BIOSORPTION MECHANISM OF MICROALGAE *Nannochloropsis oculata* AND THE EFFECT OF Pb ON ITS PHOTOSYNTHETIC ACTIVITY

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ABSTRACT: *Recent investigations on the use of microalgae for heavy metal removal have revealed immense potential for biosorption. In this study the uptake mechanism, photosynthetic activity, cell quality as well as algal density of Nannochloropsis oculata (N. oculata) were investigated extensively towards the different concentrations of lead ions and the effects of lead ions to the cultured alga. Results showed that the biosorbent used was more capable of sequestering lead ions via adsorption − which means that the mechanism of uptake was extracellular. On the other hand, the photosynthetic activity of N. oculata at different concentrations of lead was insignificantly different from each other. Regardless of the different concentrations of lead in the solution, the effects brought by the presence of lead ions to the chlorophyll content and algal density of the biosorbent were indistinguishably negligible as indicated by the greener color of the inoculated medium in contrast to its first day of culture.*

Keywords: Biosorption, *N. oculata, chlorophyll content, algal density,*

1. INTRODUCTION

Dissolved toxic metals, which are not biodegradable by nature, are the primary contaminants of wastewater. In addition, they are major pollutants in marine, ground, industrial, and even treated waters. Owing to their high toxicity, heavy metals pose a serious threat to biota [1] and the environment. As a result, it is necessary to treat or alleviate heavy metal burden of wastewaters properly before discharge [2].

For years, researchers have looked for alternative methods to remediate the problem on heavy metal-contaminated waters and thus reducing growing public health risk. Several conventional physicochemical methods for stripping toxic metals from heavy waters are neither efficient nor cost-effective [3]. They are only practical when applied to high strength wastes with heavy metal ion concentrations greater than 100 ppm, but generally, treatment for low strength heavy metal content in wastewaters is not successful with such methods [4].

Microalgae are ubiquitous in nature. They are capable of fitting to different environments ranging from aquatic environments to deserts; however, they are now increasingly cultivated and cultured [5]. *Nannochloropsis oculata* is one of the green marine microalga used extensively in aquaculture industry for growing small zooplanktons such as rotifers and fish hatcheries that show remarkable heavy metal biosorption sensitivity [6].

In this study, the application of the species *Nannochloropsis oculata* was done as a solution to the need for an efficient and economical approach in biosorbing toxic heavy metals in lead-contaminated water and wastewater from industries. The application of *N. oculata* in this study will not only impart new knowledge on the uses of this species but will also show a practicable means for the sequestration of lead in the treatment of water effluents from industrial processes economically and efficiently. On the other hand, the use of biosorbent derived from suitable biomass through the process of biosorption is an alternative to traditional methods for decontamination of liquid effluents loaded with heavy metals [7].

2. EXPERIMENTAL DETAILS

Preparation of the microalgae and culture condition

Nannochloropsis oculata strain and 10 liters of chlorinated seawater were obtained from MSU-Institute of Fisheries Research and Development (IFRD), Naawan, Misamis Oriental, Philippines. Ten liters of chlorinated seawater were aerated for 2-3 hours to ensure the removal of residual chlorine and was boiled for an hour to effectively kill all living organisms present and destroy spores or resting stages.

Enriched seawater media was prepared as soon as the Tungkang Marine Research Laboratory (TMRL) fertilizer was made. One mL of the formulated TMRL was added to every 1 liter of seawater. Dilution of the media to the desired volume was done and was placed in an acid washed container.

One liter of the stock culture of *Nannochloropsis oculata* was inoculated to 9 liters prepared enriched media. The algal culture was incubated and allowed to bloom at room temperature under natural daylight cycle for nine days with aeration to prevent clumping of the cells.

Lead treatments to cultured *Nannochloropsis oculata*

A stock solution of lead with a concentration of 5000 ppm was prepared and from this concentration, 500 mL each of 15 ppm, 10 ppm and 5 ppm solutions of lead in three replicates were prepared using the homogenized algal culture as the solvent. Each of these solutions was placed separately in previously acid washed 1 L containers and was aerated for 96 hours.

Density determination of the algal culture

The populations of the cultured microalgae before lead treatment and after four days of exposure to varying concentration of lead were determined using a hemacytometer chamber. One drop of the algal suspension was pipetted on the slide, and the mean counts of the three replicates were taken into consideration. Since *Nannochloropsis oculata* is unicellular, smaller squares were used in determining the cell number. The equation below was used in the determination of the number of cells per mL of *Nannochloropsis oculata*.

$$\text{No. of cells per mL} = \frac{\text{Total no. of cells counted}}{\text{No. of squares (25)}} \times 4\times 10^6$$

Determination of lead in *Nannochloropsis oculata*

After 96 hours, the prepared solutions were stirred and 10 mL samples from each solution were pipetted and were placed separately in 10 mL centrifuge test tubes. The samples were spun at 4000 rpm for 10 minutes in a centrifuge and the supernatant obtained was decanted and labeled as Supernatant 1. The remaining residue in the test tube labelled as Residue *1* was washed with deionized water to remove all the unbound lead ions from the cell and the washings were added to acid washed beakers containing the collected Supernatant 1 sample.

Digestion of *supernatant 1* samples

The collected supernatant 1 samples were placed over a hot plate and were digested under a fume hood with 2.5 mL concentrated HNO_3 and evaporated to dryness. The samples were then treated with 3 mL of concentrated HNO_3 and evaporated again to dryness; digested samples were then removed from the hot plate and the resulting residue or precipitate was dissolved in 5 mL 6M HCl. Lastly, the samples were diluted to 25 mL with 1N HCl and were stored in pre-labeled acid-washed containers. The resulting solutions were later subjected to the atomic absorption spectrophotometer at the Philsaga Mining Corporation to determine the amount of lead ions not accumulated by the algae.

Treatment of residue 1 samples

The collected samples at Residue 1 were washed with 0.01M EDTA to remove the surface-bound lead ions. The treated Residue 1 was spun at 4000 rpm for 10 minutes in a centrifuge and the supernatant obtained was then decanted and was placed in an acid-washed beaker labelled as Supernatant 2 and the remaining residue was labelled as Residue 2.

Digestion of *supernatant 2* samples

The collected samples at Supernatant 2 were placed over a hot plate and were digested under a fume hood with 2.5 mL concentrated HNO_3 and evaporated to dryness. The samples were then treated with 3 mL of concentrated HNO3 and evaporated again to dryness. The digested samples were removed from the hot plate and the resulting residue or precipitate were dissolved in 5 mL 6M HCl. Lastly, the samples were diluted to 25 mL with 1N HCl and were stored in pre-labeled acid-washed containers. The resulting solutions were then subjected to the atomic absorption spectrophotometer at the Philsaga Mining Corporation to determine the amount of lead ions adsorbed by the algae.

Preparation and digestion of *residue 2* samples

The residue 2 samples were dried in an oven at 110°C for 2 hours. Next, the dried samples were washed approximately with 15 mL concentrated HNO_3. The washings were collected and were placed separately in acid-washed beakers. They were then treated with approximately 15 mL concentrated HNO_3 and were placed on top of a hot plate under a fume hood and were evaporated to about 15-20 mL making sure not to boil them. The samples were removed from the hot plate and were cooled. Three mL of 1N HNO_3 and 3 mL of deionized water were then added to the cooled samples and they were placed back on top of a hot plate to continue the digestion. The addition of 1N HNO_3 and deionized water was repeated until the color of the obtained digest was pale yellow or clear. The samples were evaporated to near dryness and 3 milliliters of 1:1 v/v HNO_3 and deionized water was added to dissolve any precipitate or residue that would have resulted during the evaporation. The beaker walls as well as the watch glass covering were washed down with deionized water and the washings were filtered using a Whatman #1 filter paper to get rid of the silicates and other insoluble materials. Lastly, the volumes of the samples were diluted to 25 mL deionized water and were stored in pre-labelled acid-washed containers. The resulting solutions were then subjected to atomic absorption spectrophotometer at the Philsaga Mining Corporation to determine the amount of lead ions absorbed by the algae.

Determination of the amount of chlorophyll in algal culture

Henriques *et. al.* [9] observed that pigment content in microalgae is a specific feature of each species. It indicates the photosynthetic efficiency of the algae and its evaluation is essential as an indirect measure of cell growth.

Ten mL of the prepared solutions were pipetted and were filtered in fiber glass filter paper. The samples, held at room temperature and protected from exposure to light were placed in screw cap test tubes totally covered with carbon paper and were soaked in 2-3 mL 90% acetone and set aside overnight. The total volume of the extracted pigment was adjusted to 10 mL with 90% aqueous acetone and the optical densities (OD) of the extract at 664, 647 and 630 nm were read in the double-beam UV-Vis spectrophotometer to obtain the concentration of chlorophyll a, b and c. Since OD of the extract at 750 nm is very sensitive to changes in the acetone-to-water proportions, the OD reading at 750 nm serves as a correction for turbidity and was subtracted to each of the pigment OD values of the other wavelengths before using them in the equation below.

Concentration of chlorophyll a, b, and c extract were calculated using the corrected optical densities with the following equations:

Chl **a** = 11.85(OD664) – 1.54(OD647) – 0.08(OD630)
Chl **b** = 21.03(OD647) – 5.43(OD664) – 2.66(OD630)
Chl **c** = 24.52(OD630) – 7.60(OD647) – 1.67(OD664)

where Chl **a**, **b** and **c** are the concentration of chlorophyll **a**, **b** and **c**, respectively, in mg/L and OD664, OD647 and OD630 are the corrected optical density at respective wavelengths [8].

Quality of *Nannochloropsis oculata*

The difference of the quality of the inoculated medium at the start of culture and after its subjection to varying

concentrations of lead: 15 ppm, 10 ppm, 5 ppm and 0 ppm was determined by direct comparison of their color.

3. RESULTS AND DISCUSSION

Growth curve of *Nannochloropsis oculata* at varying concentrations of lead

Figure 1 shows the growth curve of *N. oculata* for four days of exposure to lead ions. Monitoring of the algal density of the microalgae started on the 9th day of culture (before lead treatment) during which the cultured algae started to bloom as indicated by the green color of the inoculated medium and lasted on the 12th day of culture.

As shown, the microalgae demonstrated slow production rate between 24 hours interval throughout the four days of cell density observation under a microscope. Prior to lead exposure, the *N. oculata* was determined to be 746,667 cells/mL but after 24 hours of exposure, *N. oculata* treated with different lead concentrations (5 ppm, 10 ppm, and 15 ppm) exhibited greater cell counts relative to the control. Noticeably, after the 96th hour of exposure to lead ions, cell densities were at their greatest at 10 ppm having a cell density of 1,012,800 cells/mL, followed by the algal density at 15 ppm having 960,000 cells/mL; the least is at 5 ppm with 902,400 cells/mL.

Samples treated with lead concentrations of 5 ppm, 10 ppm, and 15 ppm were not appreciably affected by increasing concentration of lead.

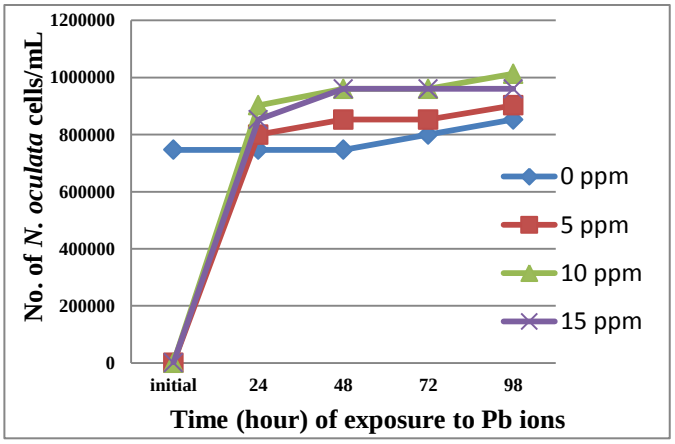

Figure 1. Growth curve of *Nannochloropsis oculata* at 24 hours interval at different concentration of lead

Lead uptake of *Nannochloropsis oculata*

The capacity of *N. oculata* to sequester lead ions was investigated and detected by an atomic absorption spectrophotometer. The mean concentration of lead ions accumulated (Supernatant and Residue 2 samples) and not accumulated (Supernatant 1 samples) by *N. oculata* at varying concentrations was determined and shown in the subsequent sections. Results were statistically evaluated using ANOVA and Duncan Multiple Range Test (DMRT) at 95% confidence interval.

Lead uptake of *Nannochloropsis oculata* at 5 ppm concentration

Table 1 shows the mean uptake of lead in Supernatant 1, Supernatant 2 and Residue 2 samples at 5 ppm lead concentration. As shown, about 0.570 ppm was the concentration of lead in Supernatant 1, 0.660 ppm in Supernatant 2 and 0.050 ppm in Residue 2 samples.

Overall, Table 1 shows significant differences among the means of the samples in 5 ppm concentration. However, there is no significant difference in the concentration of lead between Supernatant 1 and Supernatant 2. Only the concentrations in Residue 2 and Supernatant 1, and Residue 2 and Supernatant 2 have statistically different means. These results imply that the mechanism of uptake is adsorption since there is a greater amount of lead absorbed in Supernatant 2 than lead absorbed in Residue 2.

Table 1. Lead concentration in Supernatant 1, Supernatant 2 and residue 2 samples at 5 ppm concentration of biosorbing lead

Samples	Mean (ppm)	P value	Significance
Residue 2	0.050^{a}	0.000	Significant
Supernatant 1	0.570^{b}		
Supernatant 2	0.660^{b}		

Note: Means having the same letter are not significantly different at α = 0.05 DMRT.

Lead uptake of *Nannochloropsis oculata* at 10 ppm concentration

Table 2 shows the mean uptake of lead at 10 ppm in Supernatant 1 was 1.60 ppm, 2.43 ppm for Supernatant 2 and 0.257 ppm for Residue 2.

Table 2. Lead concentration in Supernatant 1, Supernatant 2 and Residue 2 samples at 10 ppm concentration of biosorbing lead

Samples	Mean (ppm)	P value	Significance
Residue 2	0.257^{a}	0.000	Significant
Supernatant 1	1.60^{b}		
Supernatant 2	2.43^{c}		

Note: Means having the same letter are not significantly different at α = 0.05 DMRT.

Table 2 shows significant differences among the means of the samples in 10 ppm concentration. Supernatant 1 and Supernatant 2, Residue 2 and Supernatant 1, and Residue 2 and Supernatant 2 samples are significantly different from each other. These results imply that the mechanism of uptake is adsorption since there is a greater amount of lead adsorbed I Supernatant 2 than lead adsorbed in Residue 2.

Lead uptake of *Nannochloropsis oculata* at 15 ppm concentration

Table 3 presents the mean uptake of lead at 15 ppm. As shown, 1.32 ppm, 3.12 ppm, and 0.263 ppm were the obtained results for Supernatant 1, Supernatant 2 and Residue 2 samples.

Overall Table 3 shows significant differences among the means of the samples in 15 ppm concentration. Supernatant 1 and Supernatant 2, Residue 2 and Supernatant 1, and Residue 2 and Supernatant 2 samples are significantly different from each other. These results imply that the mechanism of biosorption is adsorption since there is a greater amount of lead adsorbed in Supernatant 2 than lead adsorbed in Residue 2.

Table 3. Lead concentration in supernatant 1, supernatant 2 and residue 2 samples at 15 ppm concentration of biosorbing lead

Samples	Mean (ppm)	P value	Significance
Residue 2	0.263[a]	0.000	Significant
Supernatant 1	1.32[b]		
Supernatant 2	3.12[c]		

Note: Means having the same letter are not significantly different at α = 0.05 DMRT.

Effect of lead on the photosynthetic activity of *Nannochloropsis oculata*

The effects of varying concentration of lead on the chlorophyll content of *Nannochloropsis oculata* were determined. Chlorophyll a is the most predominant in all oxygen-involving photosynthetic organisms while chlorophyll b is a yellow-green chlorophyll pigment; chlorophyll c is also a chlorophyll pigment whose role is the same with chlorophyll b that is to pass on the light excitation in chlorophyll a.

Effect of lead to the concentrations of chlorophyll a, b and c content of *N. oculata*

The disparity on the concentration of chlorophyll **a**, **b** and **c** of *N. oculata* exposed to lead relative to the control was shown in **Figure 2**.

Based on the statistically evaluated result using One-way Analysis of Variance there is no significant difference among the chlorophyll **a**, **b** and **c** content per treatment. This means that chlorophyll **a**, **b** and **c** are not affected by the presence of lead ions. This also implies that *N. oculata* can survive with these concentrations and is a good biosorbent.

Based on results of the ANOVA, there is no significant difference among the chlorophyll a, b, and c content per treatment; therefore, chlorophyll a, b, and c are not affected by the presence of lead ions. The results also imply that *N. oculata* is a good biosorbent and can survive with these concentrations.

Effects of lead to the quality of *Nannochloropsis oculata*

Figure 3 presents the difference between the color of the inoculated medium at the start of culture and after its subjection to varying concentrations of lead. As shown, from the initial colorless appearance of the inoculated medium at the onset of culture, the algal culture turned to color green after nine days of inoculation.

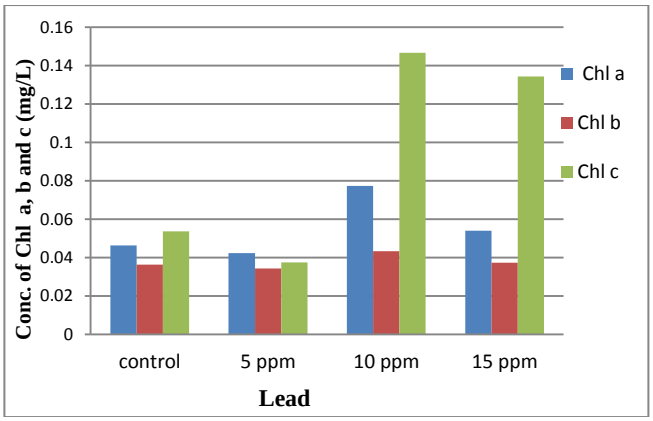

Figure 2. Chlorophyll a, b and c concentration (mg/L) in different lead treatments

Subsequently, as soon as the algal culture was subjected to varying concentrations of lead it was found out that *N. oculata* is still thriving as indicated by the greener appearance, increasing algal density and unaffected chlorophyll content of the treated samples relative to the control as the days progressed. Therefore, *Nannochloropsis oculata* is considered a good biosorbent of lead ions since it can survive even after prolonged exposure to 5 ppm, 10 ppm, and 15 ppm lead concentrations.

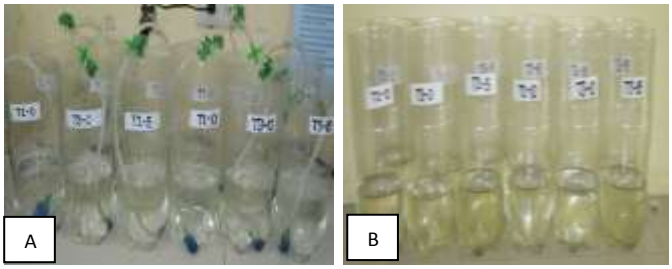

Figure 3. Color change of the samples after 4 days of exposure to lead. A-Initial color of the treated samples, B-Final color of the treated samples

DISCUSSION

The main objective of this study was to determine the mechanism by which lead is biosorbed by the microalgae, *Nannochloropsis oculata*. The alga was cultured in enriched seawater, aerated and allowed to bloom at room temperature for nine days under natural daylight cycle. Subsequently, the culture was treated with different lead concentrations: 15 ppm, 10 ppm, 5 ppm, and 0 ppm for four days. Examination of its algal density, photosynthetic activity (denoted by the resulting chlorophyll concentration) and cell quality towards different lead concentrations was done to determine the general effect of lead ions to the cultured alga. Furthermore, the uptake mechanism of lead on *N. oculata* was investigated through the determination of the different lead ion concentration not accumulated (supernatant 1), and accumulated − as adsorbed (supernatant 2) or absorbed (residue 2) by the microalgae.

Comparison of the mean lead uptake by adsorption and absorption of the microalgae was done after a four-day exposure to different concentrations of lead. Results obtained

from the concentration of lead in the supernatant and residue samples of *N. oculata* using atomic absorption spectroscopy showed that the biosorbent used was more capable of sequestering lead ions via adsorption − which means that the mechanism of uptake was extracellular. On the other hand, the acquired results for the determination of the photosynthetic activity of *N. oculata* using a double-beam UV-Vis spectrophotometer at different concentrations of lead were insignificantly different from each other. Regardless of the various concentrations of lead in the solution, the effects brought by the presence of lead ions to the chlorophyll content and algal density of the biosorbent were indistinguishably negligible as indicated by the greener color of the inoculated medium in contrast to its first day of culture. Contrary to the study conducted by Al-Homaidan [10], lead can be sequestered using a non-living mass of microalga *Spirulina platensis*; however, in this study, lead is sequestered using a living microalga *Nannochloropsis oculata.*

The application of *N. oculata* in this study will not only impart new knowledge on the uses of this species but will also show economic, efficient, and practicable means for the sequestration of lead in the treatment of water effluents from industrial processes. This study, however, is only limited to the evaluation of the lead biosorbing capacity of *N. oculata* at low concentrations. Thus, it is recommended to investigate further on the sequestration of heavy metals other than lead in higher concentrations as well as to further explore the selectivity of *N. oculata* as a developing biosorbent.

4. CONCLUSIONS

The use of *Nannochloropsis oculata* as a biosorbent in the field of bioremediation was proven to be an attractive and promising approach for removal of lead ions. In general, regardless of the concentration of lead ions present in a solution, its effect brought to the biosorbent is insignificant. Results obtained have shown that the quality, photosynthetic activity and algal density of *Nannochloropsis oculata* were not appreciably affected by the presence of the lead ions. On the other hand, comparison of the mean lead uptake of *N. oculata* in supernatant 2 and residue 2 samples at all lead treatments has shown that the biosorbent used appeared to be more capable of sequestering lead ions via adsorption − which means that the mechanism of uptake was extracellular.

5. ACKNOWLEDEGMENT

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6. REFERENCES

[1] Areco, M. M., & Afonso, M. D. S. (2007). Zinc biosorption by seaweed illustrated by the zincon colorimetric method and the Langmuir isotherm. *Journal of chemical education*, 84(2), 302-305.

[2] Volesky, B. (2001). Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy*, *59*(2), 203-216.

[3] Eccles, H. (1999). Treatment of metal-contaminated wastes: why select a biological process?. *Trends in biotechnology*, *17*(12), 462-465.

[4] Madacha, V., Apiratikul, R., & Pavasant, P. (2006, November). Heavy metals uptake by dried Caulerpa lentillifera. In *The 2nd Joint international conference on "Sustainable Energy and Environment (SEE 2006)" E-043 (P)*(pp. 21-23).

[5] Kafaie, S., Loh, S. P., & Mohtarrudin, N. (2012). Acute and sub-chronic toxicological assessment of Nannochloropsis oculata in rats. *Afr J Agric Res*, *7*(7), 1220-1225.

[6] Rocha, J. M., Garcia, J. E., & Henriques, M. H. (2003). Growth aspects of the marine microalga Nannochloropsis gaditana. *Biomolecular engineering*,*20*(4), 237-242.

[7] Romera, E., Gonzalez, F., Ballester, A., Blazquez, M. L., & Munoz, J. A. (2006). Biosorption with algae: a statistical review. *Critical reviews in biotechnology*, *26*(4), 223-235.

[8] APHA (1989) *Standard Methods for the Examination of Water and Wastewater*. ed. L. S. Clesceri, A. E. Greenberg and R. R. Trussell, 17th edn. American Public Health Association, Washington D.C

[9] Henriques, M., Silva, A., & Rocha, J. (2007). Extraction and quantification of pigments from a marine microalga: a simple and reproducible method. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology Formatex*, *2*, 586-593.

[10] Al-Homaidan, A. A., Al-Abbad, A. F., Al-Hazzani, A. A., Al-Ghanayem, A. A., & Alabdullatif, J. A. (2016). Lead removal by Spirulina platensis biomass.*International journal of phytoremediation*, *18*(2), 184-189.