# La Culebra stream, a hydrological relic in the Guadalajara Metropolitan Area (Mexico)

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**Article**

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# Abstract

In urban environments the existence of streams with ecological functionality, with an assembly of producers, consumers and degraders, with abundance governed by environmental variations and by the flow of nutrients, maintaining an aerobic process is becoming less frequent.

La Culebra stream originates in Los Colomos Urban Forest (LCUF) within the Guadalajara Metropolitan Area (GMA), which is preserved almost naturally with few human interventions in the studied section. The stream flows from springs that emerge within the Urban Forest. But its waters are discharged into an urban channel that carries drainage water and stormwater runoff. In addition, there are no records of the water quality of La Culebra stream. It is of great interest to know its characteristics to propose management alternatives or a better use.

Through the use of physicochemical techniques and test organisms, the behavior of macronutrients in the water, the existence of inhibition factors and the presence of coliforms were investigated during six months representative of the seasonality of the year 2018. In the last month of the study, it was implemented the analysis of sediment quality with the use of a specific test organism for the soil-water interface.

In general, the stream was found without an excessive load of nutrients and with a variability in its physicochemistry and biology depending on the seasonal cycle. The system maintains adequate levels of dissolved oxygen to support the respiration of organisms in the water column and in particular the upper layer of the sediment with the highest demand for this gas. It was found that the oscillation of the annual temperature in the stream presents a delay of one to two months in relation to the atmospheric temperature. The finding of the inverse behavior between temperature and pH facilitated the interpretation of the availability of nutrients that maintain a production, based on photosynthesis, proportional to their availability. Through the use of EDTA as a chelating agent of metal cations, the increase in the production of the plants was determined and in this way the presence of an inhibition factor was revealed. Although coliform levels were high in all samples, we conclude that their presence is not from human origin, but of ducks and small mammals that normally inhabit the urban forest.

We recommend conducting microbiological studies of groundwater to confirm the natural origin of coliforms. Likewise, it is recommended that studies be implemented to characterize the nature of the inhibiting agent that we have detected. Finally, it is recommended that the administrative authority of the Urban Forest manage funds for the dissemination and preservation of La Culebra stream as a subject of high environmental and cultural value in the GMA.

# Introduction

The Guadalajara Metropolitan Area (GMA) or Greater Guadalajara, is the second most populous urban zone in Mexico. There is a perennial stream with ecological functionality, which is rare in large urban communities. La Culebra stream originates in a ravine in the southern section of Los Colomos Urban Forest (LCUF). It is approximately 200 m long and is maintained throughout the year with a crystal-clear water flow of approximately 20–35 $m^3$/h. A series of springs is the main source of its water. The water of La Culebra stream is discharged into an urban channel and is mixed with sewage.

## Problem

Although LCUF has been declared a Protected Natural Area (El Estado de Jalisco, 2018), there are no historical records of the physicochemical characteristics of the water in La Culebra stream. Until before this study, it was not possible to determine, for example, whether the water was suitable for domestic use. There are also initiatives to declare the LCUF as a Ramsar site, but there is no analytical support for the quality of surface waters. Ignorance of water quality prevents determining improvement actions and their feasibility.

**Research question.** Worldwide, uncontaminated water sources are increasingly scarce and in the case of the LCUF, several questions arise about the quality of the water in La Culebra stream: Does the nutrient balance over time have adequate potential for algal growth? Are there inhibition factors for the development of primary producers? Are the dissolved oxygen levels adequate to maintain the system in aerobic conditions? Does the sediment in the stream have the ability to support test organisms? Can the water that is currently discharged outside the LCUF be used as a clean resource? If not, is it possible to provide treatment to allow its use?

**Justification**. The analysis of the water quality in La Culebra stream will help to elucidate whether the composition and structure of the biological community are modified by the temporal variations of the nutrients and the physical factors analyzed. The coliform analysis will determine the potability of the water or the existence of sources of biological contamination that must be located and controlled. Tests to evaluate the absence or presence of inhibiting factors will confirm potability or the existence of environmental chemicals: these should be characterized in later studies to determine their nature, origin and control.

**Objective**. In the present study, the protocol of the United States Environmental Protection Agency (USEPA) (Miller, 1978) was used to evaluate the algal growth potential, the limiting nutrient and the presence of inhibiting factors in La Culebra stream within the LCUF, in Greater Guadalajara, Mexico. The analysis was carried out monthly during the period from May to October of the year 2018, to cover the three seasons of the hydrological regime. Simultaneously, determinations of temperature, pH, $O_2$, nitrates, nitrites, ammoniacal nitrogen, phosphates, in addition to fecal and total coliforms were made. In the last of the samplings of October, the sediment analysis was implemented using the crustacean *Hyalella azteca* as a bio-indicator species.

**Hypothesis section**. The working hypotheses were the following. 1) High temperature value s will be obtained during the months of May and June with a gradual decrease until October due to the fact that, within the tropical strip where the GMA is located, three seasons are perceived in the annual cycle: hot dry season from March to May, rains from June to September and cold drought from November to February (Rzedowski, 2006; Ibarra-Montoya et al., 2012); 2) The concentration of dissolved oxygen will be higher in the colder months, according to Henry's law (Goldman & Horne, 1983); 3) The availability of nutrients will be greater in the warm season due to the increase in the metabolism of degrading organisms (Benjumea-Hoyos et al., 2014) and the dependence on abiotic factors such as temperature, pH and oxygen level (Chlot et al., 2011); 4) The growth of the microalgae will be greater in the warm season due to the increase in the metabolism of the degrading organisms and the greater availability of nutrients (Benjumea-Hoyos et al., 2014); 5) The limiting factor for algal growth will be nitrogen, as occurs in most warm water bodies, because the stream is located in the intertropical belt (Ramos-Higuera et al., 2008); 6) There will be no alterations in the growth of the algae with the addition of EDTA salt, as a chelating agent of heavy metals (Miller et at., 1978), due to the assumption of absence of this type of contamination because of the background of transparent waters and the constant presence of fish and invertebrate; 7) Thermotolerant coliform levels will be low due to the eventual presence of ducks in the stream (von Sperling & de Lemos-Chernicharo, 2005); 8) The mortality of the amphipod *Hyalella azteca* test organism will be low in the sediments of the stream due to the background of transparent waters and the constant presence of fish and invertebrates in the sediments (ASTM, 2004).

# Materials And Methods

**Study area.** LCUF is located in the Atemajac micro-basin at the extreme Northwest of the municipality of Guadalajara. The drainage network, to which LCUF belongs, originates in the Northeast section of the "La Primavera Forest Flora and Fauna Protection Area" (LPFFFPA) and discharges into the "Río Grande de Santiago" at the "Barranca de Oblatos" site (Fig. 1). La Culebra stream has occasional contributions during the rainy season, which come from surface runoff and from an artificial pond for ornamental use within the LCUF. The bottom of this water stream is predominantly sandy with the presence of scattered rocks; these are substrate for the development of a periphyton community (slime) dominated by filamentous green algae. There is little presence of small fish of the poeciliidae and goodeidae families. Ducks are also occasionally seen migrating freely from the artificial pond. The organic matter in the sediment comes mainly from the aerial productivity of the terrestrial vegetation that develops on the slopes. Macroinvertebrates such as earthworms, snails, and crustaceans are frequently evident in the sediment community. The existence of a stable community of microinvertebrates, protozoa, bacteria and fungi through which the processes of organic matter degradation and nutrient recirculation occur is to be expected. In the upper part of the stream, where the water film is barely forming and there is a lot of shade, the periphyton is dominated by blue-green algae. At the end of the section described, the water is piped and discharged outside the park towards the Atemajac stream, which is completely channeled for the conduction of mixed urban drainage (black water and stormwater runoff) until it is discharged into the "Grande de Santiago" river. In the LCUF there are other springs that have been piped for several decades and their waters are discharged into a large reservoir for later distribution as clean water for the surrounding neighborhoods.

The coordinates of the sampling sites were obtained with a GPS equipment (Garmin® model Oregon 600) with the WGS84 datum. The selection of the four analysis points was made according to the criterion of equidistance and accessibility for sampling (Table 1).

Table 1
UTM Coordinates from sampling places
(Datum: WGS 84).

| Site | Coordinates at 13Q | |
|---|---|---|
| A | N 2’290,178.16 m | E 667,583.34 m |
| B | N 2’290,187.53 m | E 667,597.71 m |
| C | N 2’290,239.84 m | E 667,600.09 m |
| D | N 2’290,276.56 m | E 667,582.36 m |

**Sampling**. Sampling and determinations were carried out monthly during the period from May to October 2018 to include the end of the dry season and the consequent rainy season. The laboratory glassware used for taking samples and the PET bottles, both for sampling and for transporting and storing the liquid obtained, were previously treated with acid and alkali solutions (Miller et al, 1978). The bottles were filled with water from the site, filtered at 30 microns to eliminate suspended matter that could interfere with the chemical analyses. The grab samples obtained (three replicates of 250 mL, from each of the four collection sites) were stored in a cooler until they were transferred to the laboratory to start their analyzes on the same day.

**Physicochemical of water**. For *in situ* analysis, a multisensor (Pasco® Xplorer model GLX) was used with electrodes for measuring pH, dissolved $O_2$ and temperature. The following analyzes were done under laboratory conditions: for the evaluation of nitrogen concentrations (nitrates, nitrites and ammoniacal nitrogen) and phosphates, the HACH methodology indicated in the technical data sheets of each of the different reagents was used; for nitrates, NITRIVER 5 (code: 2106169); for nitrites, NITRIVER 3 (code: 2107169); for ammoniacal nitrogen AMMONIA SALICYLATE (code: 2653299) followed by AMMONIA CIANURATO (code: 2653199); for phosphates, PHOSVER 3 (code: 2106069). Then, a spectrophotometer (Thermo Scientific® model GENESYS 20) was used to read the absorbance values. The readings were converted to concentration units using the specific calibration curves for each analyte. The chemical residues of the determinations were emptied into plastic containers for proper disposal according to local regulation (NOM-052, 2006).

**Statistical analysis**. Polynomial regression analyzes were done with the physicochemical data of the study sites, with respect to time; determination coefficients ($r^2$) and their respective graphs were reported. To locate the limiting factors of algal growth, statistical analyzes were done comparing averages of the maximum values of each of the experimental groups; normality and homoscedasticity analyzes were done to choose between ANOVA or Kruskal Wallis as the analysis method. Subsequently, multiple range tests of LSD were carried out in cases where significant differences were found. In all cases, 95% was used as the reliability level.

**Bioindicators**. The three bioindicators, referred to in the objectives, were used for the analysis of the water and sediment samples taken at site D (Table 1).

Microalgae. Limiting nutrient and inhibition factors were evaluated with the microalgae *Pseudokirchneriella subcapitata* (Miller et al., 1978). In each of the sampling months, 3000 mL of water were collected (five 600 mL containers). The water samples were processed by microfiltration with cellulose acetate membranes of 0.8 and 0.45 microns, consecutively. The filtered water was used to establish the necessary experimental groups in order to identify the limiting factor and the presence of inhibition (see Table 2). The microalgal inoculum for the bioassays with the test microalgae was obtained from the Mexican Institute of Water Technology; the algal concentrate was propagated and used as inoculum in each of the monthly bioassays.

Table 2
Algal bioassay. Experimental groups evaluated monthly.

| Group | Contents |
|---|---|
| 1 | Control EPA[1] |
| 2 | Control site water |
| 3 | Site + Phosphorus[2] |
| 4 | Site + Nitrogen[3] |
| 5 | Site + Phosphorus + Nitrogen |
| 6 | Site + EDTA[4] |
| 7 | Site + Phosphorus + EDTA |
| 8 | Site + Nitrogen + EDTA |
| 9 | Site + Phosphorus + Nitrogen + EDTA |

1 EPA (culture medium of the “U.S. Environmental Protection Agency”). 2 load of 0.05 mg/L phosphorus as $K_2HPO_4$; 3 load of 1.00 mg/L nitrogen as $NaNO_3$; 4 load of 1.00 mg/L of $Na_2EDTA$ (ethylenediaminetetraacetic acid, disodium salt).

Five replicates per group (10 mL test tubes) were used. The incubations were done for fifteen days with a photoperiod of 12 hours (4,304 lumens) and the cultures were shaken once or twice a day. The bioassays were carried out at laboratory temperature that remained stable around 21 ± 1°C during the study period. The daily follow-up of the cultures was done by fluorescence (Turner® model 450). Filters of 440 nm (excitation wavelength) and 665 nm (emission wavelength) were used. The fluorometer was operated in an arbitrary range from 0 to 550 units, according to preliminary studies (Zavala-Aguirre et al., 2007).

Crustacean. The evaluation of the sediment was made with the amphipod *Hyalella azteca* (ASTM, 2004). The organisms were obtained from the Laboratory of Hydrobiology and Aquatic Ecotoxicology of the AUG where they are maintained on a regular basis. In the present study, 80 adults were used, with whom two groups were formed: 5 repetitions for the control group and 3 repetitions with the evaluated sediment; each culture vessel (replicate) contained 10 adults (300 mL beakers). During the ten days of the bioassay, the records of mortality and dissolved oxygen were made, as well as the change of water and food. The change of water for each container was implemented 2 times a day (every 12 hours); artificial aeration was not provided. The $O_2$ record was made every day before the water change (Xplorer® GLX). The organisms were fed with commercial flakes for marine fish at a ratio of 0.0087g per day (TetraMarine®, Large saltwater flakes). The exchange of water and removal of dead organisms was done with a 10 mL automatic pipette (CLP®, POSEIDON-BETA-PETTE model). The trial with *Hyalella azteca* was implemented in the last of the study months.

Coliforms. Fecal bacteria analyses were done to evaluate the potability of the water (NOM-127, 1994). A water sample was taken monthly for analysis of fecal coliforms and total coliforms in 100 mL sterile bags (Nasco® WHIRL-PAK); On each occasion, the samples were stored with ice until they were transferred to the environmental laboratory analysis (Grupo ECOTEC, S.A. de C.V.) certified in accordance with local regulation (NOM-001, 2021).

**Maps production.** We used hydrology shapefiles downloaded from the Mexican Institute of Statistics, Geography and Informatics (https://antares.inegi.org.mx/analisis/red_hidro/siatl/) that were transformed to KML format, with a Geographic Information System (gvSIG) for the final maps production at GoogleEarth platform.

# Results

**Physical chemistry of water**. The results obtained from the four sites in La Culebra stream are summarized in Table 3.

Table 3
Point values, averages and standard deviations of the physicochemical analyzes of La Culebra stream in the study period.

| Factor | Site | May '18 | Jun '18 | Jul '18 | Aug '18 | Sept '18 | Oct '18 |
|---|---|---|---|---|---|---|---|
| ºC | Site A | 19.7 | 22.4 | 22.4 | 22.2 | 21.2 | 21.6 |
| | Site B | 23.0 | 23.5 | 23.7 | 23.3 | 23.3 | 22.9 |
| | Site C | 22.1 | 22.8 | 23.3 | 22.7 | 22.8 | 21.7 |
| | Site D | 21.4 | 22.5 | 23.1 | 22.4 | 22.5 | 21.2 |
| **Average** | | **21.6** | **22.8** | **23.1** | **22.7** | **22.5** | **21.9** |
| **Stand. Dev.** | | **1.40** | **0.50** | **0.54** | **0.48** | **0.90** | **0.73** |
| pH | Site A | 8.4 | 7.8 | 7.5 | 7.4 | 8.2 | 7.8 |
| | Site B | 7.4 | 7.4 | 7.2 | 7.1 | 7.3 | 7.2 |
| | Site C | 7.4 | 7.3 | 7.1 | 7.1 | 7.2 | 7.2 |
| | Site D | 7.5 | 7.3 | 7.1 | 7.0 | 6.3 | 7.0 |
| **Average** | | **7.7** | **7.4** | **7.2** | **7.2** | **7.2** | **7.3** |
| **Stand. Dev.** | | **0.49** | **0.27** | **0.21** | **0.19** | **0.78** | **0.33** |
| $O_2$ mg/L | Site A | 4.4 | 6.5 | 3.8 | 3.4 | 4.6 | 3.8 |
| | Site B | 4.2 | 6.5 | 7.3 | 6.6 | 5.4 | 7.1 |
| | Site C | 4.8 | 7.6 | 7.9 | 7.2 | 5.9 | 7.8 |
| | Site D | 4.3 | 6.8 | 7.8 | 7.1 | 5.7 | 7.9 |
| **Average** | | **4.4** | **6.9** | **6.7** | **6.1** | **5.4** | **6.7** |
| **Stand. Dev.** | | **0.26** | **0.52** | **1.95** | **1.80** | **0.57** | **1.93** |
| $NO_3^{-1}$ mg/L | Site A | 3.903 | 5.230 | 5.422 | 6.229 | 4.864 | 5.556 |
| | Site B | 7.979 | 6.806 | 6.595 | 6.854 | 5.566 | 6.402 |
| | Site C | 6.883 | 7.085 | 7.565 | 7.162 | 6.921 | 6.393 |
| | Site D | 8.450 | 6.595 | 7.469 | 7.373 | 6.768 | 6.306 |
| **Average** | | **6.804** | **6.429** | **6.763** | **6.905** | **6.030** | **6.164** |
| **Stand. Dev.** | | **2.042** | **0.824** | **0.995** | **0.498** | **0.985** | **0.408** |
| $NO_2^{-1}$ mg/L | Site A | 0.027 | 0.085 | 0.014 | 0.021 | 0.021 | 0.020 |
| | Site B | 0.059 | 0.019 | 0.007 | 0.009 | 0.009 | 0.009 |
| | Site C | 0.078 | 0.031 | 0.011 | 0.013 | 0.013 | 0.011 |

| Factor | Site | May '18 | Jun '18 | Jul '18 | Aug '18 | Sept '18 | Oct '18 |
|---|---|---|---|---|---|---|---|
| | Site D | 0.080 | 0.040 | 0.013 | 0.020 | 0.013 | 0.012 |
| **Average** | | **0.061** | **0.044** | **0.012** | **0.016** | **0.014** | **0.013** |
| **Stand. Dev.** | | **0.025** | **0.029** | **0.003** | **0.006** | **0.005** | **0.005** |
| $NH_3^+$ mg/L | Site A | 0.027 | 0.011 | 0.030 | 0.022 | 0.038 | 0.026 |
| | Site B | 0.011 | 0.008 | 0.031 | 0.015 | 0.036 | 0.014 |
| | Site C | 0.015 | 0.011 | 0.031 | 0.017 | 0.076 | 0.010 |
| | Site D | 0.022 | 0.010 | 0.033 | 0.024 | 0.036 | 0.013 |
| **Average** | | **0.019** | **0.010** | **0.031** | **0.019** | **0.047** | **0.016** |
| **Stand. Dev.** | | **0.007** | **0.001** | **0.001** | **0.004** | **0.020** | **0.007** |
| $PO_4^{-2}$ mg/L | Site A | 0.288 | 0.223 | 0.142 | 0.204 | 0.091 | 0.124 |
| | Site B | 0.270 | 0.204 | 0.157 | 0.249 | 0.128 | 0.145 |
| | Site C | 0.184 | 0.408 | 0.157 | 0.182 | 0.117 | 0.148 |
| | Site D | 0.200 | 0.189 | 0.162 | 0.201 | 0.125 | 0.131 |
| **Average** | | **0.236** | **0.256** | **0.154** | **0.209** | **0.115** | **0.137** |
| **Stand. Dev.** | | **0.051** | **0.102** | **0.009** | **0.028** | **0.017** | **0.011** |

Temperature. The initial average was 21.6°C in the month of May; the maximum value was 23.1°C in the month of July; from then on, there was a decrease to the value of 21.9°C in the month of October (Fig. 2). For the evaluation of the statistical differences over time, the ANOVA test was implemented; there were no significant differences ($P = 0.1128$).

pH. In this analysis, an initial and maximum average of 7.7 was obtained in the month of May; the following three months the values fell until reaching the minimum of 7.18 in the month of August; from there, an increase was observed until having a value of 7.3 in the month of October (Fig. 3). The Kruskal-Wallis test was used to compare the medians, and no significant differences were found ($P = 0.238373$).

Dissolved oxygen. In this analysis, an initial average of 4.4 mg/L was obtained in the month of May (being the minimum value); the maximum value was presented in June with 6.9 mg/L; there were oscillations in the values of the following months (Fig. 4). The Kruskal-Wallis test was used to compare the medians, and no significant differences were found ($P = 0.195987$).

Nitrates. These presented an oscillatory behavior through time; the maximum concentration occurred in August (6,905 mg/L) and the minimum in September (6,030 mg/L); the behavior was very stable (Fig. 5). An ANOVA analysis was used without finding significant differences ($P = 0.4905$). To compare each of the sites between them over time, a bifactorial ANOVA was applied, finding stability over time, with a value of $P = 0.2533$.

Nitrites. These showed some oscillation, but with a tendency to decrease over time (see Fig. 6). The maximum concentration occurred in the month of May (0.061 mg/L) and the minimum in the month of July (0.012 mg/L). The Kruskal-Wallis test showed significant differences ($P = 1.16566 \times 10^{-8}$). To compare each of the sites between them over time, a bifactorial ANOVA was applied, finding significant differences in this variable, with a value of $P < 0.00001$.

Ammoniacal nitrogen. An oscillatory behavior was observed over time (Fig. 7), with its maximum concentration in September (0.047 mg/L) and the minimum in June (0.001 mg/L). A Kruskal-Wallis analysis was applied, obtaining significant differences ($P = 6.16804 \times 10^{-10}$). To compare the sites with each other over time, a bifactorial ANOVA was done and significant differences were found with a value of $P < 0.00001$.

Phosphates. An oscillatory behavior was observed over time (Fig. 8); showed their maximum average concentration in June (0.2558 mg/L) and the minimum in September (0.1152 mg/L). When applying the Kruskal-Wallis test, significant differences were found ($P = 5.74983 \times 10^{-8}$). To compare the sites with each other over time, a bifactorial ANOVA was performed and significant differences were found with a value of $P < 0.00001$.

**Bioindicators.** The results of this section correspond to the analyzes made exclusively at site D of La Culebra stream in terms of microalgae, crustaceans and coliforms.

Microalgae. In all the months analyzed, it was observed that the algal growth was higher in group 3 (site water + phosphorus) in relation to group 2 (only site water). The result points to the prevalence of phosphorus limitation in the stream (Table 4). It was also observed that in all months, group 4 (site water + nitrogen) was statistically equal to group 2 (site water). The result indicates the absence of nitrogen co-limitation.

In relation to the inhibition factors to be detected in group 6 (site water + EDTA), the results show that in 50% of the months (May, July and September) the increase in fluorescence was evident. Therefore, the existence of variables that interfere slightly with the growth of the test species is admitted. In group 7 (site water + phosphorus + EDTA) a synergistic effect on algal growth was observed in all months to the extent of reaching levels statistically similar to control group 1 (control with EPA medium); this confirms the role of phosphorus as a limiting element and the existence of some factor that probably inhibits the growth of the algae (Table 4).

Table 4
Average values of maximum fluorescence in the different experimental groups for each month. The statistical method used and the P value are indicated. The homogeneous groups are the result of an LSD test.

| Date | Experimental groups, Average, Grouping | | | | | | | | | Test | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May 2018 | 4 | 2 | 8 | 6 | 3 | 5 | 7 | 9 | 1 | | |
| | 50.2 | 65.8 | 89.4 | 91.7 | 130.8 | 138.5 | 159.1 | 167.0 | 179.1 | ANOVA | 1.00E-05 |
| | a | a | b | b | c | c | d | de | e | | |
| Jun 2018 | 2 | 4 | 8 | 6 | 5 | 3 | 9 | 7 | 1 | | |
| | 53.4 | 56.1 | 62.9 | 63.0 | 98.4 | 103.4 | 122.3 | 134.0 | 172.9 | K.W | 5.90E-05 |
| | a | a | a | a | b | bc | cd | de | e | | |
| Jul 2018 | 4 | 2 | 8 | 6 | 3 | 5 | 7 | 9 | 1 | | |
| | 70.9 | 78.4 | 89.9 | 100.9 | 175.2 | 197.6 | 250.7 | 261.7 | 266.1 | K.W. | 1.20E-05 |
| | a | ab | bc | cd | de | e | f | f | f | | |
| Aug 2018 | 4 | 2 | 8 | 6 | 3 | 5 | 1 | 7 | 9 | | |
| | 64.2 | 67.5 | 68.0 | 74.3 | 128.8 | 138.7 | 165.9 | 185.2 | 204.9 | K.W. | 1.17E-04 |
| | a | a | a | a | b | b | bc | bc | c | | |
| Sept 2018 | 4 | 2 | 8 | 6 | 5 | 3 | 1 | 7 | 9 | | |
| | 95.1 | 97.5 | 112.4 | 122.9 | 239.0 | 242.1 | 253.5 | 268.7 | 274.9 | ANOVA | 1.00E-05 |
| | a | a | b | b | c | c | cd | de | e | | |
| Oct 2018 | 4 | 2 | 6 | 8 | 7 | 3 | 5 | 9 | 1 | | |
| | 50.9 | 51.1 | 52.6 | 55.6 | 152.4 | 152.9 | 159.2 | 159.4 | 196.2 | K.W. | 1.06E-05 |
| | a | a | a | a | b | b | b | b | c | | |

Crustacean. The average mortality of the Hyalellae during the ten days of cultivation was significantly lower ($P = 0.0034$) in the sand substrate (1.96%) compared to that of the organisms maintained in the stream sediment (3.30%). The analysis of mortality, day by day between the two types of substrate (Fig. 9), showed statistical equality during the first six days ($P > 0.05$, Kruskal-Wallis).

Regarding the comparisons of dissolved oxygen in the bioassay with *Hyalella* (Fig. 10), it was found that the average concentration was statistically higher (P = 0.0098, ANOVA), in the group with sand substrate (5.23 mg/L), compared with the stream sediment group (3.93 mg/L).

Coliforms. In all the analyzes that were done, the results obtained for fecal and total coliform counts were greater than 1,800 cells (Most Probable Number, MPN) per 100 mL.

## Discussions

**Study area.** Surface runoff in the Colomos area has historically been used as clean water by the surrounding urban settlements, but not in the case of La Culebra tributary: for years it has been discharged into the Atemajac stream. The springs present in the Colomos park have their recharge sites in the upper basin of the Atemajac stream, in the "Bajío de la Arena" nano-basin and in the rest of the valley (García-Becerra and García-Estrada, 2009). The altitude variation in Colomos is in the range between 1,527 and 1,586 meters above sea level, so the recharge sites of the springs must be located within the hydrological basin and at altitudes above the indicated range.

According to the geological models of types of springs (Manga, 2001), it is estimated that the origin of the outcrop in Colomos is due to one of the following options: due to exposure of the aquifer; due to fracture of the upper confinement layer in an area underlying the urban forest or due to the heterogeneous permeability of the soil in the area (Fig. 11).

There are still gaps in information about the exact location of the recharge sites for the water that emerges in the park. García-Becerra and García-Estrada (2009) suggest the injection of trace elements in possible sites as an alternative to specify the trajectories of groundwater flows. But the use of this method is controversial when it comes to water for urban use (Kreye et al., 1996) so other approaches should be used. These studies would also be useful to quantify the contribution percentages of the different infiltration sites and therefore to establish priorities in the establishment of protection and maintenance programs.

Following the methodology of Strahler (1964), we determined that the Atemajac stream corresponds to a “middle stream” because the order number of the runoff at the discharge site to the Río Grande de Santiago has a value of 5 (Fig. 12). García-Becerra and García-Estrada (2009) classify the Atemajac as order 7, without showing the data on which they were based, but streams with an index greater than 6 are classified as “river” (Cole, 1983). The runoffs that originate within Parque Colomos are of order 1.

# Physicochemical of water

Temperature. The water temperature variations during the study months were not statistically significant (P = 0.1128). Even so, a trend similar to what had been hypothesized was observed, but with a subsequent lag of one to two months: the maximum temperature of the stream occurred in July. The meteorological records of maximum atmospheric temperature in the GMA of the last 50 years indicate that 80% of the cases occurred in the months of April and May.

Temperature & pH. The inverse behavior of the temperature and pH showed at Figs. 2 and 3 respectively, is in accordance with the hypothesis of increase of the metabolism at the warmer season (Benjumena-Hoyos et al., 2014); the increase of the metabolism of benthic microorganisms, mainly decomposers, liberate carbon dioxide that transforms to carbonic acid. A consequence of the trend to acidify the sediments and the rest of the aquatic environment is the availability of nutrients, as will be shown in the section related to the algal growth in control site water.

Dissolved oxygen & limiting nutrient. The results of temperature and dissolved oxygen showed a parallel behavior during the study months (Figs. 2 & 4), which is striking, because from the physical point of view the dissolved oxygen levels must decrease with an increase in temperature (Goldman & Horne, 1983). Taking into account that throughout the study the limiting nutrient was phosphorus, the explanation for the increase in $O_2$ levels was due to the increase in algal productivity, propitiated by the interaction of the decrease in pH (Fig. 3), and the phosphorus bioavailability (Boyd, 1979). The lack of correspondence between dissolved oxygen levels and water temperature, contrary to what had been hypothesized according to Henry's Law, is another indicator of biological dynamism in the stream. Due to the geographical location of the stream in the intertropical zone, we believed that the limiting factor would be nitrogen (Ramos-Higuera et al., 2008), which could be true under natural conditions; but the constant dumping of the excess volume of the “lago de aves” during the rainy season causes nitrogen to be present in abundance in the excreta of the ducks, giving rise to phosphorus behaving as the element that governs the dependent primary production of photosynthesis.

Availability of nutrients. The behavior obeys the aerobic model of consumption and degradation of organic matter, followed by the conversion of ammoniacal nitrogen to its nitrate form (Gaudy and Gaudy, 1980); these processes are mediated by invertebrates and microorganisms in the sediment. The monthly averages of NH4+ (0.024 mg/L) were lower than those of $NO_2^-$ (0.026 mg/L) and in turn these were lower than those of $NO_3^-$ (6.516 mg/L) which reveals an efficient microbial action of oxidation of nitrogen and explains the reason why this nutrient was limiting in none of the months. The low values of ammoniacal nitrogen and high nitrites, which were detected in the months of May and June, can be explained by the alkaline pH values that occurred in those months, which favored the bacterial activity of *Nitrosomonas* in ammonium oxidation (Rivera-Gutierrez, 2016). Regarding the availability of nutrients in general, it had been hypothesized that it would be greater in the warm season due to the greater metabolic activity in the system (Benjumea-Hoyos et al., 2014). The discussion will focus on phosphorus, which turned out to be the limiting nutrient and therefore the one that regulates the production based on photosynthesis: the nutrient had its highest concentrations in the first two months of sampling (0.246 mg/L) and from then on it showed a gradual decrease until October (0.137 mg/L), which is explained by the dilution effect that occurs during the rainy season. The concentrations of nitrogenous nutrients and phosphate in La Culebra stream are within national and international standards for the use of clean water (see Table 5).

Table 5
Summary of nutrients and coliforms of the present study with reference to national official documents.

| Water uses | Nitrates (as N) mg/L | Nitrites (as N) mg/L | $NH_4^+$ mg/L | Phosphates mg/L | Fecal Coliforms MPN/100 mL | Total Coliforms MPN/100 mL | Source |
|---|---|---|---|---|---|---|---|
| "La Culebra" stream | 1.35 1.58 | 0.003 0.018 | 0.001 0.039 | 0.11 0.26 | ≥ 1800 | ≥ 1800 | This study |
| National Standards | 10 | 1 * | 0.5 | - | Not detectable | Not detectable | CONAGUA, 2016 |
| Water for use and human consumption | 10 | 0.05 | 0.5 | - | Not detectable | 2 | NOM-127, 1994 |
| Water for urban public use | 5 | 0.05 | - | 0.1 ** | 1000 | - | CDHCU, 2018 |
| Water for agricultural irrigation | - | - | - | - | 1000 | - | CDHCU, 2018 |
| Clean water supply | 5 | 0.05 | 6 | 0.1 | 100 | - | CCA, 1989 |
| Recreational with primary contact | 6 | - | 6 | 6 | 1 | - | CCA, 1989 |
| Agricultural irrigation | 6 | - | 6 | 6 | 1000 | - | CCA, 1989 |
| For livestock | 90 | - | 6 | 6 | 6 | - | CCA, 1989 |
| Clean water | 50 | 3 | - | - | Not detectable | Not detectable | WHO, 2017 |

Algal growth in control site water. The bioassay with the microalgae allowed the evaluation of the productive potential of the waters in the stream. As shown in Table 4, in group 2 the site water was evaluated each month. Although the production levels, indirectly measured by fluorescence units (FU), presented the lowest values in relation to the other experimental groups, the results are sufficient to evaluate the behavior through the months. The working hypothesis indicated that the growth of the microalgae would be greater in the warm season due to the increase in the metabolism of the degrading organisms and the greater availability of nutrients (Benjumea-Hoyos et al., 2014). The results are compatible with the hypothesis: there was an oscillatory increase starting in May and reaching the maximum value in September. In the month of May it began with a value of 66 FU followed by a decrease in June to 53 FU. In July it rose to 78 FU and in August it fell to 68 FU. In September the maximum value of 97 FU was achieved and from there was a decrease to 51 FU in October. In Fig. 2 it can be seen that the maximum temperature of the water in the stream was maintained in the interval from June to September, favoring the processes that lead to algal growth. In October there was a decrease in temperature and in turn a proportional drop in algal growth.

Inhibiting factors. The evaluation of inhibition factors through the use of EDTA as a chelating agent was a decision based on the protocol of Miller et al., (1978) and we really did not expect to find this type of evidence, considering the apparently good state of the ecosystem. In the results of Group 6 (site water + EDTA) in Table 4, the favorable and proportional effect of algal growth is observed in time: in May a value of 92 FU was obtained followed by a value of 101 FU in the month of July and finally a value of 123 FU in the month of September. Since the additions of EDTA were always constant (1.00 mg/L of Na2EDTA), it can be assumed that the inhibition agent decays over time, possibly due to the action of dilution through the rainy season. This same line of thought suggests that the agent causing the inhibition is not caused by the runoff, but rather an underground contribution. The Miller et al. (1978) protocol is not designed to characterize the agent causing the inhibition; The most that could be suggested is that it is an ionic agent, possibly metallic, which is why the present study sets the guidelines for subsequent analyzes that allow elucidating both the identity and the concentration of the inhibiting agent. The same study should be of interest to the local authorities in charge of extracting clean water from the Colomos Forest and distributing it to the surrounding neighborhoods.

Crustaceans. The higher mortality of *H. azteca* found in the group with natural sediment from the stream was contrary to what had been hypothesized, but it is explained by the respiration of the benthos community: during the determinations of the % survival of the amphipods, the presence of of a community of macroinvertebrates such as: annelids, gastropods, copepods and flatworms. Benthic community respiration is evident by lower oxygen levels in the sediment group (3.93 mg/L) compared to the sand substrate group (5.23 mg/L). The discovery of a dynamic community of macroinvertebrates in the sediment opens a line of research that had not been proposed before for La Culebra stream. Rodríguez-Barrios et al., (2011) in their study on functional groups point out the need to integrate information to obtain ecological models on the structure of the community and the role of macroinvertebrates in fluvial systems. The role of macroinvertebrates in the sediment is associated with that of the mineralization of organic matter (Oseguera et al., 2016).

Coliforms. The standards for evaluating the sanitary quality of water in Mexico require the quantification of total and fecal coliforms (CONAGUA, 2016a, Barrera-Escorcia et al., 2013), and the Federal Law of Rights (CDHCU, 2018) establishes as permissible limits the values of 1000 NMP/100 mL, of fecal coliforms for urban public use and for agricultural irrigation. The rules are different in each country; In Europe, intestinal enterococci are also evaluated (DOUE, 2006), which are associated with warm-blooded organisms and therefore help to discriminate the origin of the contamination. According to the guide for the assessment of the quality of drinking water of the World Health Organization (WHO, 2017), coliform organisms are excreted by both humans and animals and have the ability to live and multiply in natural waters, especially when there is presence of biofilms; For this reason, it is indicated that they are not useful to evaluate contamination by fecal pathogens and that their use as indicators is more valuable to evaluate the cleanliness and integrity of water distribution systems.

Due to the fact that there is usually a positive correlation between sewage discharges and nutrient concentrations (Burford et al., 2012), and to the low values of nitrogen and phosphorus in the water, we believe that the origin of the coliforms detected in La Culebra stream is originated by the fauna present in the park, especially by birds, and by the presence of biological conditions that favor their permanence, such as the benthic community of the periphyton, especially present in the upper part of the stream.

To complete the sanitary characterization of the stream, it is suggested to make specific studies of enteric bacteria of importance, such as Salmonella and Shigella. Studies should be done on groundwater to rule out leachates originated from broken drainage pipes and also from biofilm scrapings to confirm the new hypothesis about the natural origin of coliforms.

# Declarations

**Availability of data and material.** Upon request from the authors.

**Author's contribution.** KACG and JLZA conceived of and designed the study and performed field and labwork. KACG and JLZA made the statistical analysis, wrote the main manuscript text. KACG made the figures. JLZA made the tables. KACG made the Spanish to English translation.

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**Ethics approval.** Not applicable.

**Concent to participate.** Not applicable.

**Concent for publication.** The authors give concent to publish this work in Urban Ecosystems, if accepted.

**Conflicts of interest/competing interests.** The authors declare that they have no conflict of interest.

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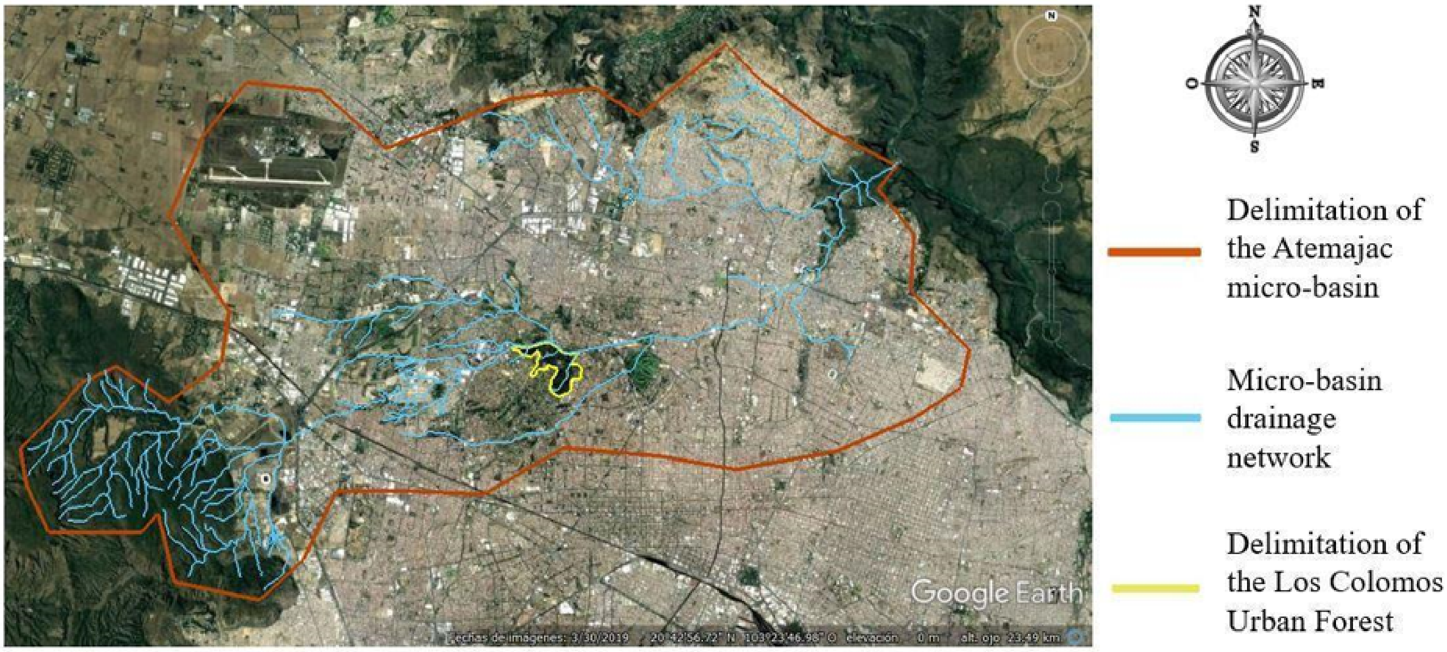
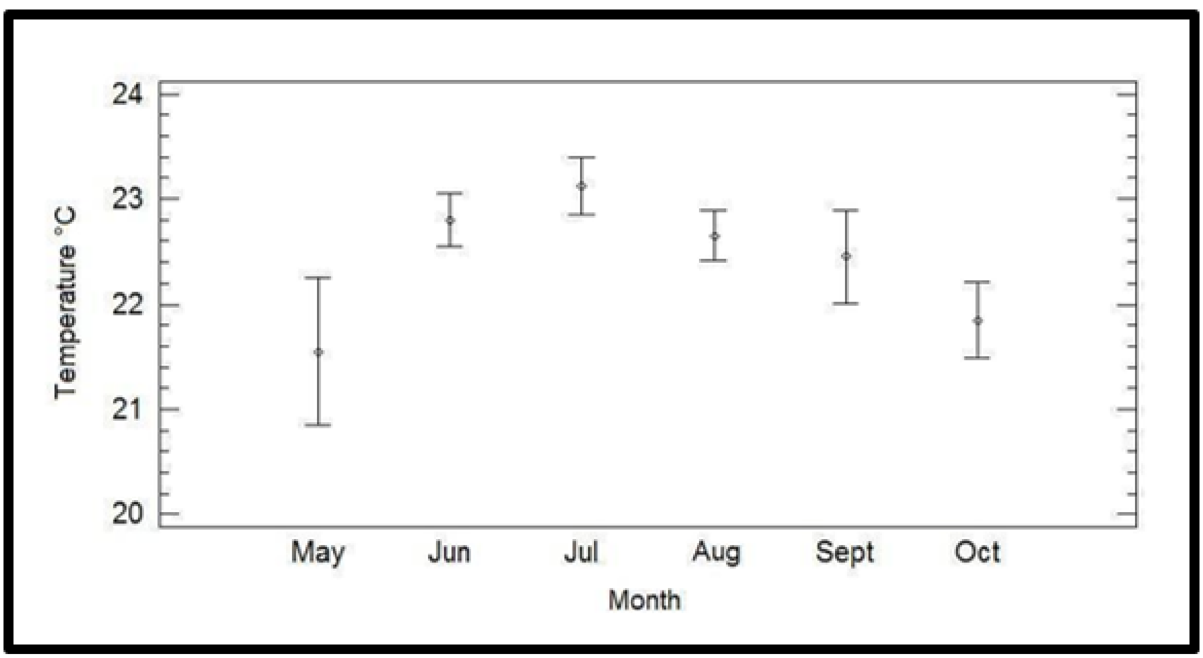
# Figures




## Figure 1

Google earth image showing the delimitation of the Atemajac micro-basin with brown, representation of the drainage network with blue color, and the delimitation of LCUF with yellow




## Figure 2

Average values and standard errors of temperature

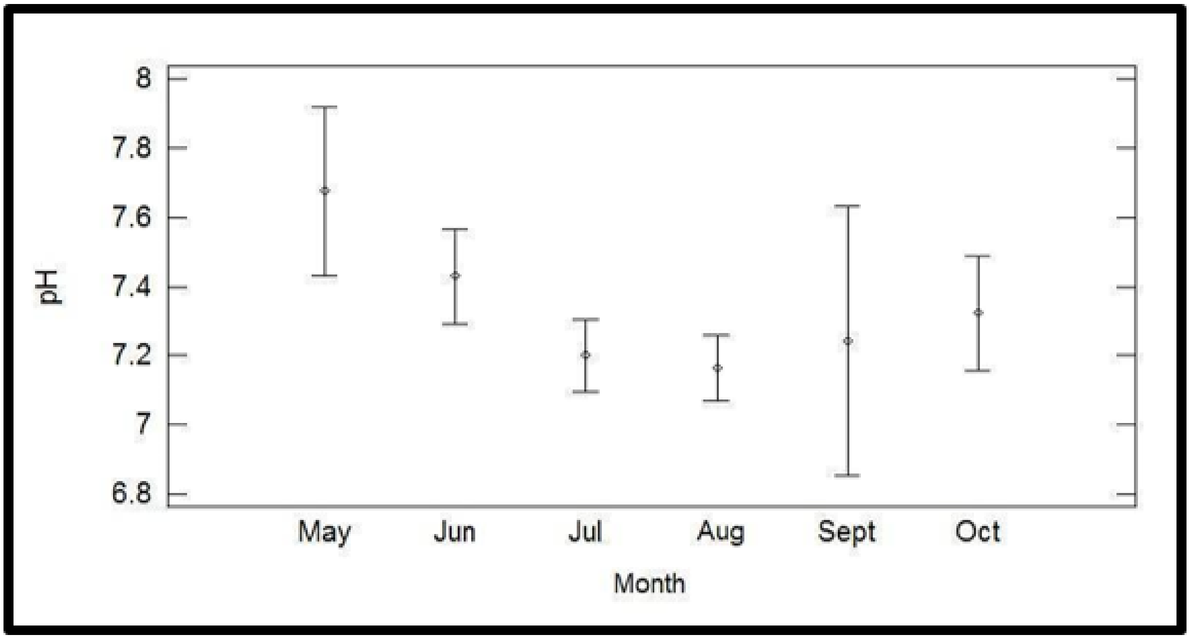



**Figure 3**

Average values and standard errors of pH

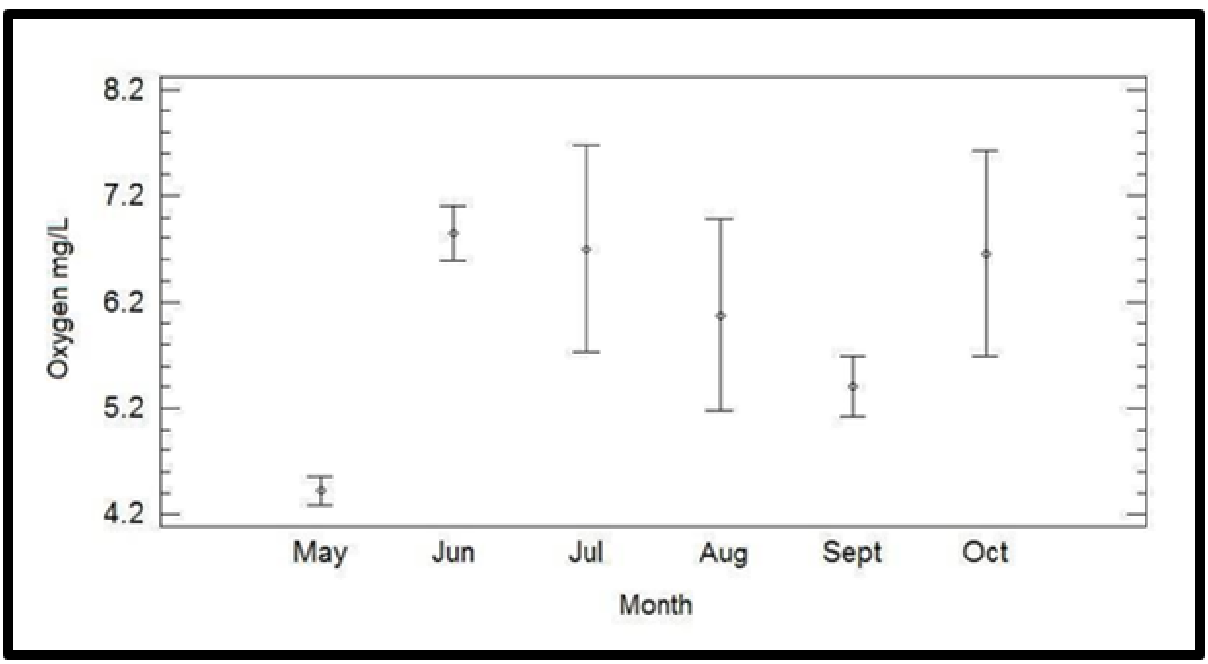
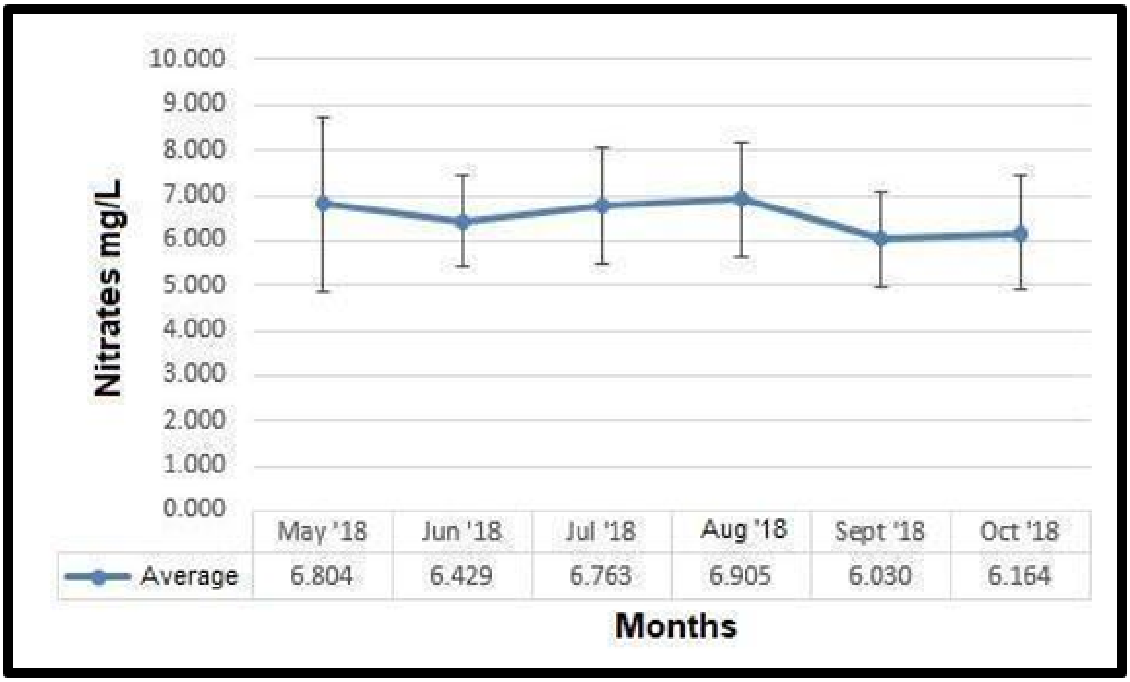


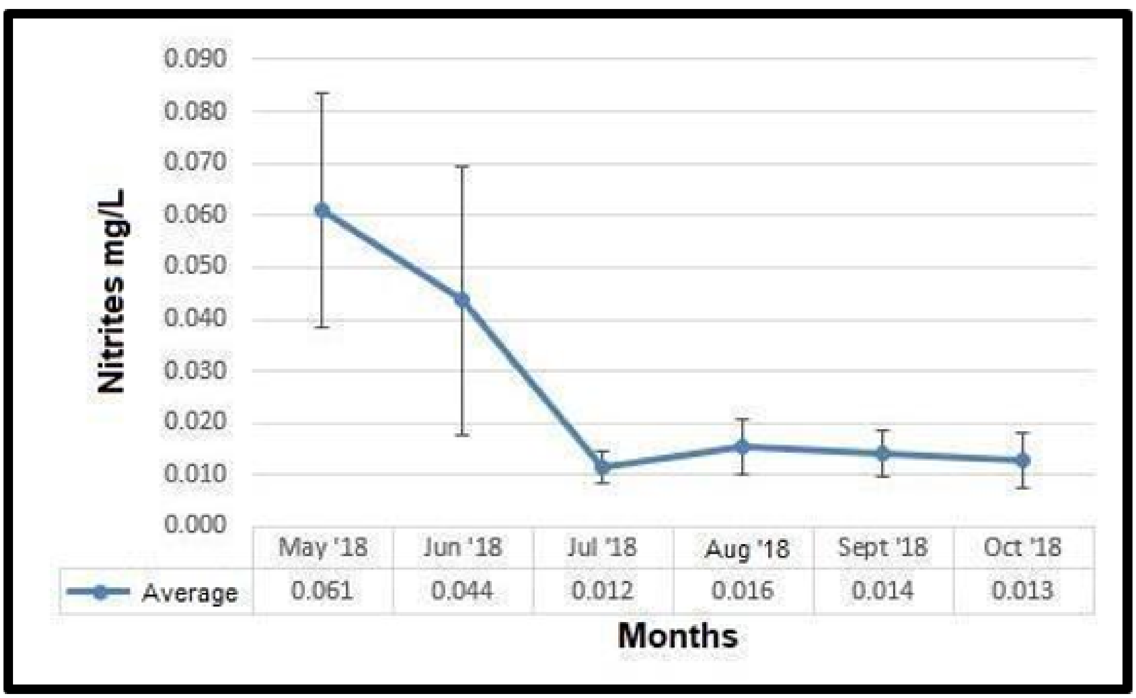

**Figure 4**

Average values and standard errors of dissolved oxygen



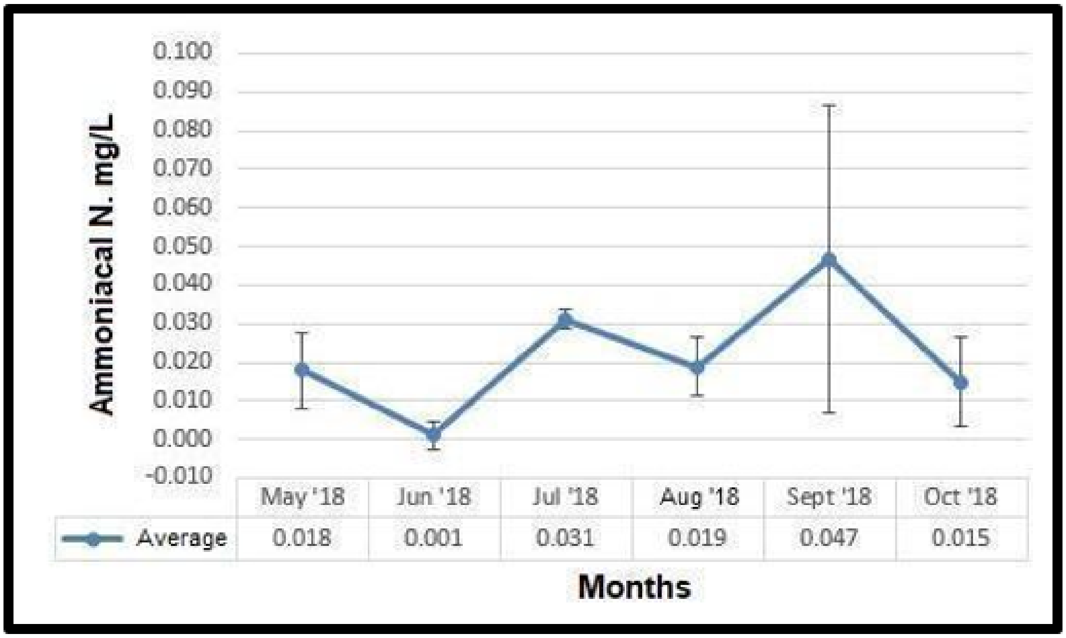

## Figure 5

Average variation and standard deviation of nitrates.



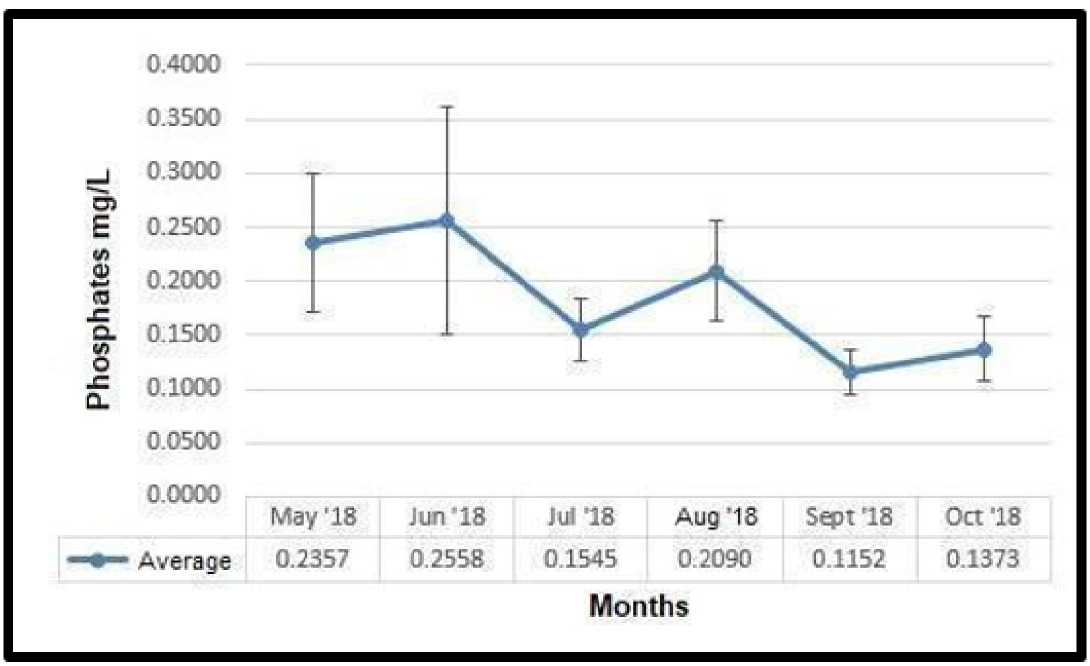

## Figure 6

Average variation and standard deviation of nitrites.




## Figure 7

Average variation and standard deviation of ammoniacal nitrogen.




## Figure 8

Average variation and standard deviation of phosphates.

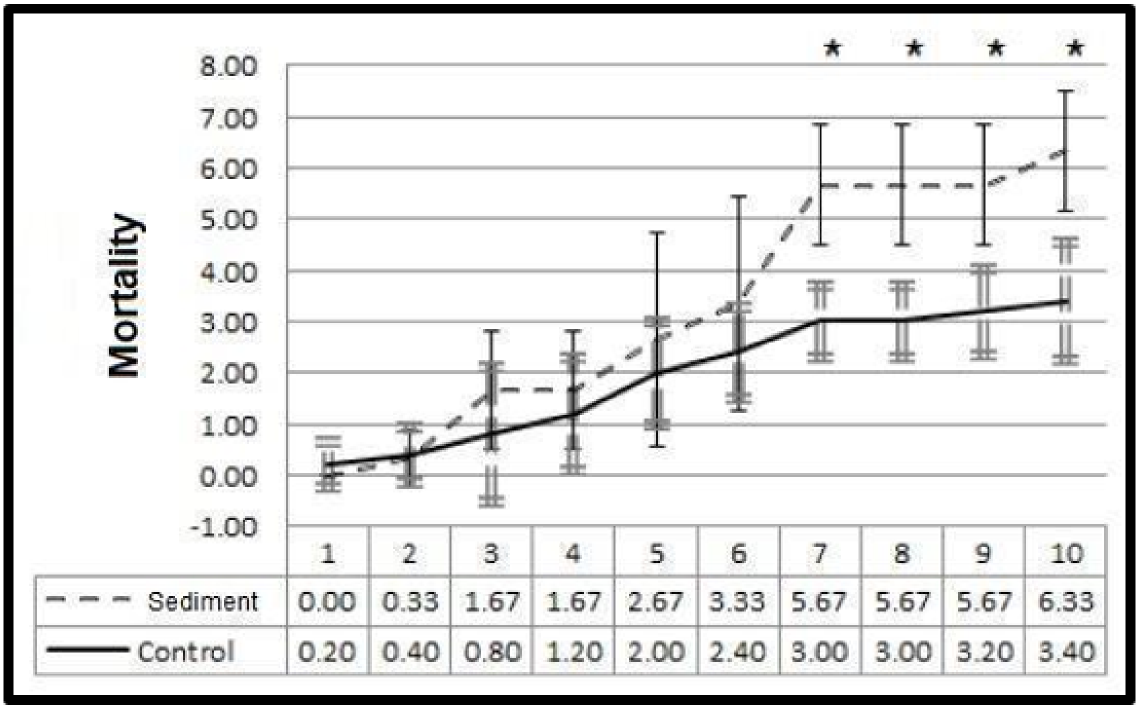



**Figure 9**

Average values and standard deviation of mortality between the evaluated groups.

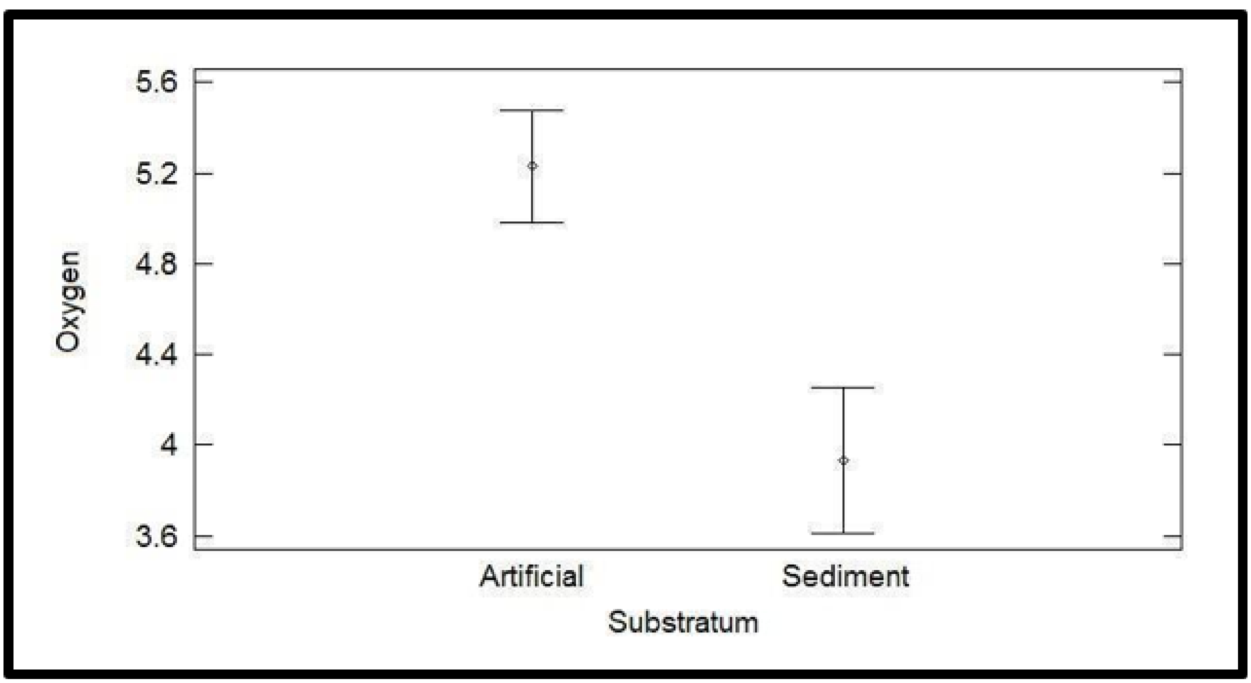



**Figure 10**

Dissolved oxygen concentrations in the different bioassay substrates.


**Figure 11**

Google earth image showing the delimitation of LCUF with yellow, representation of the drainage network with blue color, and correction of La Culebra stream within the LCUF in white.

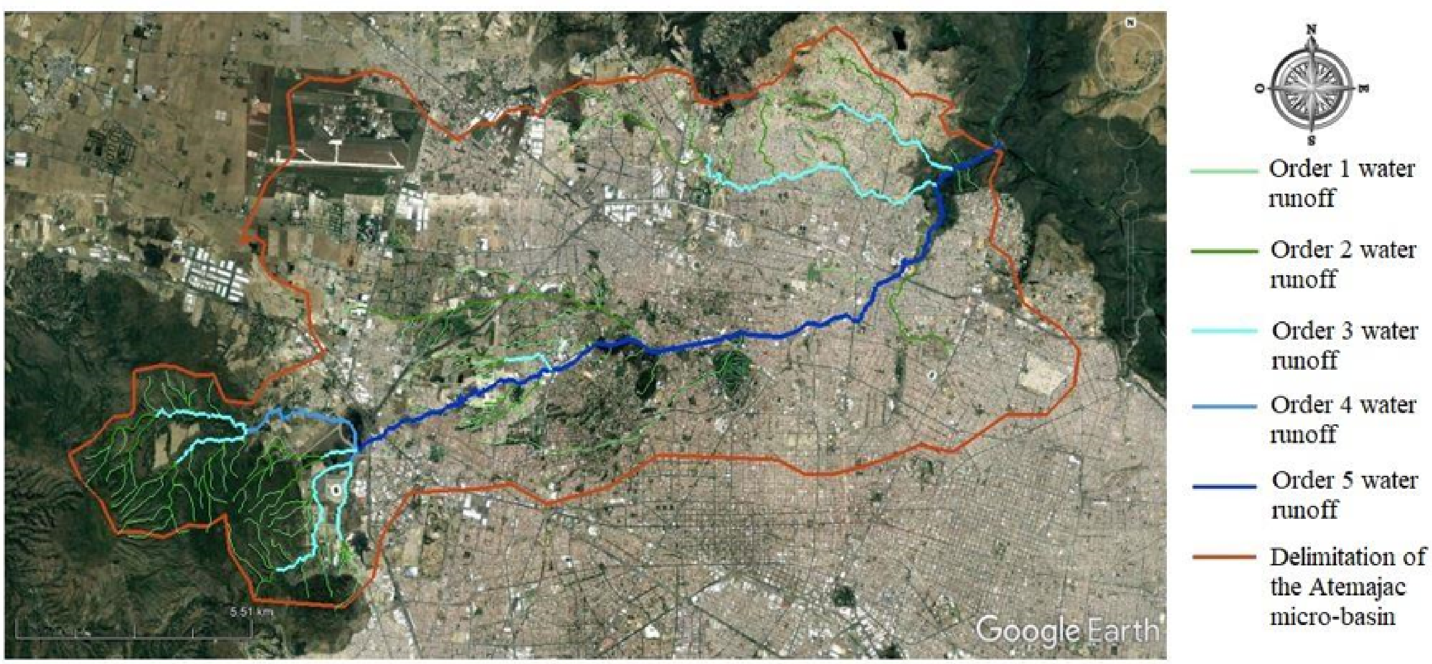



**Figure 12**

Google earth image showing the classification of the drainage network of the Atemajac stream based on Strahler's criteria (1964), marking runoff of different order in colors: order 1 in light green, order 2 in dark green, order 3 in light blue, order 4 in medium blue, order 5 in dark blue. The border of the micro-basin in brown color.