30 normalized samples from La Sabana with a bidimensional stress of 0.09. The ellipses marked

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significant groups identified by SIMPROF. Symbols identified each lake zone (▲ North, ● Center, ▼ South, ■ Wetland), with dry season samples represented by unfilled symbols and rainy season samples represented by filled ones. Labels identified the number of samples of each lake zone and season.

PERMDISP showed that in the case of the season factor multivariate distances from each group's centroids were homogeneous (F = 3.024, p perm = 0.131), but in the case of the lake zone factor, dispersions were heterogeneous (F = 7.637, p perm = 0.001). Two pairwise compared lake zones resulted with significantly different dispersions after Benjamini-Hochberg correction [52]: center-wetland and south-wetland (p perm = 0.001 and p perm = 0.002, respectively). Given that multivariate dispersions were not homogeneous and that the design was unbalanced, the pseudo-F ratio as well as the permuted p value of PERMANOVA were computed using Type III sums of squares and permuting residuals under a reduced model [53]. For pairwise PERMANOVA tests, Monte Carlo simulations were also computed taking into account the limited number of permutations available in some cases.

The results of PERMANOVA showed that significant differences existed among the levels of the lake zone factor and between the levels of the season factor ($p \leq 0.001$ in both cases; Table 3; S2); in the interaction between both factors significant differences were detected as well ($p \leq 0.01$; Table 3). In the pairwise tests, under the term lake zone, all comparison with wetlands resulted significant at $\alpha = 0.05$ level after Benjamini-Hochberg correction, both with Monte Carlo and permuted values of p (S3), while north-center and north-south tests resulted not significant taking Monte Carlo values of p, albeit were significant regarding the permuted probabilities (Table S2). As expected from the PERMANOVA main test, the only pairwise test under the term Season (dry-rainy) was significant ($p \leq 0.001$) with both Monte Carlo and permuted values of p (S3). Under the interaction term lake zone × season all the comparisons for pairs of levels of the lake zone factor, both within dry and rainy levels of the season, resulted significant against wetland at α = 0.05 after Benjamini-Hochberg with Monte Carlo values of p (Table S2); due to the reduced number of unique possible permutations in only one of those comparisons (north-wetland within level dry) the test was also significant using the permuted p value (Table 3).

Under the interaction term lake zone × season for pairs of levels of the season factor, although the number of unique permutations available were very restricted in three out of four cases, taking Monte Carlo p values instead of permuted p values after Benjamini-Hochberg correction, all the comparisons between dry and rainy seasons within any zone level, except one (within south level) were found to be significant at α = 0.05 (Table 3).

Table 3. Table of the principal results of Permutational Multivariate Analysis of Variance (PERMANOVA) test. The total variation is partitioned according to four sources: Lake zone, Season, lake zone × season – interaction between lake zone and Season factors, Res – residual. Bold values indicate significant results (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Source	df	SS	MS	Pseudo-*F*	*p* perm	Unique perms
Lake zone	3	100.25	33.418	10.834	**0.001***	998
Season	1	70.617	70.617	22.893	**0.001***	999
Lake zone × Season	3	23.175	7.725	2.504	**0.003**	999
Residuals	22	67.862	3.085			
Total	29	261				

The first two principal components obtained by the Principal Components Analysis (PCA) captured 66.9% of the total variation (PC1: 35.6%, PC2: 31.2%). The variables total coliforms, BOD_5, transparency, and fecal coliforms were the most correlated to PC1 and contributed to segregate the samples under the lake zone factor; samples of the wetland zone appeared to be associated to higher values of transparency, while the remaining zones, particularly in rainy season and remarkably samples from the center zone, showed

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a strong negative association to transparency and strong positive association to total coliforms, BOD5, and fecal coliform values (Figure 5). The most correlated variables to PC2 were temperature, pH, conductivity, and TSS, and they strongly contributed to segregate the samples under the two levels of the season factor; samples obtained during dry season appeared to be associated to high pH, conductivity and TSS values, in contrast to samples obtained in rainy season, which instead showed higher temperatures (Figure 5).

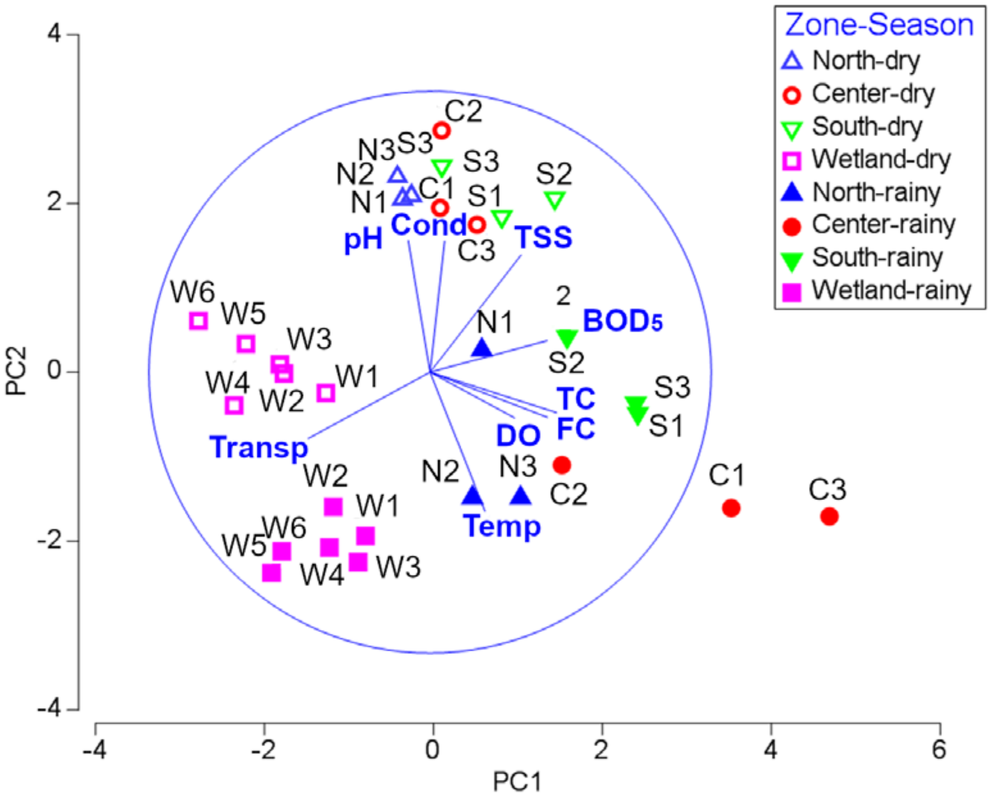

Figure 5. Bidimensional plot with the first two principal components obtained by the Principal Components Analysis (PCA). Symbols identify each zone (▲ North, ● Center, ▼ South, ■ Wetland), with dry season samples represented by unfilled symbols and rainy season samples represented by filled ones. Labels identify the number of samples for each lake zone and season. Vectors represent the variables included (BOD_5: biochemical oxygen demand, TC: total coliforms, Cond: conductivity, FC: fecal coliforms, DO: dissolved oxygen, TSS: total solids, Temp: temperature, Transp: transparency).

4. Discussion

4.1. Anthropogenic alterations in La Sabana: water quality, heavy metals and environmental risk

La Sabana is a shallow lake that does not exceed, in the main basin, 1.9 m during the dry season and 2.3 m in the rainy season. Shallow conditions are typical of the Bacalar hydrological system as surrounding lakes such as lakes Milagros and Bacalar do not exceed 5 and 20 m, respectively [54,55]. Southern basins corresponding to wetlands (Figure 5) are more sensitive to flooding and desiccation [22], whereas in the northern basin, the increase of water levels forms a temporal water outlet that discharged toward the Chetumal bay (Caribbean Sea). The overall shallow conditions in La Sabana suggests that it may be sensitive to anthropogenic activities, particularly to contaminant loads. The NSF-WQI did show bad to medium water quality conditions within La Sabana (Figure 2e, f). The center and south showed predominant bad quality conditions through most of the year (Table S1) with the lowest values of the NSF-WQI. The PCA revealed that total coliforms, BOD_5, low transparency, and fecal coliforms are the most relevant variables of the center-south of La Sabana (Figure 5). Fecal and total coliform values were substantially high in this same zone during dry and rainy seasons, largely exceeding the threshold of the Mexican standard NOM-210-SSA1-2014 of <16,000 MPN 100 ml^{-1} for total and <450 MPN 100 ml^{-1} for fecal coliforms, which indicates maximum values for waters of recreational uses (Table S1). High values in fecal coliforms originated from anthropogenic activities which are usually associated with the presence of other enteric pathogens [56] and therefore, the direct or indirect interaction with waters of the center-south of La Sabana must be done with caution as it may cause waterborne diseases such as gastrointestinal diseases, ear, eyes and wound infections, typhoid fever, or dysentery [57]. The area of continuously bad water quality and maximum amount of bacterial content coincides with the discharge area of the wastewater treatment plant "El Centenario". Contrastingly, in the wetlands corresponding to southern basins, where the influence of "El Centenario" is limited, the NSF-WQI shows better water quality (medium quality) and the bacterial concentration drastically dropped in both climatic periods, compared with the center-south of the lake. PCA illustrated that wetlands are associated with waters of higher transparency and lower levels of variables related with contamination such as fecal coliforms and BOD_5 [58]. Wetlands are the section of the lake, where anthropogenic influence is most limited. Extensive zones surrounding the system still maintain native vegetation and irregular settlements have not been developed yet. Man-made dams contribute to isolate wetlands from water exchange from polluted zones of the lake, favoring better water quality.

Wastewater treatments plants discharging to lakes and even coastal areas, are known to represent source of contaminants and organic matter [59,60]. In Latin America, one of the most important problems for wastewater management is the lack of infrastructure for wastewater treatment plants, consequently, only about the 20% of wastewaters are properly treated, and the remaining 80% is partially or not even treated in urban centers [61,62]. In Chetumal city, "El Centenario" processes about 120 lt s^{-1} of wastewaters and provide services to more than half of the population of the city, but the treatment capacity is exceeded, and waters are only partially treated. Nitrogen and phosphorous are main constituents of wastewaters and promoters of eutrophication in lakes [63,64]. In La Sabana, the nitrogen available in the form of nitrites+ nitrates (NN, Table S2) revealed relatively high concentrations (> 1.6 mg l^{-1}) particularly in the south, and phosphorus in the form of orthophosphates (0.19-0.23 mg l^{-1}, Table S1) exceeding limits of international Standars such as USEPA (0.05mg l^{-1}) [65] suggesting that water conditions in La Sabana is above the maximum acceptable (0.1 mg l^{-1}) and susceptible to rapid eutrophication. Currently, La Sabana is categorized within eutrophic to hypereutrophic based on the N and P concentrations [25]. Bad water quality and over-enrichment (eutrophication) of La Sabana, may therefore be, a consequence of the insufficient capacity of "El Centenario" to process wastewaters and release partially treated water to the lake.

Heavy metals concentration was relatively low in both, sediments, and fish tissue (Table 2), with almost all values not exceeding national norm or standard, such as the NOM-242-SSA1-2009, and internationals standard thresholds, such as the CSQG. Most values of the Risk index (RI) and the Pollution index (PI) of sediments and fishes, similarly indicates low environmental risk. The RI, however, demonstrated moderate ecological risk for Hg in sediments, and metal pollution index showed values of severe pollution of Hg in 26% of fish individuals. The presence of Hg in aquatic ecosystems is a global concern as it can be toxic even at low concentrations [66,67]. The Hg is easily incorporated into trophic chains and can also be potentially toxic to humans through fish consumption [68–70]. In La Sabana, fishing is an important activity, with products being locally traded. The consumption of fish of La Sabana must be carefully evaluated, as the results obtained in our study were conducted in liver, an organ not typically consumed, that may display relatively higher values of Hg than muscle [71,72]. Concentrations of Hg in muscle and liver, however, can be equaled or even reverted given the overall Hg availability in the lake system [73,74]. For instance, in our study putative juvenil fishes (with smaller length) display higher values of Hg, which is indicative that the Hg contamination may be extended to other wildlife and lower levels of trophic chain as juvenile stages of *O. niloticus*

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are more specialized in zooplankton, insects and detritus, compared with adults characterized by more herbivorous feeding habits [75,76].

Incorporation of heavy metals in La Sabana was a priori considered to be related with leachate of the open-air landfill of the Chetumal city located less than 5 km of the northern basin. In our study, low values or absence of heavy metals typically generated by landfills such as Cd, Zn, Pb, Cr [77–79] were detected, suggesting that the leeching influence of the landfill is minor. This can be explained by the hydrological dynamic of the zone, as the landfill is located in the drain area of the lake during high water levels. Therefore, in case of availability, leachate may be moved toward the Chetumal Bay to the Caribbean Sea. The source of Hg incorporation to La Sabana, is relatively difficult to determine, as activities that increase its incidence [80,81] such as metallurgical processes, chlor-alkali industries, battery production and agriculture is absent in the surrounding areas. Fossil fuel burning and airborne emissions from activities in landfills such as compaction of solid waste and burning [82–84] may be the most important sources, but it remains to be proved.

The hydrological connectivity in the region, including the groundwater flow, the surficial aquatic environments and their interactions with the Caribbean Sea, suggests that the anthropogenic impact and contaminants of La Sabana may not only be focalized in the core area, but largely extended in the region, affecting a broad range of ecosystems. For instance, both coliform bacteria [85] and heavy metals in sediments and aquatic species [41] have been recorded at a regional scale in the Chetumal bay (Caribbean Sea).

4.2. Ecosystem effects of the anthropogenic activities in La Sabana, direction for effective environmental management

Lake La Sabana is relevant for the hydrological balance in the region and particularly for Chetumal city. Although its waters are not used for water supply, most meteoric water received by the city is discharged to both La Sabana or Chetumal Bay and therefore these systems control flooding. The growth of the city caused that the lake was partitioned into four sub basins, leading to changes in the lake dynamic and functionality. The nMDS and SIMPROF tests revealed the presence of five well-defined groups, corresponding either to basins, or climatic periods. Wetlands were the most different region in comparison with any other zone of the lake. Climatic variables such as temperature, pH, conductivity, and TSS were more variable between climatic periods, coinciding with what has been observed for the region by different authors [86,87]. The relative isolation of the basins thus focalizes contaminants and drives different biological structure. The north-center-south of the lake are the zones more drastically altered by anthropogenic activities, partially treated wastewater loads in these zones can be tracked back to at least 10 years ago, during the city population growth. First effect of such anthropogenic influence was the change in the trophic status of the lake. Natural mesotrophic conditions progressively change to hypereutrophic conditions [25]. In hypereutrophic basins, zooplankton community exhibited population changes, likely as a response of increase nutrients. Currently, no-native species dominates the northern basin, as a typical response of highly perturbed aquatic environments, with groups such as Asplanchidae (Rotifera), Cyclopidae (Copepoda) and *Cypridopsis* (Ostracoda), whereas in the southern basins, regional distributed groups are more common, e.g., *Alicenula*, *Chlamydotheca* (Ostracoda) [25]. In this same basin, we were unable to collect native fish species and local fisheries only recover *O. niloticus*, suggesting an additional effect on fish assemblage.

The coverage of *P. stratoites* on La Sabana is most likely an additional and sequential consequence of waters over enrichment. *Pistia stratoites* is an invasive species in tropical and subtropical regions of the world, that colonize water bodied rich in N and P [88,89] and with prevailing eutrophic conditions [90–92]. Due to its geographic location and climate, the Yucatán Peninsula, is susceptible to invasion of aquatic plants, such as *P. stratoites*. The region is, however, almost free of this plant species given the overall oligotrophic conditions of most water bodies [93–95]. In La Sabana, the colonization of *P. stratoites* from

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2017 to 2019 ranged from absent to more than 14 hc [22], revealing the high risk of the region to aquatic plants invasion, if aquatic bodies become enriched by anthropogenic activities.

The mitigation of environmental alterations of Lake La Sabana is necessary to preserve ecosystemic services and maintain ecological functionality at a regional scale. Based on our data and available literature, it has become evident that actions toward restauration of La Sabana must be address integrally to understand the underlying causes and the effects of the environmental alteration, and establish criterias for sustainable development. We propose three main directions to mitigate environmental alteration in La Sabana: i) reduction of nutrients and contaminants loads from the wastewater treatment plant; ii) management of *P. stratoites*; and iii) delineate regulations for land use and sustainable development of the city in the surroundings of La Sabana. One of the projected actions of the Quintana Roo state government in favor of these three actions, is the modernization of the wastewater treatment plant, increasing the treatment capacity from 120 to 180 lt s^{-1} [96]. This action is expected to mitigate the input of nutrients and organic matter to the lake. There is still, however a high percentage of people inhabiting around La Sabana, discharging wastewaters directly to the ground (not connected to sanitary drainage system). Therefore, must be mandatory for people, to use the city sanitary drainage system for wastewater management, as it is currently optional for them. The management of *P. stratoites* is necessary because is a plant that can alter the ecological stability of an aquatic system [97,98], but it can also provide opportunities for nutrients and contaminant remotion [91,99,100]. Strategies for viable management for the region, include its use for heavy metal and nutrient removal from the water and sediments and the biomass transformation for energy productions in the form of methane [101].

In La Sabana exists a high uncertainty in regard to the agency in charge of surveillance and what are the applicable laws for lakes protection. This uncertainty is motivated by incipient guidelines for local inland aquatic system management and the lack of a formal regulation of the municipality and state. The clear determination of the institution in charge of La Sabana, and its competences for lake protection is a pre-requisite for an integrative plan for the sustainable development of the city.

5. Conclusions

Lake La Sabana is a fundamental system for the ecological dynamic and the hydrological balance in the region, particularly benefiting the management of meteoric water in Chetumal city. Lake La Sabana diagnosis highlighted that within its four basins environmental condition are variable spatial and temporally, with water quality going from medium in wetlands to bad in the northern basin, in accordance with the National Sanitation Foundation water quality index. Heavy metals were relatively low in sediments and fish tissue, except for mercury in which high potential environmental risk were determined by ecological indices. The wastewater treatment plant "El Centenario" was recognized as the major source of contamination on La Sabana, because of partially treated wastewaters loading to the lake, thus contributing to increase pathogenic bacteria in surficial waters and altering the balance of N/P related with eutrophication. Three actions were recognized as key for lake management: 1) reduction of nutrient input, 2) management of invasive plants, and 3) establishment of local regulations and define institutions in charge of lake protection for an orderly and sustainable growth of the city. The anthropogenic alteration on La Sabana, particularly the over enrichment generates a snowball effect of environmental alterations such as modification of ecological relationships and appearance of invasive plant species. Sequential alterations in La Sabana must be considered a baseline model of what can be expected in aquatic systems of the Yucatán Peninsula in case of incipient aquatic system management.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Physical, chemical, and microbiological variables from Lake La

Sabana during rainy and drying season in Yucatán Peninsula; Table S2. Table of pairwise Permutational Multivariate Analysis Of Variance (PERMANOVA) tests.

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