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GROWTH CURVE OF THE MICROALGAE *Chlorella Vulgaris* IN THREE MERCURY CONCENTRATIONS

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ABSTRACT
Despite the existence of physicochemical decontamination methods, these have proved to be ineffective and costly, so biological treatments such as phytoremediation are currently gaining ground as they are low-cost and environmentally friendly. The aim of this work was to evaluate in vitro the growth capacity of the microalga Chlorella vulgaris for 25 days in the presence of Hg^{2+} and subsequently subjected to concentrations of 1.0, 2.0 and 3.0 mg/L of $HgCl_2$. The results show the efficiency of the microalgal *Chlorella vulgaris* to grow with different behaviours in the three concentrations of mercury in the form of $HgCl_2$. The data obtained indicate the ability of this microalga to remediate mercury in aquatic environments contaminated with this metal.

Key words: Contamination, aquatic environment, mercury, microalgae,

INTRODUCTION

Numerous approaches have now been studied for the development of more economical and effective technologies, both to decrease the amount of wastewater produced and to improve the quality of the treated effluent (Barakat, 2011). Adsorption has become one of the alternative treatments; in recent years, the search for low-cost adsorbents with metal-binding capabilities has intensified (Leung et al., 2000). For this reason, the cultivation of microalgae in wastewater is spreading widely for nutrient removal and control of physicochemical parameters of wastewater, and as a feedstock for biofuel production (Wang et al., 2010).

In addition, the use of living and non-living microalgae is a low-cost and environmentally friendly technique, which is increasingly being used to remove various toxic substances such as heavy metals, antibiotics (Xiong et al., 2017; Santaeufemia et al., 2016), phenol and aromatic-polycyclic compounds from wastewater (El-Sheekh et al., 2012; Gao et al., 2011).

Phytoremediation technology has great advantages over conventional methods implemented in wastewater treatment, because it has lower costs in its implementation and is ecologically viable for

aquatic and terrestrial environments (Chabukdhara et al., 2017), in addition to a decrease in energy consumption, the non-implementation of chemicals, the ability to generate photosynthetic oxygen and fix CO_2 (Abinandan and Shanthakumar, 2015; Yao et al., 2015).
The aim of the present study was to evaluate the growth behaviour of the microalga Chlorella vulgaris in the presence of high concentrations of the metal mercury.

MATERIALS AND METHODS

Growth of Chlorella vulgaris

The pure microalgae were donated by the Microalgae Biotechnology, Applied Physicochemistry and Environmental Studies Research Group of the Universidad del Atlántico. Subsequently, it was bioaugmented in the Microbiological Research Laboratory of the University of Sucre in 2.5 L of Nutrifoliar culture medium at a concentration of 4 mM, following the recommendations on the label. This culture medium contains as macronutrients K, Mg, S, P, Cl and micronutrients Fe, Cu, Zn, Mn, B and Mo, necessary for normal cell growth. *Chlorella vulgaris* cells were inoculated in each vessel at a concentration of $1x10^6$ CFU and an optical density of 0.1 absorbance measured with a 647 nm band (Infante et al., 2012). The cultures (phyco-reactors) were kept under constant agitation to avoid cell sedimentation at a temperature of 28 ± 1°C and presence of light for 24 d and photoperiod of 12 h of light and 12 h of darkness (Sánchez et al., 2008).

Growth curve of Chlorella vulgaris in the presence of mercury chloride.

The growth curve of *Chlorella vulgaris* was determined by growing the microalgae in culture medium with mercury concentrations of 1.0, 2.0 and 3.0 mg/L $HgCl_2$ daily for up to 25 d. Aliquots of the microalgae culture were taken and growth measurements were made by optical density readings using a Merck Spectroquant Pharo 300 UV-vis spectrophotometer at a wavelength of 647 nm (Infante et al., 2012). The results of microalgae growth at different concentrations of mercury in the form of mg/L $HgCl_2$ were compared with the behaviour of *Chlorella vulgaris* without the presence of the metal.

RESULTS AND DISCUSSION

Figure 1 shows the growth behaviour of *Chlorella vulgaris* in the presence of 1.0 mg/L $HgCl_2$ compared to the growth of the control (without $HgCl_2$). The figure shows that Chlorella vulgaris had a growth up to 17 days of 2.1 nm and from 22 days onwards went into dormancy between days 18 and 22 and after this time passed into the death stage. The results also show an adaptation phase in the presence of up to five days 1.0 mg/L $HgCl_2$ compared to the growth behaviour of the control microalgae treatment.

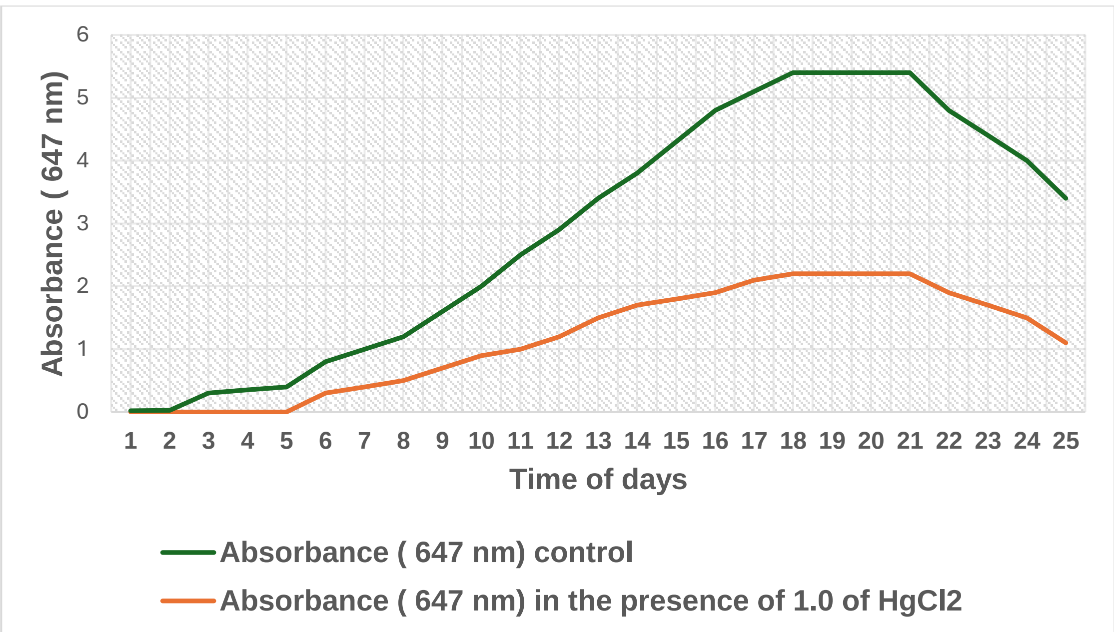

Figure 1. Growth curve of the microalga Chlorella vulgaris in the presence of 1.0 mg/L $HgCl_2$.

Figure 2 describes the growth behaviour of *Chlorella vulgaris* in the presence of 2.0 mg/L $HgCl_2$ compared to the control of the same microalgae species without the presence of the metal. The maximum growth shown by *Chlorella vulgaris* was up to 17 days and from that moment onwards it entered the stationary phase and finally from day 22 it entered the death phase. The results infer growth of the microalgae at concentrations of 2.0 mg/L $HgCl_2$.

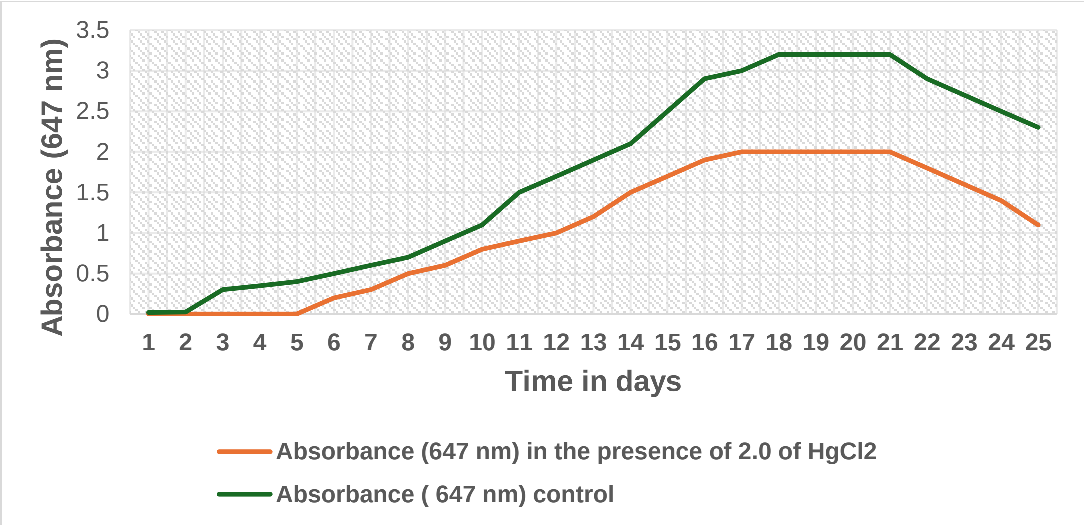

Figure 2. Growth curve of the microalga *Chlorella vulgaris* in the presence of 2.0 mg/L $HgCl_2$.

Figure 3 shows the growth performance of *Chlorella vulgaris* in the presence of 2.0 mg/L $HgCl_2$ compared to the growth performance of the control (without $HgCl_2$). In figure 3, the growth behaviour of Chlorella vulgaris in the presence of 3.0 mg/L $HgCl_2$ compared to the control of the same microalgae species without the presence of $HgCl_2$ is described. The maximum growth shown by *Chlorella vulgaris* was up to 17 days and from that moment it entered the stationary phase and finally from day 22 it entered the death stage. The results inferred growth of the microalgae at concentrations of 3.0 mg/L $HgCl_2$.

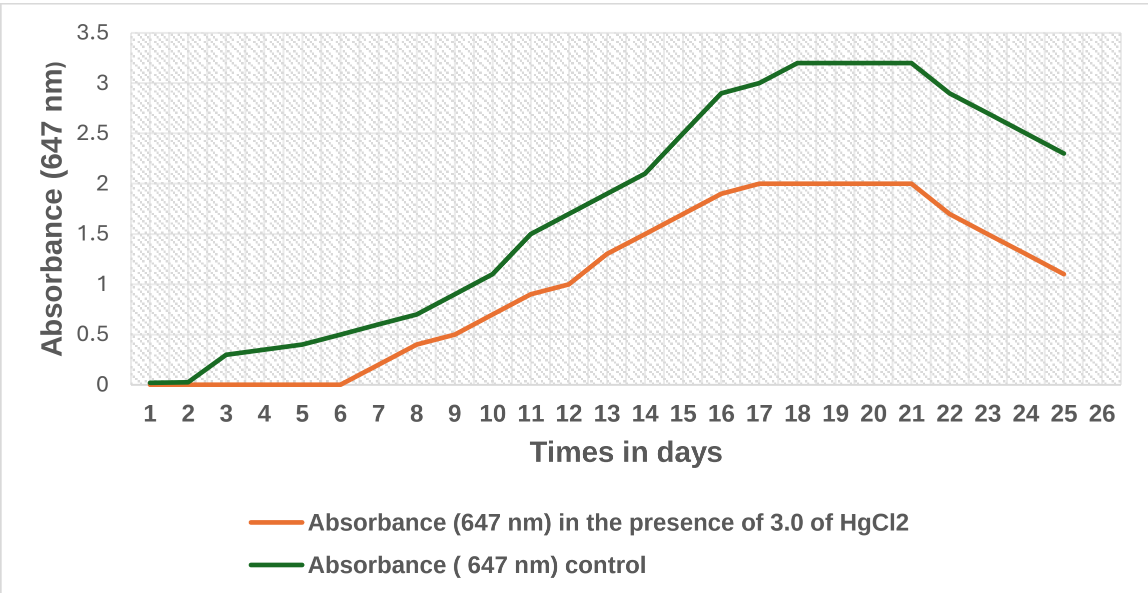

Figura 3. Growth curve of the microalga *Chlorella vulgaris* in the presence of 3.0 mg/L $HgCl_2$.

According to the results obtained by Vitola et al, (2022), regarding desorption, Chlorella vulgaris presented the highest averages with 97.29 ± 1.93 % of Hg, 96.86 ± 2.14 % of Cd and 95.48 ± 1.19 % of Pb; in comparison with the microalgae species Scenedesmus obliquus which showed a desorption of 96.74 ± 2.14 % of Hg, 95.15 ± 2.90 % of Cd and 93.82 ± 2.68% of Pb. These results demonstrate that the application of immobilized microalgae biomass for the biosorption of heavy metals is a bioremediation alternative.

Microalgae show affinity for polyvalent metals, which makes them important cleaning agents for water and wastewater containing dissolved metal ions (de-Bashan and Bashan, 2010). Furthermore, *Chlorella vulgaris* was chosen among 10 microalgae for the removal of dyes from textile wastewater, as it showed higher growth in this medium and higher pollutant removal (Lim et al., 2010).

As evidence of results related to the activity of microalgae against different concentrations of mercury, we have those obtained by Benítez et al. (2018) who evaluated the mercury removal capacity of the microalgae Chlorella sp. immobilized on fragments of scouring pad (*Luffa cylindrica*), finding the highest average mercury removal at 24 h when the microalgae was exposed to a concentration of 3.0 mg/L of $HgCl_2$, although from 8 h of exposure to 4.0 mg/L the removal was higher than 89 %.

CONCLUSIONS

The use of *Chlorella vulgaris* as a biological alternative for heavy metal biosorption techniques is efficient, since the microalgae used in this research grew and showed different growth behaviour against mercury concentrations of 1,0; 2,0 and 3.0 in the form of $HgCl_2$.

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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.

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

REFERENCES

1. Abinandan, S., & Shanthakumar, S. (2015). Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: A review. *Renewable and Sustainable Energy Reviews. 52*, 123–132.
2. Barakat, M.A. (2011). New trends in removing heavy metals from industrial wastewater. *Arab. J. Chem. 4*(4), 361–377.
3. Benítez, S., Pérez, A. & Vitola, D. (2018). Removal and recovery of mercury in vitro using immobilized live biomass of Chlorella sp. Indian Journal of Science and Technology, 11(45), 1-8. https://doi.org/10.17485/ijst/2018/v11i45/137575.
4. Chabukdhara, M., Kumar, S., & Gogoi, M. (2017). Phycoremediation of Heavy Metals Coupled with Generation of Bioenergy. *Algal Biofuels*. DOI 10.1007/978-3-319-51010-1_9.
5. de-Bashan, L.E., & Bashan, Y. (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresour. Technol. 101*(6), 1611-1627.
6. El-Sheekh, M., Ghareib, M., & El-Souod, G. (2012). Biodegradation of phenolic and polycyclic aromatic compounds by some algae and cyanobacteria. *J. Bioremed. Biodegrad. 3*, 1-9.
7. Gao, Q., Wong, Y.S., & Tam, N. (2011). Removal and biodegradation of nonylphenol by different *Chlorella* species. *Mar. Pollut. Bull. 63*, 445-451.
8. Hernández, Y., Pérez, A. & Vitola, D. (2018). Biosorption of mercury and nickel in vitro by microalga Chlorella sp. in solution and immobilized in dry fruit of squash (Luffa cylindrica).

Indian Journal of Science and Technology, 11(41), 1-8 https://dx.doi.org/10.17485/ijst/2018/v11i41/131111.

9. Infante, C., Angulo, E., Zárate, A., Florez, J.Z., Barrios, F., & Zapata, C. (2012). Propagación de la microalga *Chlorella* sp. en cultivo por lote: cinética del crecimiento celular. *Av. cien. ing. 3*(2), 159-164.
10. Leung, W.C., Wong, M.F., Chua, H., Lo, W., & Leung, C.K. (2000). Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Water Sci. Technol. 41*(12), 233-240.
11. Lim, S.L., Chu, W.L., & Phang, S.M. (2010). Use of *Chlorella vulgaris* for bioremediation of textile wastewater. *Bioresour. Technol. 101*(19), 7314-7322.
12. Santaeufemia, S., Torres, E., Mera, R., & Abalde, J. (2016). Bioremediation of oxytetracycline in seawater by living and dead biomass of the microalga *Phaeodactylum tricornutum. J. Hazard. Mater. 320*, 315-325.
13. Sánchez, E., Garza, M., Almaguer, V., Sáenz, I., & Liñán, A. (2008). Estudio cinético e isotermas de adsorción de Ni (II) y Zn (II) utilizando biomasa del alga *Chlorella* sp. inmovilizada. *Ciencia UNAL. 11*(2), 168-176.
14. Vitola, D. Pérez, A., Montes, D. (2022). Utilización de microalgas como alternativa para la remoción de metales pesados. Revista de Investigación Agraria y Ambiental, 13(1), 195 – 203. DOI: https://doi.org/10.22490/21456453.4568
15. Wang, X., Chen, J., Yan, X., Wang, X., Zhang, J., Huang, J., & Zhao, J. (2015). Heavy metal chemical extraction from industrial and municipal mixed sludge by ultrasound-assisted citric acid. *J. Ind. Eng. Chem. 27*, 368-372.
16. Xiong, J. Q., Kurade, M. B., & Jeon, B. H. (2017). Biodegradation of levofloxacin by an acclimated freshwater microalga, *Chlorella vulgaris. Chem. Eng. J. 313*, 1251-1257.
17. Yao, L., Shi, J., & Miao, X. (2015). Mixed wastewater coupled with CO_2 for microalgae culturing and nutrient removal. *PLoS ONE. 10*(9), 1-16.