Characterization of physico-chemical parameters of water with the metabolism of cultivable bacteria in the Rochester School's artificial reservoir

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Abstract

Small reservoirs are prevalent landscape features that affect the physical, chemical, and biological characteristics of headwater streams and land around them. We reported a four months characterization of the artificial reservoir of Rochester School in Chia, Colombia. The site was sampled weekly for water quality parameters (pH, dissolved oxygen, turbidity, alkalinity, total phosphates, total nitrate with the use of protocols proposed by GLOBE and the analysis kit for water La Motte). The most fluctuated parameters were the concentration of nitrates and dissolved oxygen. Procedures for cultivating and characterizing bacteria and their metabolism were made, finding 23 isolates with both fermentative and oxidative metabolic capacities, low motility and great capacity to reduce nitrates, possibly as an adaptive measure to changes in chemical conditions of water and nutrient availability. All of this in order to first know the properties of the sample site and in a future evaluate whether or not it was possible to have complex animals.

General objective

Correlating variations in the physico-chemical parameters of the water in the presence of cultivable bacteria that are in the reservoir school.

Specific objectives:

Evaluating physico-chemical parameters of pH, concentration of phosphates, nitrates, dissolved oxygen, alkalinity, conductivity and turbidity using kits La Motte.

Cultivating and isolating the culturable bacteria present in the reservoir by seeding exhaustion.

Characterizing the morphology and metabolism of the bacteria isolated by Gram staining and biochemical tests.

Introduction

In order to develop this project, we analysed an artificial reservoir located on Rochester School, Chia, Colombia through the timespan of six months and took weekly samples of various physical and chemical properties. The purpose is to characterize the physical chemical parameters and the metabolism of the microorganism that live there so to guide future studies to the evaluation of the possibility of having superior organisms, either plants or fish, living there. The properties analyzed were the following: alkalinity, which is defined as the water's Buffer capacity or its ability to receive protons, representing the addition of the bases that can be titrated in a water sample. Dissolved oxygen, which measures the amount of molecular oxygen in the water, but not the amount of oxygen itself in the water. The turbidity of a water body, which refers to how clear or how murky the water can be, therefore, clear water has a lower turbidity while cloudier water has a higher level of it. The pH, defined as the measurement of the hydrogen ions in a solution and it indicates the acidity or alkalinity of it. It is a parameter to be considered when determining the characteristics of water body and can define which organisms could live there. Phosphates, in which phosphorus is one of the elements that plants require in relatively large quantities, which are essential because they are a part of the energy carrying phosphates, phospholipids, nucleic acids and several essential coenzymes. Nitrates, which are an inorganic form of nitrogen which play an important role in an ecosystem as their properties come to serve as nutrients for plants, microorganisms and other living beings that can be found in water. And finally the metabolism of the bacterias present in the aforementioned water body, which according to a study conducted by Xavier Mora 2012, was measured by the Gram stain technique, that is a type of differential staining used in microbiology that allows bacteria to be differentiated quickly and easily according to their morphological characteristics separating them into two groups: Gram+ and Gram-. The development of these studies are very important since we believe that they contribute to the sustainable development of the area by: preserving a high biodiversity in wild flora and fauna (even migratory species), offering protection against erosion, mitigating the floods, favoring the recharge of underground water, the purification the of water and stabilizing the the local temperature and rain conditions (Pérez-Castillo, et al. 2008). Having in consideration that our school promotes the conservation of the native biodiversity, we can say that is also of dire importance to the school.Once the research and analysis are finished, our goal is to be able to answer what type of organisms are ideal to live in the experimented location and why. This would be helpful because then we would be able to conduct a future research where we can conclude why those species are the best suited and then change the conditions in which these organisms live under. Other than investigation contributions, later on we could see into expanding this region's wildlife and modifying its parameters so more developed animals such as fish or frogs can make this artificial reservoir their new living environment.

Methodology

The following Figure 1, shows the location of our school where we measured the physico-chemical parameters,this location as seen is located in the outskirts of Bogotá, near the municipality of Chía with coordinates : Latitude 4.82, Longitude -74 with an elevation of 2612 m. In this environment is common a cold weather with variances of hot temperature in the midday.

Figure 1. Spatial view of Rochester School, a) In the circle is shown the location of the reservoir at Rochester School

To carry out the measurement as always we had in mind our goals to measure both the physical and chemical parameters which in turn will serve us later to understand how they acted upon these organisms in the environment and the parameters and microorganisms. To achieve this weekly measurements of physico-chemical parameters such as : hydrogen ion concentration (pH), concentration in parts per million (ppm) of nitrate, phosphate, dissolved oxygen (DO), alkalinity, electrical conductivity and turbidity with a Secchi disk. This as proposed by the Water Quality Monitoring Kit La Motte code 5870 and recommended by The Globe Program time protocol. With this information besides also performing titrations for both the dissolved oxygen measurement as for alkalinity, the modified Winkler method was used to measure OD and alkalinity titrations were performed using readings turn phenolphthalein (P) and total alkalinity (T) in terms of calcium carbonate $CaCO3$. The detection ranges titrations were 0.2 to 10.0 ppm (intervals of 0.2 pmm) and 0.0 to 200 ppm respectively. Colorimetric tests also using parameters such as pH, nitrates and phosphates were measured. pH was considered a range from 3 to 10; nitrate concentration was analyzed by the modified method and finally the phosphate concentration were determined from the different shades of blue generated by reacting ascorbic acid with different phosphate concentrations present in the sample, both nitrate to phosphate ranges from 0.2 ppm handled - 1.0 ppm (Figure 2).

Figure 2. Sampling and Measurement of physico-chemical parameters

These measurements described above (with its data entry in Figures 4A and B have an important use in understanding aspects of microorganisms and organisms in general living or can live in this environment, in our case specifically focuses on microorganisms as these serve as an integral measurement to understand that substances or elements they can lead to the expansion of this. Therefore we rely on research on the taxonomy of bacteria and studies conducted worldwide on the subject to measure the characteristics of the bacteria found in the reservoir and compare the type of bacteria that were the results of the parameters physico-chemical. Used to measure the growth of culturable microorganisms reservoir which a soil sample was allowed to dry at room temperature, 10 grams of this soil was taken and resuspended in 90 ml sterile peptone water 0.1% was taken. A resuspension were performed serial dilutions of 1 ml resuspension 9ml peptone water until a dilution of 1x10-21 and finally were seeded in a petri dish with agar plate count (APC) the last five dilutions and incubated at 37 ° C for 8 days. This process culminated in a demonstration and / or separation of each type of bacteria which were tested, all using the process of staining bacteria in positive and negative grams, which separates into two groups according to their morphological characteristics (Zapata, H. A. V. 2004).

Each colony grown in petri dishes were isolated and labeled and characterized by gross morphology using a stereoscope considering parameters such as shape, and edge lifting and microscopic morphology was performed by Gram stain (Figure 3 and 5) (Zapata, H. A. V. 2004).

Figure 3. A) Macroscopic morphology for bacterial colonies and B) cell morphology for bacteria .

A) B)

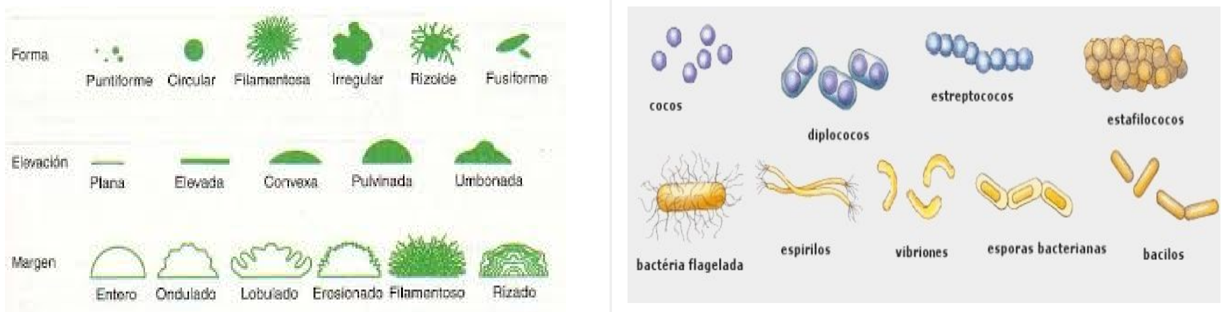


Figure 4. A)Data entry menu in GLOBE website and B) Example of pH data entry

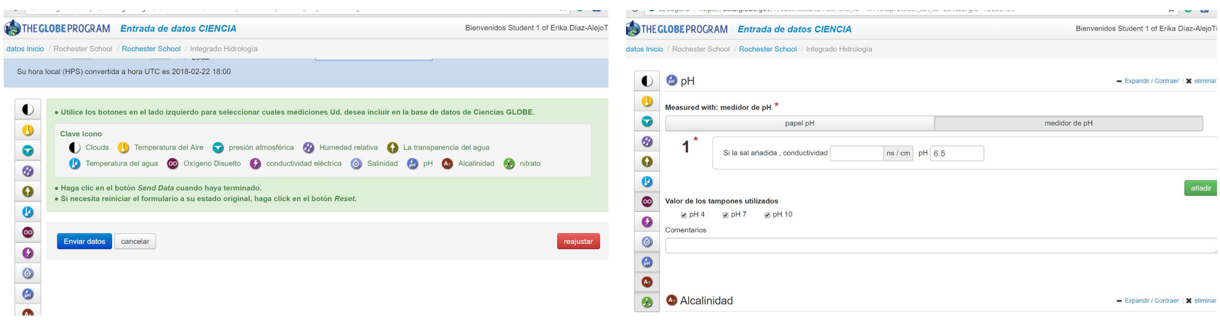
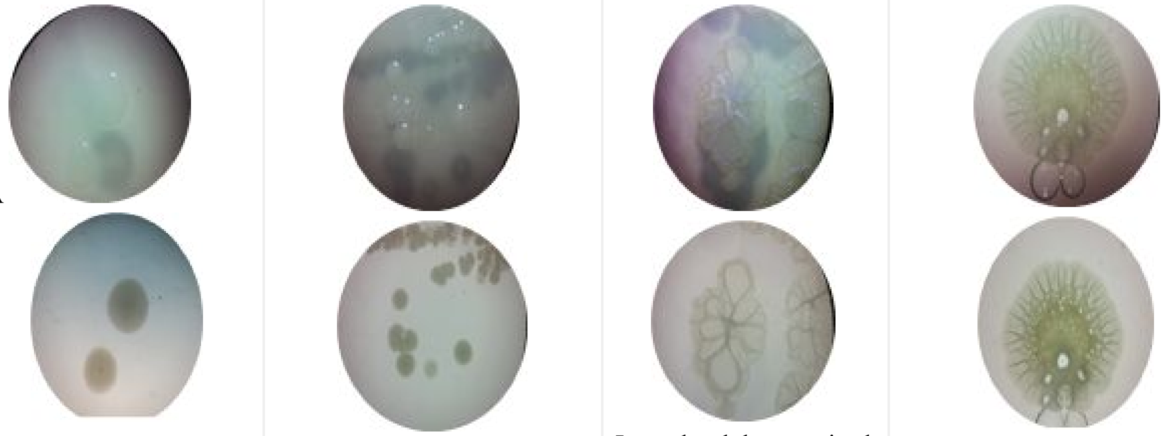


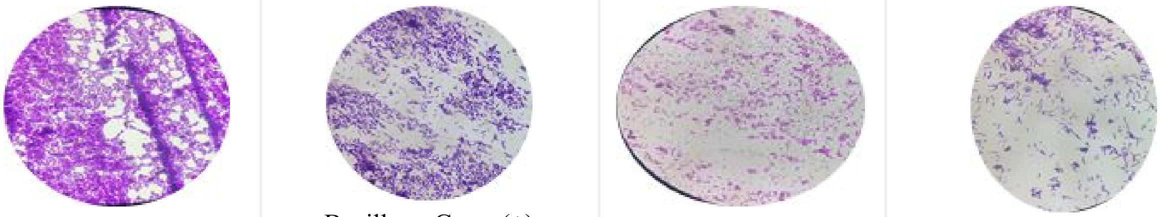
Figure 5. A) Main macroscopic features of bacterial Culture, wole colony, edge and elevation and B) Gram staining and cell morphology of 23 bacterial aislations in the reservoir

A



Circular, entire, convex | Punctiform,entire, umbonate | Irregular, lobate , raised | Filamentous, filamentous, raised

B



Coccus - Gram (+) | Bacillus - Gram (+) | Bacillus - Gram (-) | Bacillus - Gram (+)

Results and Discussion

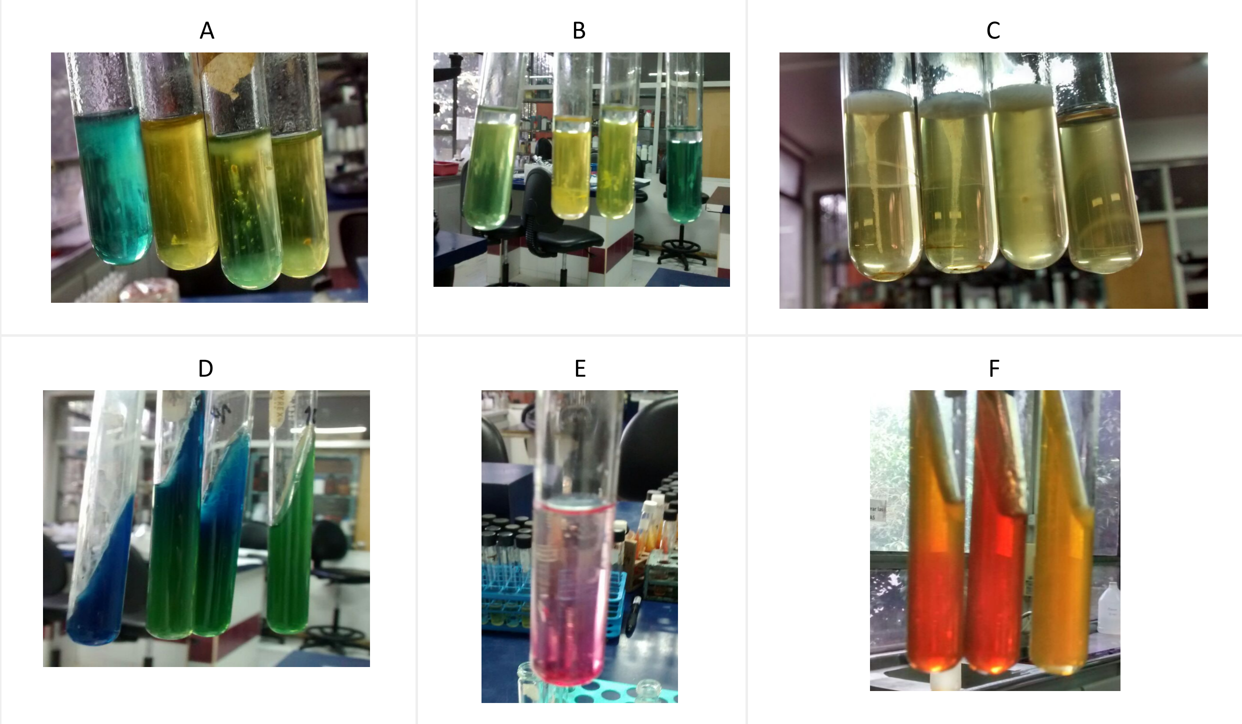
The results observed in this work are the average of triplicate data of each of the physico-chemical and biochemical tests. The data was added through the app and through GLOBE's official webpage (Figure 4). We consider as a source of error the fact that some data wasn't collected (during December) due to school vacation. In general, the data was taken fridays of every week around noon, but sometimes that hour wasn't possible for academic compromises.

Table 1. Results of physico-chemical measurements

Date	Nitrates (ppm)	Dissolved Oxygen (ppm)	Alkalinity (ppm)	Phosphates (ppm)	pH	Turbidity (cm)
17/11/17	1	4,6	40	0	7.5	25
1/12/17	2	3	60	0,2	6	25
11/12/17	2	3,4	78	0,2	7	20
14/12/17	10	4,9	58	0,2	6.5	25
12/01/18	4	3,4	76	0,2	7	30
17/01/18	10	2,3	20	0	6	25
19/01/18	10	3,2	64	0,2	7	22
02/02/18	8	3.6	124	0	6.5	20
22/02/18	8	4,1	78	0.2	6	10

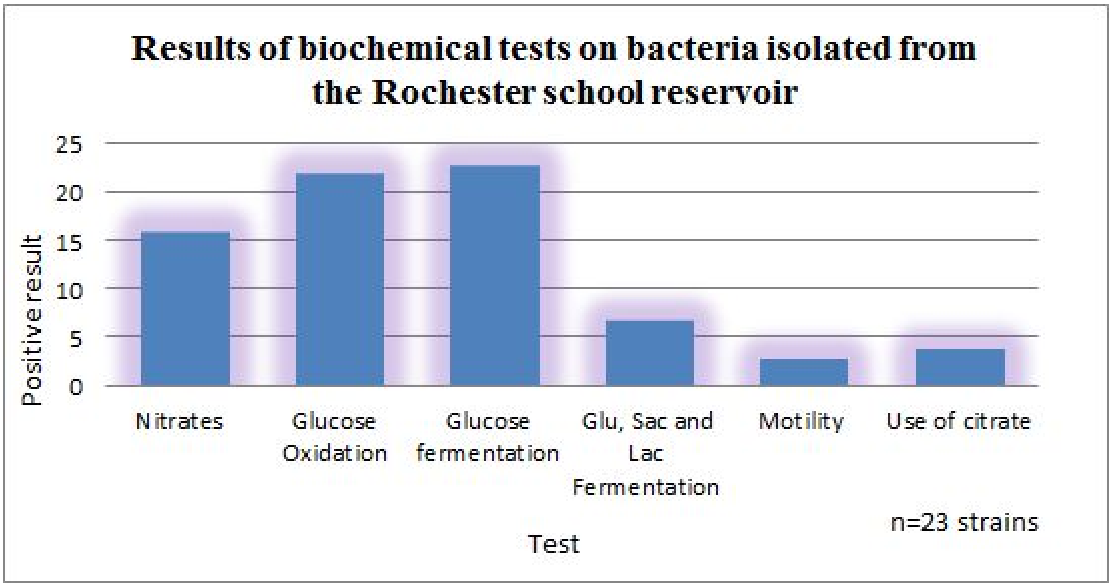
In the characterization of the culturable bacteria of the reservoir, 23 bacterial strains were isolated whose macroscopic morphology oscillated between the punctiform, circular, irregular and filamentous forms. The elevations were convex, umbilicated and elevated, regarding the margins, there were found the following types: whole, lobed and filamentous. Regarding the Gram stain, it was evidenced that the majority (20 out of 23) were Gram positive and 3 were Gram negative (Figure 5).

Figure 6. Results of biochemical test A) Glucose oxidation, B) Glucose fermentation, C) Motility test, D) Nitrate reduction capacity E) use of citrate as the only carbon source and F) Glucose, lactose and sucrose fermentation



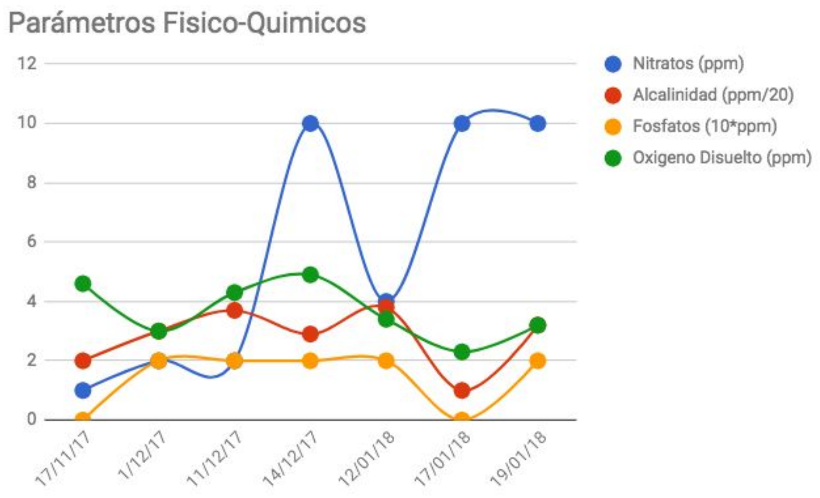
In the Figure 6, it can be observed the reading of the biochemical samples, which relied on the preparation of a specific method of cultivation. For the test, it was used a positive control and one negative, in most cases the change of coloring indicated a positive result and in the case of motility samples, the turbidity of the sample tube was evidenced. The results of the metabolic analysis aspects such as nitrates reduction, the use of citrates as the only source of carbon, sugar fermentation (glucose, sucrose and lactose) were evaluated as well as the use of glucose as carbon for fermentative ways as for oxidative, as observed on the Graph 1, most bacteria use some as carbon sources, such as lactose, sucrose and glucose not only for oxidative but also fermentative methods, demonstrating the versatility and adaptive capacities concerning the fluctuations in quantities of substrates in the reservoir.

Graph 1. Results of biochemical tests on bacteria isolated from the Rochester school reservoir



As for the physico-chemical samples of the reservoir's water observed (graph 1 and table 1) we can see for example that the average amount of nitrates found in the reservoir was approximately 6 ppm, 10 ppm being the highest value and 1 ppm the lowest. We can see that after the fourth data recollection, that is to say since mid December, the concentration has been increasing, though not constantly. Usually this parameter is very fluctuant in water bodies, the contribution of different forms of nitrogen in water bodies, for the best part of it are of anthropogenic type, whether it is for domestic wastes, the use of fertilizers and so on. In our case, the land where the school has been built counts with an inclination that allows all superficial water and rain to get to the reservoir, which could drag substance rich in nitrogen, which can be converted in simpler forms such as nitrates and nitrites by bacteria actions, which collaborates with the evidence in the analysis of the bacterial metabolism (Graph 1). Another factor to keep in mind and that we have observed is that with the decrease of rain and water levels in the reservoir, this compounds tend to increase in the water body (Graph 2) (Pacheco Ávila, et Al. 2002). The high concentrations of nitrates create an anoxygenic environment, the concentrations over 0,9 ppm tend to stimulate the growth of seaweeds and indicate eutrophication, values higher than 20 ppm indicate a high contamination rate in the water body. (Pérez-Castillo, et al. 2008),(Peña, O.S et al. 2006).

Graph 2. Results of the physico-chemical Parameters in units of ppm



Dissolved oxygen like nitrates are considered to be an indication of contamination by organic matter due to the residual flushes, either domestic or industrial, in water bodies. The low concentrations of dissolved oxygen can be located where the organic matter is decomposing, which means that bacterias use the oxygen to decompose waste, they are also low in warm water of slow movement. The waters with dissolved oxygen concentrations above 4,1 mg/L are considered to be of good quay, in our study, the levels of diluted oxygen were between 3 and 4,9 mg/L the which affects the life of aquatic organisms that require it to live (Peña, O.S et al. 2006). In the year of 2017, a Rochester School student did a project of the evaluated parameters between her own work and the Fúneque Lagoon, place where a fish species in danger of extinction, Capitán Sabana lives. Once observed that there wasn't a significant difference between these two evaluated places, it was concluded that those type of fish could live in our reservoir. (Rodríguez, P.V. 2017)

We observed that the decrease in the amount of dissolved oxygen is connected to the increase of the nitrate concentration in the reservoir (Graph 2 and table 1), this goes also to a microbiological level, where the bacteria cultivated showed having both metabolisms (oxidative and fermentative) (Graph 1). Although the levels shown in the reservoir aren't alarming, it is necessary to keep monitoring and studying ways to improve said parameters since it has been documented that the decrease of dissolved oxygen not only affects the nitrates but also the concentrations of manganese hydroxide, iron and sulfates. The fact of propitiating the reducing conditions in the environment can be toxic for fish and superior organisms, in addition these reduced compounds maintain the anoxic sediment-water interface and prevent the accumulation of layers of iron hydroxide. With the loss of iron in the water that covers it, the capacity of retention of phosphorus of the sediment decrease. The previously mentioned can also explain the results we obtained. The results given for phosphates were considerably constant throughout the data recollection (They had a range of 0-0,2 and its average was 0,2 ppm) (Friedl, G., & Wüest, A. 2002)

As shown in the results, the levels of transparency varied between 22 cm to 30cm. The average transparency was of 24.6 cm. We used a handmade secchi disk to make this measurements. The average alkalinity levels found were of 56 ppm, the highest value being 78 ppm and 20 ppm being the lowest. We can probably infer that the lowest value (20ppm) may be an outlier given its difference from the other data. Alkalinity is a measure that has a direct relationship with pH as both measure the changes of acids in water, extreme changes in Alkalinity have a direct impact towards the pH levels; as a higher alkalinity level in water will help prevent mayor impacts changes in pH levels, and as consequence help the propagation of life as it helps protect the environment from these great changes. Alkalinity is also determined by the soil and terrain that surrounds the body of water as its components can alter the water's chemicals when diluted. (Mr. Brian Oram, P. 2018)

The pH of reservoir showed to have a range between 6.0-7.5, though the average pH levels were of approximately 6.7. are almost neutral and could indicate that it is fit for holding life of other organisms in the reservoir, since the pH is considered as an indicator of the water quality in general, how much it is being affected by external agents. The changes in pH can indicate the insertion of fertilizers, particularly when continuous measures are registered along the conductivity of the water body and, eutrophication processes, if associated with the photosynthesis process and algae respiration. Furthermore, the pH affects the toxicity of some compounds such as the amoniac when controlling its ionization, like the biological availability of certain contaminants, as the heavy metals. Values higher than 9.0 and lower than 5.8 produce limitations in development and physiology of the aquatic organisms. (Pérez-Castillo, et al. 2008).

Conclusions

- During the measurement time the amount of dissolved oxygen decreased progressively while the nitrate concentration was increased, this tendency was also observed that was linked to increased temperature, which is crucial for the retention of oxygen in water and although oxygen levels did not drop drastically their levels at the moment are in the upper limit for any organism can live in our reservoir.

- Parameters such as phosphates, pH and alkalinity showed no significant variations with time and changing weather conditions, both pH and the alkali showed no dependence on the other parameters analyzed, and showed that the reservoir is feasible to support life other organisms. the low concentration of the phosphates as mentioned above may be related to the presence of nitrates although further studies to determine why the low phosphate concentrations.

- Bacteria isolated and characterized shown to be in its large majority Gram positive , both oxidative metabolic capacities and fermentative, and preferential use of carbohydrates against substrates such as citrate, also showed a high capacity to reduce nitrates to nitrites, and this could show how the excesses of available organic matter is utilized by microorganisms and why this parameter is so fluctuating.

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