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In vitro testing of the efficiency of *chlorella vulgaris* in the removal of organic load in contaminated water using different light source conditions

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ABSTRACT

The present study aimed to use Chlorella vulgaris as a biological alternative to remove organic load present in stream and bog water. For this purpose, bioaugmentation of *Chlorella vulgaris* and in vitro evaluation of organic load in stream and swamp water was carried out using Chlorella vulgaris biomass subjected to different light sources. The results obtained after the evaluation of the organic load of the samples analyzed (marsh and stream water), show that the treatment (T2) with *Chlorella vulgaris* using artificial light source presented a percentage of organic load removal represented in BOD_5 of 56.5% of the marsh water. Meanwhile, for the stream water sample test, it was observed that the maximum percentage of removal for the treatment (T6), where *Chlorella vulagaris* was used in dark growth. This test demonstrates the capacity of *Chlorella vulagaris* after 10 days to remove organic load in the form of BOD_5, which shows the capacity of microalgae to carry out photosynthesis and position themselves as primary producers in the trophic chains, becoming an ideal organism to remove organic pollutants from wastewater.

Key words. Microalgae, wastewater, organic load, BOD_5, removal, phycoremediation.

1. INTRODUCTION

According to Brennan and Owende (2010); Stager, (2012), the presence of climate change at the planetary level is currently undeniable. Likewise, as stated by Chiu et al. (2011); Stager, (2012), the dimension of this phenomenon is such that it forms part of the political agenda of states and international organizations, (2011); Stager, (2012), climate change involves, among others, droughts, floods and/or acidification of the oceans, both locally and globally, with human activity being the main culprit, especially the combustion of fossil fuels (Olguín

2003, Chen et al. 2011, Chiu et al. 2011, Stager 2012), which is responsible for 80% of energy production (Chen et al. 2011).

With the ongoing demographic expansion and industrial development, the demand for water is steadily increasing. This increase puts significant pressure on existing water resources, pushing towards the use of sources that may be subject to contamination or vulnerable to industrial pollution, with important consequences for human health and the environment. Therefore, water and wastewater quality monitoring is essential to identify pollutants, assess their impact and implement measures to minimize pollution, ensuring the security of water resources.

According to García et al., (2015), industrial activity is generating waste discharges to water bodies influencing their quality, due to poor or absent treatment of their wastewater. The composition of wastewater is a reflection of society's lifestyles and production technologies.

Chlorella vulgaris is a single-celled, non-motile alga of the class Chlorophyceae. It has a single cup-like chloroplast with or without a pyrenoid and a single small nucleus. Its mode of reproduction is asexual, with each haploid cell dividing mitotically two or three times to give rise to four to eight non-motile cells. Cellular organization in these microalgae is in colonies, forming a cellular aggregate (Silva, 2006). Their growth cycle is rapid, as they are autotrophic microorganisms and, through photosynthesis, they transform solar energy into chemical energy; their reproduction is asexual, division of a single cell or fragmentation of a colony. The productivity of microalgae is determined by parameters such as light, nutrients, temperature, pH and salinity (López, 2017).

One of the parameters of this monitoring is biochemical oxygen demand (BOD_5), which helps to assess organic pollution levels and to protect human and animal life from harmful pollutants. Knowing BOD_5 is essential for assessing water and wastewater quality, as it reflects the microbial activity and biodegradability of organic pollutants (Lecca et al., 2014).

In the present study, the microalga *Chlorella vulgaris* was used, as it presents rapid growth, high photosynthetic power, easy adaptability and low cost, resulting in a good alternative for obtaining biomass, nutrients, to reduce the amount of BOD_5 present in wastewater from a stream and from the discharge point of this to the mouth of the swamp in the municipality of San Benito Abad in the department of Sucre, Colombia under three sources of light (natural light, artificial light and darkness).

2. MATERIALS AND METHODS

Sampling. Water sampling was carried out in the marsh ecosystem and in the stream that discharges its waters into the water body of the marsh in the municipality of San Benito Abad. The sampling methodology was carried out as described in figure 1.

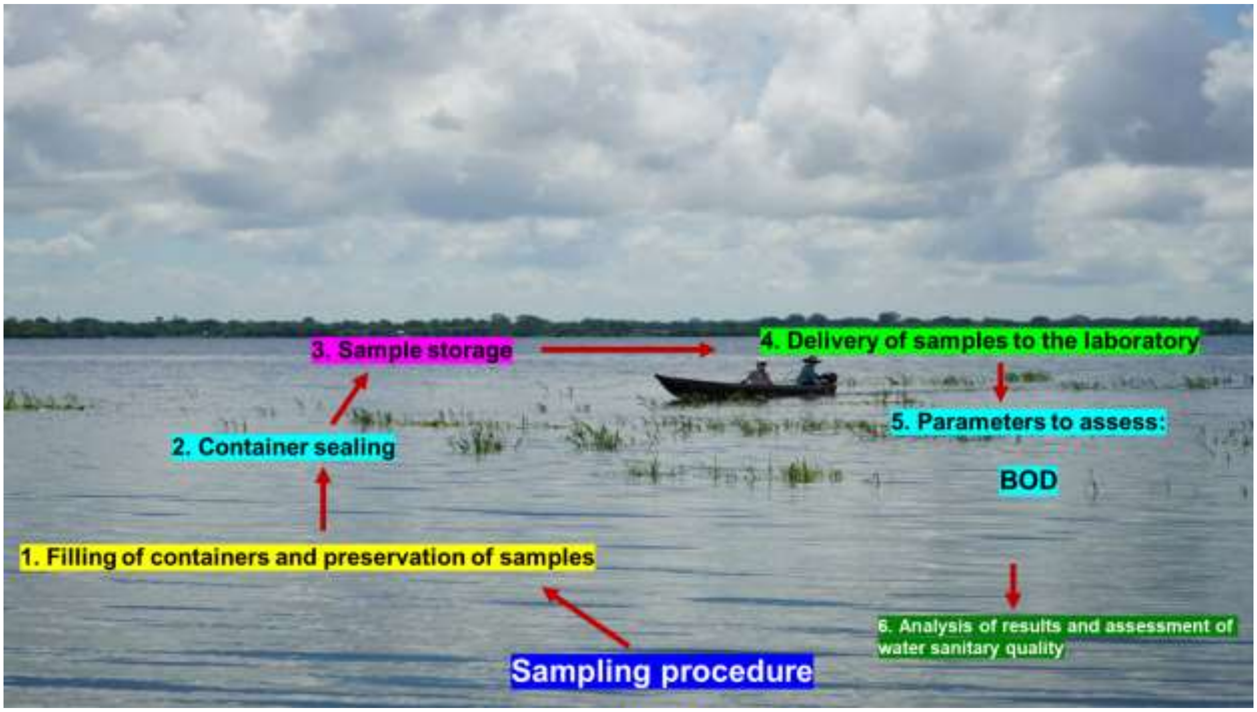

Figure 1. Schematic of water sampling stage in stream and bog.

Bioaugmentation *of Chlorella vulagaris*. Biomass of *Chlorella vulgaris* (CVLINM 99 %), isolated from the Santiago Apostol swamp complex in the department of Sucre (Colombia), which are part of the germplasm bank collection of the Microbiological Research Laboratory of the University of Sucre, immobilized on dried fruit of *Luffa cylindrica* as a support and previously washed with detergent to remove impurities (Nabizadeh et al., 2008), was used. Subsequently, microalgae fragment 30.0 ± 1 mm in diameter and 35.0 ± 1 mm thick were cut and sterilized and impregnated with nutrifoliate culture medium (Colinagro 4.0) containing 200 g/L total nitrogen, 100 g/L phosphorus and nutrients such as K, Mg, S, Cl, Fe, Cu, Zn, Mn, B and Mo for 24 h (Hernández et al., 2018, vitola et al., 2022). They were then placed in bioreactors with the different treatments and replicates to carry out the organic load removal test as described in the following item.

Organic load degradation tests. In the organic load degradation test, water samples taken from the stream and marsh sampling campaigns were used. The evaluation of the organic load removal capacity was carried out during 15 days, since this is the growth period of Chlorella vulgaris according to the evaluation of the growth kinetics of the microalgae species and the biosorption and removal capacity of the greatest amount of pollutants and other compounds, carried out in the microbiological research laboratory attached to the Agricultural Bioprospecting group of the University of Sucre, as described in figures 2 and 3 below. The organic degradation tests of the AGC water sample in the presence of the microalgae consortium were carried out in phyco-reactors designed in the microbiological research laboratory of the University of Sucre, as shown in the following experimental design in figure 2 and 3.

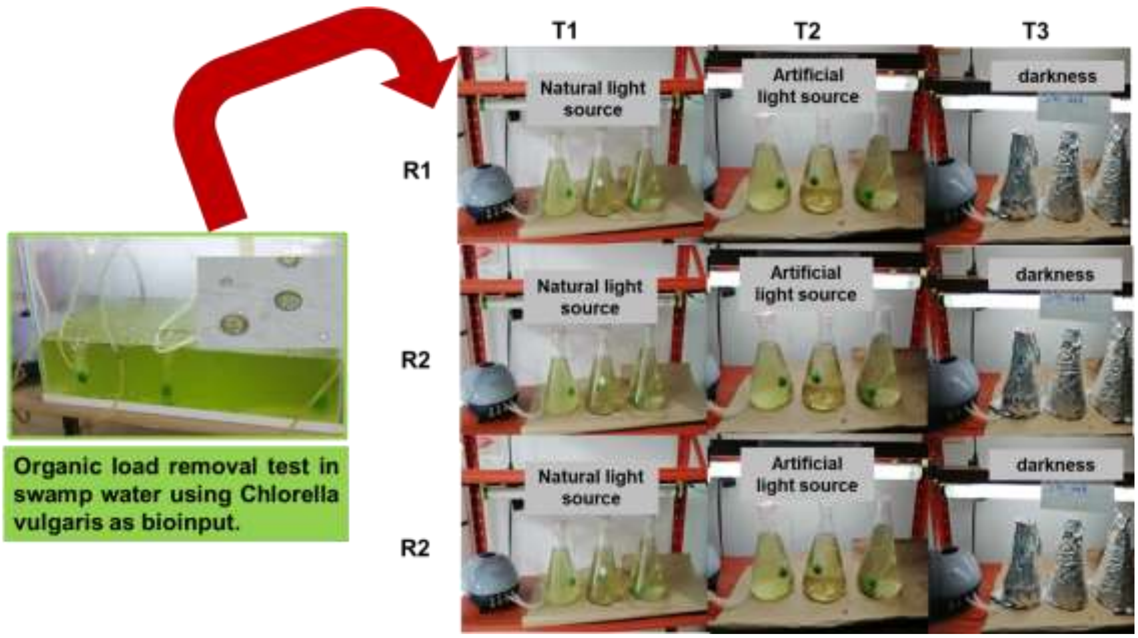

Figure 2. Organic load removal test of bog water using Chlorella vulgaris biomass in the presence of three light sources.

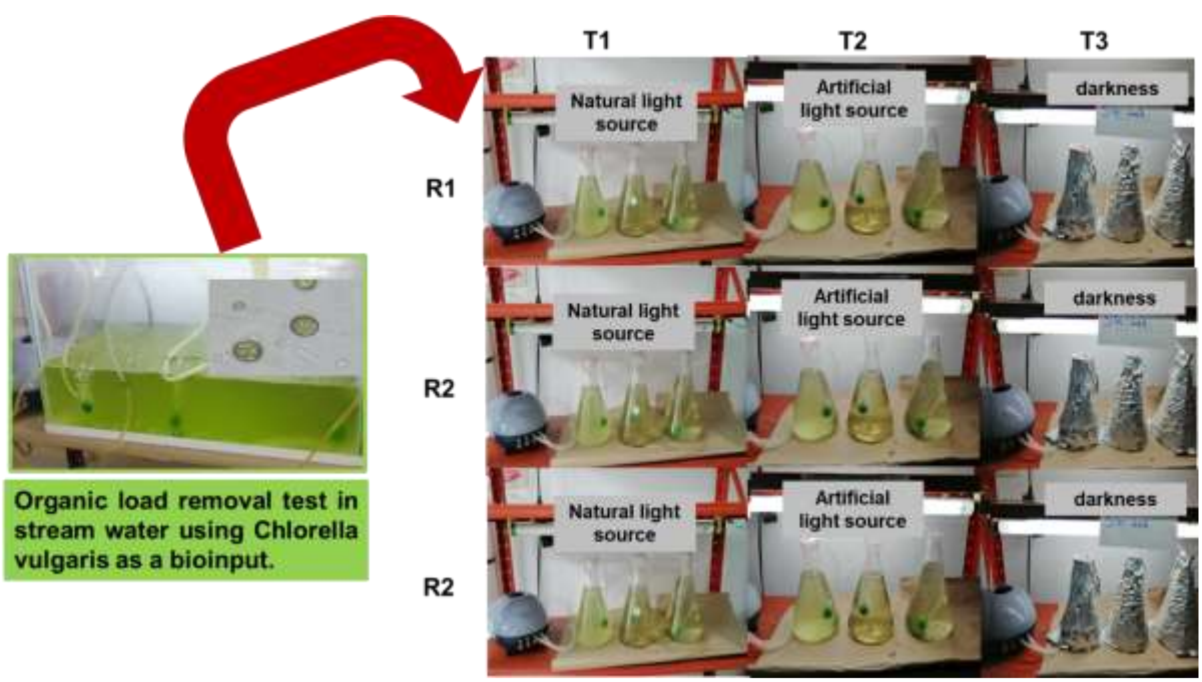

Figure 3. Stream water organic load removal test using Chlorella vulgaris biomass in the presence of three light sources.

3. RESULTD AND DISCUSSION

For the evaluation of the organic load degradation test in water samples, two types of samples were used: one water sample was taken from a stream that discharges its water into the swamp and the other sample was taken from the swamp or the point where the stream discharges its water. According to the results, the initial analysis of the Biological Oxygen Demand values showed values of 22.72 for the stream water and 12.67 mg O_2/L, while dissolved oxygen showed values of 9.45 in the stream water and 5.83 mg/L for the bog water (Table 1).

Table 1. BOD_5 and dissolved oxygen parameters of swamp and stream water samples in the department of Sucre-Colombia.

Parameters	**Units**	**Swamp**	**Stream**
Dissolved Oxygen (In situ)	mg/L	5,8	9,4
BOD_5	mg O_2/L	12,6	22,7

Ten days after initiation, BOD_5 analyses were carried out and it was found that for the treatments where *Chlorella vulgaris* microalgae biomass was used with different light sources (natural sunlight, artificial light from a white light wavelength fluorescent lamp and darkness without the presence of light). The results obtained after the evaluation of the organic load of the samples analyzed (bog and stream water), infer that in the treatment (T2) with Chlorella vulagaris using artificial light as a source of light, a percentage of organic load removal represented in BOD_5 of 56.5% of the water from the bog and less in the treatment (T3) with Chlorella vulagaris in the dark. Meanwhile, for the stream water sample test it is observed that the maximum percentage of removal was presented for the treatment (T6), where *Chlorella vulagaris* was used in dark growth. It is also observed that it was lower for Ch. vulgaris under artificial light (table 2).

Table 2. Percentage efficiency of organic load removal in stream and marsh water using *Chlorella vulagaris* subjected to three light sources.

Treatments		**DOB_5 (initial)**	**DOB_5 (end)**	**% BOD_5 removal efficiency**
T1	*Cv* in naturally light swamp water	12,6	6,0	52,5
T2	*Cv* swamp water with artificial light	12,6	5,5	56,5
T3	*Cv* in Darkness in swamp water	12,6	8,2	35,2
T4	*Cv* stream water with natural light	22,7	11,2	50,7
T5	*Cv* stream water with artificial light	22,7	12,6	44,2
T6	*Cv* stream water in Darkness	22,7	9,5	57,8

Cv= Chlorella vulgaris

According to Contreras-Flores et al. (2003), light intensity is one of the main parameters to be considered in a crop. In the absence of nutrient limitation, photosynthesis increases with increasing light intensity, until the maximum species-specific growth rate is reached at the light saturation point (Park et al. 2011). After this point, the point of photoinhibition is reached, with detrimental results for the cell itself and even death, involving loss of photosynthetic efficiency and crop productivity (Contreras-Flores et al. 2003, Richmond 2004, Martinez 2008, Park et al. 2011).

As reported by Martinez, (2008), outdoor microalgal cultures often suffer from photoinhibition in the main daylight hours due to high light intensity. Under certain conditions, cultures with higher cell density (> 3 g L-1) are able to use incident light more efficiently compared to dilute conventional cultures (Contreras-Flores et al. 2003). This is due to self-shading, where cells closer to the surface shade the lower layers, with cells further away from the surface (Contreras-Flores et al. 2003, Markou and Georgakakis 2011, Park et al. 2011).

Figure 4 and 5 show the mean BOD5 values found in the experiment using bog and stream water containing *Chlorella vulagaris* subjected to three light length sources. Note that the lowest BOD_5 values were found in the microalgae treatments using bog water with all three light sources.

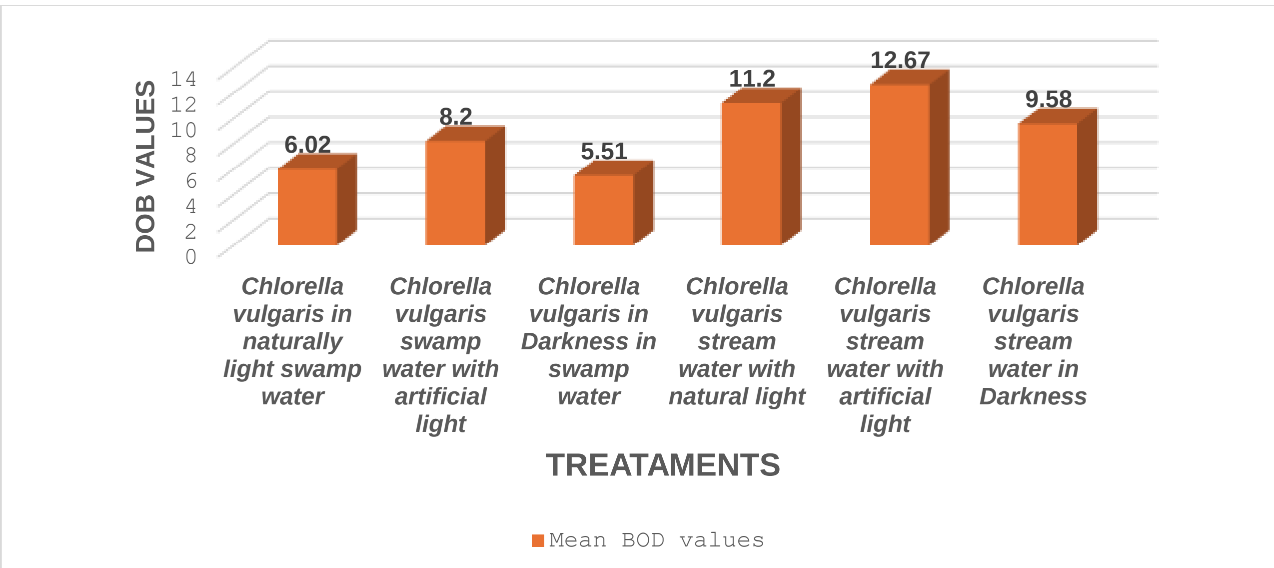

Figure 4. Organic load removal test of stream and bog water using Chlorella vulagaris biomass subjected to three light sources.

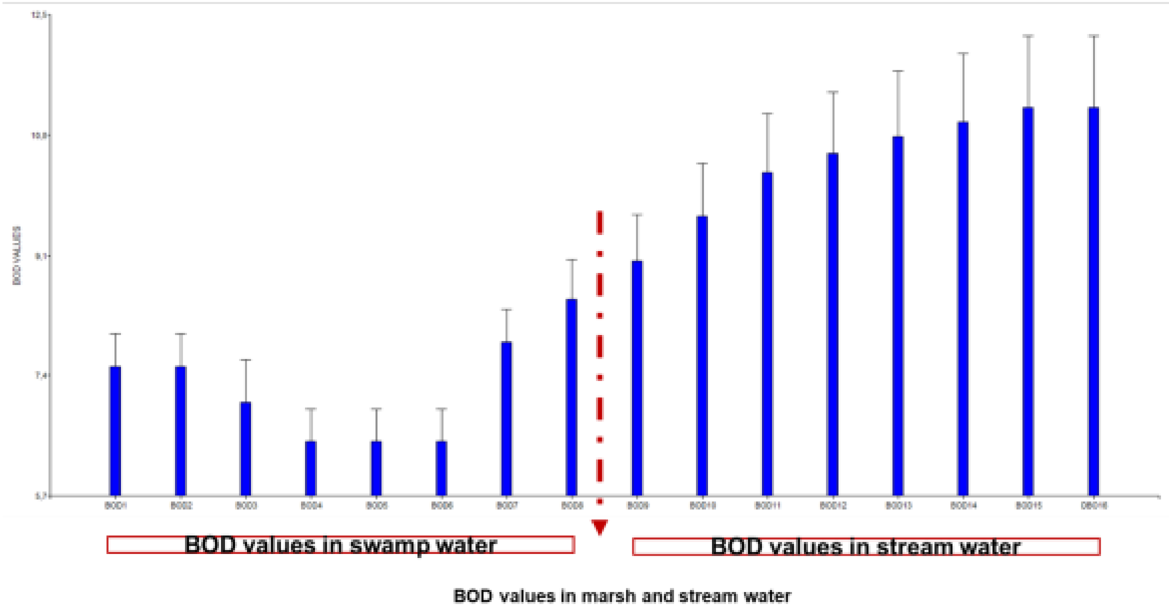

Figure 5. Results of Biological Oxygen Demand (BOD_5) found in bog and stream water after testing with microalgae cells.

In relation to the Biological Oxygen Demand (BOD5), which corresponds to the amount of oxygen that micro-organisms, especially bacteria (aerobic or anaerobic), fungi and plankton, consume during the degradation of the organic substances contained in the sample. It is used to measure the degree of contamination.

According to Abdel-Raouf et al. (2012), BOD_5 reduction values are variable as they depend on various environmental factors, and studies on the use of microalgae in urban wastewater showed moderate BOD_5 removal values of 68.4%. In 2008, similar results by Brennan and Owende (2010), observed a removal of 67.4% in Scenedesmus cultures fed with wastewater from olive oil production. While Leon and Chaves (2010) achieved an appreciable reduction of 91.4% in treatments with stabilization ponds and microalgae.

Organic pollutants result in oxygen depletion, which is a product of the biological degradation of the compounds. In the case of inorganic pollutants, the result is their possible toxic effect. Biological degradation of organic substances produces fatty acids, carbohydrates, amino acids and hydrocarbons; and inorganic substances in the case of toxic metals, particulate matter such as clays and sediments; and microorganisms such as bacteria and protozoa (Lecca et al., 2014).

Surface waters are highly susceptible to contamination, being the traditional dumping ground throughout the history of industry and populations. In the case of oxygen-demanding waste pollutants, they affect streams as well as standing water. Organic matter requires oxygen to be degraded in a watercourse. High organic content favors the growth of bacteria and fungi. Oxygen used for the oxidation of organic matter consumes oxygen used for the development of aquatic flora and fauna. Effects to the ecosystem include a change in water quality, and possible elevation of pH, leading to the disappearance of fish and plants.

Under suitable conditions, as indicated by Park et al., (2011); Rawat et al., (2011); Prajapati et al., (2013), macroalgae have been used for water purification, this is known, as phycoremediation (defined as the use of macroalgae and/or microalgae for the removal or biotransformation of pollutants, from wastewater and gaseous media (Olguín 2003, Dominic et al. 2009, Doušková et al., 2010, León and Chaves 2010, González-López et al., 2011, Hongyang et al., 2011, Rawat et al. 2011, Abdel-Raouf et al. 2012, Infante et al. 2012; Prajapati et al. 2013; Maity et al. 2014). Many factors are involved in the growth and composition of microalgal species and their depurative capacity (Park et al. 2011, Rawat et al. 2011), so their capabilities must be verified under specific local environmental and pollution conditions.

4. CONCLUSION

In wastewater treatment, the aim is to remove biochemical oxygen demand (BOD5), suspended solids, nutrients, coliforms and toxicity (Dominic et al., 2009, Park et al. 2011, Rawat et al. 2011, Abdel Raouf et al., 2012). In the present study, a reduction of BOD5 up to 56.7% was achieved in bog water using Chlorella vulgaris and 57.8% for stream water using the same microalgae species.

5. ACKNOWLEDGEMENTS

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6. AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

7. CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflictof interest

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