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Biosorption of Copper (Cu^{2+}) and Lead (Pb^{2+}) Ions by *Chlorella* sp. and *Navicula* sp. Isolated from Addalam River, Quirino, Philippines

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***Abstract*:** *Adverse ecological and health effects of heavy metals have increased ever since the second part of 20th century. The discharge of these metals into freshwater ecosystems as a result of pedogenetic processes and anthropogenic activities has become a serious problem all over the world. Biosorption is a cost-effective method of removing metal ions from aqueous solutions using biological materials. Microalgae are versatile biosorbents that can grow under a wide range of conditions. In this study, local strains of freshwater microalgae from Addalam River were successfully cultured under the optimal conditions of 28 ±2 °C temperature and light/dark duration of 12:12 h in Bolds Basal Medium (BBM). Two microalgae species were identified in their genera: Chlorella and Navicula, these were chosen as candidate species for this study. Biosorption capacity of Chlorella sp, and Navicula sp. was evaluated for the removal of Cu and Pb ions from aqueous solutions. Experiments were performed in BBM containing the selected heavy metals (10 mg L-1) with 1.00 mL of living algae at 28 ±2 °C and pH 6.6 ±0.1. The final metal ion concentrations after biosorption were analyzed using Atomic Absorption Spectrophotometer (AAS) for Cu and Electrothermal Atomic Absorption Spectrophotometer (ETAAS) after Microwave-Assisted Digestion for Pb. The results indicated that Chlorella sp. was a more competent species for the removal of Cu at 99.73% and Pb at 64.40% while Navicula sp. also showed a remarkable biosorption capacity at 87.10% for Cu and 62.60% for Pb. Taken together, Chlorella sp. and Navicula sp.; two microalgae from Addalam River—and possibly other microalgae species—may emerge as potentially more ecofriendly and economical alternative for the removal of toxic metals in wastewaters and probably offset the cost of eutrophication.*

Keywords: *AAS, Biosorption, Heavy Metals, Microalgae*

1. Introduction

Rivers, large and small, and all of their tributaries, are often referred to as the circulatory system of the planet: the arteries and veins of our landscape—quenching, nourishing, filtering, transporting, supporting life, providing places of beauty, serenity, and spirit. They provide fisheries, agricultural lands, tourism, water for farms, towns and cities, and they support populations, cultures, and economies. This is no mere metaphor, as people have seen what happens when too much or too little water damages entire ecosystems and affects all of the species that depend on them for life and livelihood. If water is the life-blood of the planet, then certainly its stewards constitute the immune system of its liquid pathways [1]–[2].

Majority of the world's rivers are deteriorating. They are either dying or are now endangered. We now face a situation where rivers are seriously unable to perform their valuable function of providing water and food, and many other services to humanity—to all life [2]. As specified by the Philippines Environment Monitor (PEM) of 2003, Region II has the highest potential source of groundwater of 2, 825 Million Cubic Meters (MCM) but then

the same report projects that by year 2025, water availability deficits would take place in several river basins in Cagayan Valley, and in all other regions in Luzon [3]. The productive potential of aquatic organisms has remained largely unexplored in this region; thus, it is important that species in these areas are documented before irreversible environmental damage sets in.

The Cagayan River, the longest river in the Philippines and one of the biggest resources of the region, is being threatened by urbanization and unsustainable economic activities. The apparent reasons are the visible anthropogenic effects such as increased water consumption, domestic (also called municipal), agricultural, and industrial wastewater discharges, and inorganic processes such as mining concessions near the headwaters of the tributaries, that degrade surface waters declining water quality and affect the biodiversity, and biological integrity of freshwater ecosystems [3]–[4]. The degradation of river systems and functional watersheds that lie dangerously close to sites of mining activities has been the object of serious concern in many countries throughout the developing world. Furthermore, the Mines and Geosciences Bureau (MGB) has attributed small-scale mining operations as the culprit behind the chemical contamination of rivers in the province of Nueva Vizcaya [5].

Consistent with the technical report of the Environmental Investigation Mission (EIM) of 2014 on the impacts of heavy metal contamination in Nueva Vizcaya, exceeded levels of copper concentration were observed in Brgy. Didipio, Kasibu. Copper concentrations in sediment samples ranged from 90.91–196.4 mg Kg-1 in Surong River, 509.2–924.1 mg Kg-1 in Didipio River, and 769.3–885.8 in Dinaoyan River which exceeded the Severe-Effect Level of 110 mg Kg-1. Also, the level of copper in water samples from Didipio River are 50 mg L-1 and 200 mg L-1 which exceeded the maximum level for both survival of aquatic organisms and irrigation use, respectively, indicating the water and sediments in the rivers are heavily polluted which could adversely affect the health of benthic or sediment-dwelling organisms and could be transported to agricultural lands that might result to the inhibition of plant growth and development as well as the reduction of grain yield [6].

Nevertheless, enrichment of water quality is based only on the physical and chemical parameters formulated by the Department of Environment and Natural Resources (DENR) for both surface and coastal water [3]. The mine site is usually situated in a remote, mountainous location where there are significant seasonal variations in rainfall. After high rainfall, there is a marked increase in rain run-off matter from wash-down sediments, impacting on tailings slurry and ore levels in solids, adding to the complexity [7]. In conventional wastewater treatment systems, the main aim is to treat the existing tailings storage facility outflow in order to reduce total suspended solids (TSS) and/or remove metals precipitated from solution to safely reintroduce into the adjacent river. However, industrial effluents are conventionally treated using a variety of hazardous chemicals for water quality correction. Extensive use of these chemicals for effluent treatment results in huge amounts of sludge or hazardous solid wastes generated by the industry and finally disposed by depositing them into the adjoining river [8].

Hence, to solve this water crisis and to meet the growing population's demand for freshwater, sustainable efforts have been and are being undertaken to address the growing concerns about deteriorating water quality and ecosystem health which lead to an increased interest in integrated development of freshwater systems and sustainable use and management of natural resources and a robust design to cope with fluctuating water quality and quantity. Such a cutting-edge approach is bioremediation.

Bioremediation is a biological treatment system of using specific microorganisms to transform or reduce the concentration of hazardous contaminants in water to nonhazardous waste products [9]. Bioremediation is, therefore, a process in which microorganisms are employed to rectify and reestablish water quality altered by contaminants to make them free from heavy metals and trace elements. However, many pollutants (e.g., heavy metals and persistent organic pollutants/POPs) can bind physically and chemically with water and persist for long periods of time to become bioavailable depending under certain hydrological conditions and exert adverse effects on aquatic organisms [10]. So it is very important to overcome this problem by investigating a suitable and economically feasible technique to remove these contaminants using biological systems [11]. Removal of

heavy metals from wastewater has traditionally relied on conventional techniques like sedimentation, flocculation, absorption and cations and anion exchange, complexation, precipitation, oxidation-reduction. Thus, the search for new cheaper technologies for the removal of heavy metals has been focused towards biosorption [12]. Biosorption is a cost-effective method of removing metal ions from aqueous solution using microbiological activity and uptake but plants and fungi are used more often. Until recently, the use of aquatic plants especially algae have received much attention due to their ability to sorb metals and take up toxic elements from environmental impact studies [13].

Microalgae (also called microphytes or phytoplankton) are photosynthetic organisms that lack true roots, stems or leaves and are part of the aquatic biomass. They vary from small, prokaryotic forms to complex eukaryotic forms and exhibit a wide range of reproductive strategies from simple asexual cell division to multifaceted forms of sexual reproduction. There are hundreds of thousands of microalgae species existing in freshwater where they form the basis for most food chains [14]–[15]. The use of biological organism such as algae for removing and recovering heavy metals from contaminated aquatic ecosystem has emerged as a potential alternative method to conventional techniques [14]. An inherent property of microalgae that make them attractive candidates for use in bio-industrial applications is their photosynthetic capability. Another advantage on the use of microalgae in bioremediation is the high biomass production by these species leading to high sorption of heavy metals [16]. Also, it has been reported that microalgae are so efficient in uptake of heavy metals from effluent water [17]. Biosorbent materials such as naturally occurring algae are generally less costly than existing technologies and remove pollutants by physical sorption, complexation, chemisorption as well as metabolically mediated physico-chemical pathways of uptake. In particular, algae have been proven to possess high metal binding capacities due to the presence of polysaccharides, proteins or lipid on their cell wall surfaces [18]. Algae removes heavy metals directly from polluted water by two major mechanisms: the first is a passive process (adsorption), and the second is an active process (absorption), a metabolism-dependent uptake [19].

Therefore, the use of microalgae for removal of contaminants from different wastes has been described by a number of authors worldwide: Manuel and Neyra-Tanabe, (2010) isolated Scenedesmus sp., Chlorella sp., and Oscillatoria sp. in Kematu River, T'boli, South Cotabato, Philippines and showed potential of these microalgae isolates for Hg removal capacity [14]. Imani et al. (2011) discussed that Dunaliella sp. is highly tolerant to the ascending concentrations of Hg, Cd, Pb and their absorption in aquatic media [20]. Brahmbhatt, Patel, and Jasrai (2013) have shown promise in the biological management of pollutants by Oscillatoria sp. from industrial wastewater at Ankleshwar GIDC area because of its high potential to absorb Cr and Pb [21]. Abd El Monsef, Ragab, and Shalaby (2014) confirmed that Eichhornia sp. collected from the Nile River at the El-Zomor canal (Giza) had high capacity for bioremoval of Pb and Cd [22]. However, different species have different sensitivities to chemical stress; therefore, the combination of a battery of contact tests with different organisms from the same organizational and trophic levels allows a better assessment of heavy metal uptake [10].

In view of such reports on the use of microalgae for removal of heavy metals from contaminated sites, the present study focuses on recent developments in bioremediation techniques with the aim of generating site-specific microalgae data for Addalam River, Brgy. Dibibi, Cabarroguis, Quirino. Additionally, new approaches such as high-throughput algal bioassay and modification on the methods of heavy metal elimination to achieve the goal of bioremediation in a cheaper and safer way were also discussed.

2. Procedure

2.1. Study Area and Sampling Station

The study was performed in Addalam River, Brgy. Dibibi, Cabarroguis, Quirino (impoundment reach) located at 16°25'34"N latitude and 121°31'28"E longitude on January 22, 2017. The selected site covers a substantial area of the Addalam Sub-River Basin, as well as a rich set of socio-ecological conditions. Addalam River is a tributary of the Cagayan River but forms a smaller-sized watershed consisting of 18 sub-watersheds. Addalam River has a total area of 104, 525 hectares. The largest portion of the Addalam River Watershed

consists of residual forest (34%) followed by grass land (16.3%), old growth (15.4%), and agricultural land (15.2%). Reconnaissance of Addalam River was undertaken in order to assess the actual situation, recent activities, and existing development of the area along the river system. Humidity forecast level reached to 79%, temperature was 24 °C, and pH was 8.36 at the time of sampling.

2.2. Collection of Water and Sediment Samples

Water and sediment samples were collected in Addalam River and shipped via overnight express at the Center for Natural Sciences Laboratory, Saint Mary's University. Using the Winogradsky Column, an enclosed self-sustaining microcosm for microbially driven linked global elemental cycles for culturing a large diversity of microorganisms, the samples were prepared by filling high density polyethylene containers with sufficient amount of sediment samples supplemented with organic carbon (filter paper), CaSO4, and CaCO3. The remainder of the sediments was added and then the overlying water sample. The space in the vessel contained sediment, water, and air in a 2:1:1 ratio. The vessels were covered for evaporation control and incubated at room temperature illuminated under a 40-watt fluorescent lamp for 2-4 weeks allowing growth of algae until test initiation for high throughput isolation.

2.3. Media Preparation

Bold's Basal Medium (BBM) was used in the isolation and biomass production of microalgae as well as in the biosorption process. BBM as culture medium was used for the entire research duration. The concentrations of nutrients in BBM (per 400 mL distilled H2O) are as follows: Six different stock salt solutions were prepared using KH2PO4, 6.00 g; CaCl2•2H2O, 1.00 g; MgSO4•7H2O, 2.00 g; NaNO3, 10.0 g; K2HPO4, 4.00 g; NaCl, 1.00 g. To 936 mL distilled water, 10.0 mL of each stock salt solution and 1.00 mL each of the following micronutrient solutions (per 1000 mL distilled H2O) were added: EDTA stock was prepared using Na2EDTA•2H2O, 50.0 g and 85% KOH, 31.0 g; H-Fe stock was prepared using FeSO4•7H2O, 4.98 g and 1 mL conc. H2SO4; Boron stock was prepared using H3BO3, 11.4 g; H-H5 stock was prepared using MnCl2•4H2O, 1.44 g; ZnSO4•7H2O, 8.82 g; Na2MoO4•2H2O, 0.71 g; CuSO4•5H2O, 1.57 g; Co(NO3)2•6H2O, 0.49 g; and 1 mL conc. H2SO4. pH was adjusted to 6.6 ±0.1 with KOH and autoclaved at 121 °C (15 psi for 15 min.) to dissolve. 1 mL of sterile vitamin stock: Cyanocobalamin, 0.001 g; Biotin, 0.001 g; and Thiamine, 0.200 g were then added after the medium had cooled to room temperature prior to use. For BBM plates, 15.0 g agar was added before autoclaving.

2.4. Isolation and Purification of Microalgae

Algae grown in the vessels were isolated and inoculated in 250 mL Erlenmeyer flask containing 150 mL of BBM under aseptic conditions. Three replicates were made for every sample. The cultivation of microalgae was conducted under optimized conditions at 28 ±2 °C, for a 12:12 h constant light cycle, under a 40-watt fluorescent lamp to establish a critical biomass. Culture vessels were agitated at least three times a day to achieve adequate mixing and enhance aeration. Once the desired biomass is achieved, 150 µL of mixed culture samples were inoculated near the periphery of a Petri plate containing 25 mL of BBM. Streak plating techniques were made to the whole surface of the agar plate. Plates were then incubated at a constant temperature of 28 ±2 °C. The second stage of culture involved the purification of algal cultures. Colonies were isolated one at a time and streaked onto BBM plates for further isolation. Sub-culturing was done until a pure culture was obtained. Stock cultures of all microalgae were maintained in BBM in the CNS Laboratory prior to experimental testing.

2.5. Morphological Characterization and Identification

Characterization of pure isolates using conventional description method was done via optical microscope observation of their morphological characteristics such as shape and dimension of cells (area) and length of the filament, cell type (unicellular or filamentous), presence or absence of heterocyst (for filamentous), presence or absence of akinetes (for filamentous), color, and pigment accumulation using the book of Bellinger and Sigee (2010) for the more frequently occurring freshwater algae [24]. Microscopic examination using Bestscope BS-

2036c Biological Microscope was done to assess the morphological diversity of colonies on BBM plate and selection was employed by choosing the microalgae isolates. Two microalgae species were initially identified based on their genera. Images were captured with ScopeImage 9.0 (X5). The identified isolates were chosen as candidate species for the biosorption process.

2.6. Biosorption Process

The ion exchange of heavy metals on microalgae species was carried out using the methods developed by Manuel and Neyra-Tanabe (2010) and Shamshad et al. (2015). All chemicals used in this study were supplied by Unilab as analytical-grade reagents (or the highest purity available) and distilled water were used in preparing all the solutions [14], [23].

Stock solutions of heavy metals were prepared by a synthetic solution of Cu2+ and Pb2+ from the correspondent chloride salts, CuCl2•2H2O and PbCl2, respectively, to final concentrations of 10.00 mg L-1 at pH 6.8 by using either 0.1 M NaOH or 0.1 M HCl in order to prevent precipitation. Microalgae were grown in 150 mL BBM for 3 days. 1.00 mL of the three-day old cell suspension was inoculated further in BBM containing Cu and Pb ions. One set without heavy metals was included as a control treatment. Cultures were incubated for 1 week at 28 ±2 ºC with constant aeration. After 7 days, the cell suspension was then filtered using Whatman filter paper (No. 42). Filtrates were used for metal analysis.

2.7. Analysis of Heavy Metals

The treated samples were analyzed based on the standard operational analysis set by the Department of Science and Technology (DOST) Cordillera Administrative Region, Regional Standards and Testing Laboratory, Km. 6, La Trinidad, Benguet. The total concentrations of residual metal ions in the liquid samples were determined using GBC SensAA Atomic Absorption Spectrophotometer (AAS) and PinAACle 900Z Electrothermal Atomic Absorption Spectrophotometer (ETAAS) after Microwave-Assisted Digestion, for Cu and Pb respectively. This is in accordance with the Standard Methods for the Examination of Water and Wastewater, (2012), 22nd edition, Methods 3030 K and 3113 B and Milestone Microwave Digestion Methods Manuals.

The biosorption capacity was calculated by the following equation: % Heavy Metal Removed = A/B x 100; where, A is the concentration of heavy metals removed in the aqueous solution after biosorption and B is the concentration of heavy metals introduced in the medium.

TABLE I: Determinations of CU and PB in BBM by Spectrophotometric Methods

Sample	Concentration of Cu (mg L^{-1})	Concentration of Pb (mg L^{-1})
BBM + *Chlorella*	ND	ND
BBM + *Navicula*	ND	ND
BBM + HM + *Chlorella*	ND	3.56
BBM + HM + *Navicula*	1.29	3.74

Copper. Atomic Absorption Spectrophotometric Method after Microwave-Assisted Digestion. Lead. Electrothermal Atomic Absorption Spectrophotometric Method after Microwave-Assisted Digestion. The results given in this report are those obtained at the time of test and refer only to the particular sample submitted. BBM = Bolds Basal Medium, HM = Heavy Metals (Cu and Pb), ND = Not Detected.

3. Results and Discussion

3.1. Isolation and Identification of Microalgae

Microalgae 1 was unicellular with spherical shape. It was pigmented green and about 40 µm in diameter and was without flagella. Microalgae 2 was unicellular with lanceolate or boat-shaped usually free-floating frustule covered with minute striae. The cell was motile with a diameter of about 20 µm. Microalgae 1 belongs to Family Chlorellaceae while Microalgae 2 is a diatom which belongs to Family Naviculaceae.

Based on the identification guide for the more frequently occurring freshwater algae by Bellinger and Sigee (2010), actual descriptions matched Chlorella sp. and Navicula sp [24]. The identification was further referred to available guides for the identification of freshwater algae. The microalgae in this study are well represented in the freshwater environment with the species occurring as unicellular organisms.

Chlorella sp. is a unicellular, spherical green alga and part of the Phylum Chlorophyta (Fig 1A). Its cell size is around 20–40 µm. The genus Chlorella is placed below the Order Chlorellales and Family Chlorellaceae. These include non-motile vegetative cells (autospores) which reproduce asexually and rapidly or they multiply by autosporulation, which is the most common asexual reproduction in algae [25]. This microorganism grows competently under non-strictly specified conditions meaning, it can grow under wide ranges of aeration rate, light intensity, and temperature, making it as one of the most versatile strains in algal culture industry [26]. Hence, Chlorella is one of the most attractive species owing to its fast growth and easy cultivation.

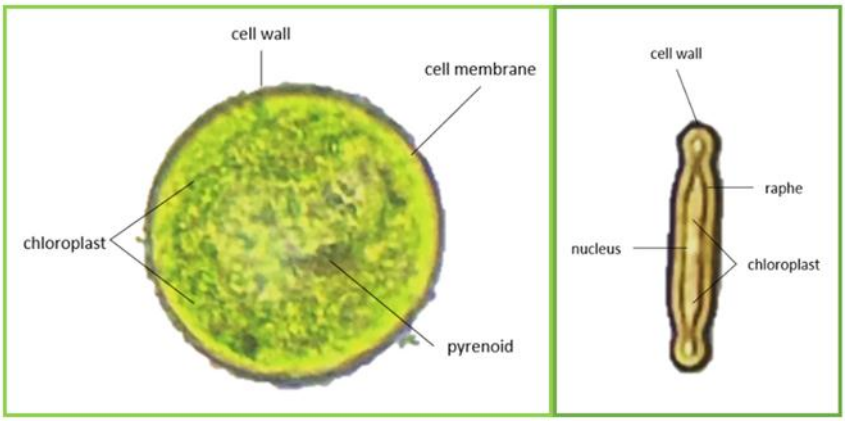

Fig. 1 (A) *Chlorella* sp. Cell detail, showing parietal chloroplast and apical clear region, (B) *Navicula* sp. A live cell with two elongate olive-brown chloroplasts

Chlorella sp. has many structural elements similar to plants. A thin layer as the first visible structure of the cell wall was observed. Its rigid cell wall consists of cellulosic material, and the rest is formed by saccharides and unknown substances [27]. The rigidity preserves the integrity of the cell and is basically a protection against acetolysis. Chlorella has chloroplast which stores a cluster of fused thylakoids where the dominant pigment chlorophyll is synthesized. The pyrenoid (a collection of starch granules) contains high levels of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) and is the center of carbon dioxide fixation [28].

Navicula sp., a pennate, siliceous diatom is placed into the Order Naviculales and Family Naviculaceae (Fig 1B). Its cell size is around 10–20 µm. Navicula became a genus that contained a large number of unrelated lanceolate, biraphid species. Hence, many of these unrelated species have been separated recently into new genera or replaced into the older, originally described genera. Diatoms of the genus Navicula reproduce both asexually as well as sexually in the form of auxospores. Cells of Navicula sp. are boat-shaped, motile, and solitary. These are elliptical in girdle view, and broadly-lanceolate in valve view. Valve ends are capitate and both valves have a central longitudinal raphe with a nodule (bump). The raphe is straight and filiform with unilaterally deflected distal ends. Each cell contains a definite nucleus in the middle and two chloroplasts, one on each side of the raphe [29].

Until now, there has not been mention of the biosorption capacity of these microalgae in the Cagayan Valley literature. Hence, the purified and identified isolates were further evaluated on their proficiency in sorbing metal ions. In this case, two metals have been tested: Cu and Pb. The environmental damage produced by these type of heavy metals is a worrying problem that has resulted in the promulgation of strict regulations by the Department of Environment and Natural Resources (DENR) and other national agencies to guarantee the quality of waters being disposed of from industrial processes of extracting metals [27].

Microalgae has been reported to have high metal sequestering ability and this fact had been confirmed in this study. Biosorption of Cu and Pb ions by Chlorella sp. and Navicula sp. were determined on the seventh day of incubation.

3.2. Biosorption of Cu and Pb using Chlorella and Navicula

Table 1 shows the report of analysis for the removal of Cu and Pb by Chlorella sp. and Navicula sp. grown at a concentration of 10.00 mg L-1. Moreover, a simple, fast, and sensitive method for the direct determination of heavy metals in BBM by AAS and ETAAS after Microwave-Assisted Digestion has been adopted in this study.

The Required detection Limit (RdL) or ND in Table 1 is expressed as the minimum concentration of the analyte that was detected by the instrument with a known level of confidence for a given analytical procedure [30]. The ability of microalgae to sequester metals within their cellular machineries has led to their widespread use as biomonitors of metal availability in freshwater ecosystems [19]. Hence, the microalgae species that were identified in the Addalam River do not respond to the copper and lead gradients suggesting that there was no bioaccumulation in the microalgae for both metals. The initial results revoked the indication that the isolated Chlorella sp. and Navicula sp. play a role in the copper and lead cycle in Addalam River.

The results of the biosorption of Cu and Pb in aqueous solution using Chlorella sp. and Navicula sp. are presented in Figure 2.

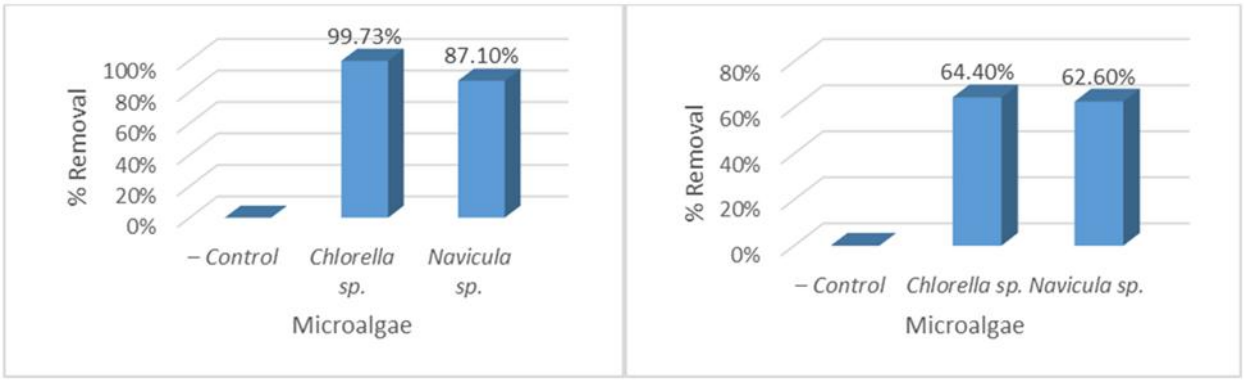

Fig. 2 Biosorption Capacity Expressed as, (A) Cu Removal and (B) Pb Removal in Percentage

Chlorella sp. has higher biosorption capacity as expressed in its Cu removal of 99.73% (0.0269 mg L-1 left in the solution) and Pb removal of 64.40% (3.56 mg L-1 left in the solution) compared to Navicula with 87.10% (1.29 mg L-1 left in the solution) and 62.60% (3.74 mg L-1 left in the solution). Similarly, Chlorella sp. has higher capacity for taking up each Cu from aqueous solutions. Although Chlorella sp. showed better removal of Cu, Navicula sp. also showed a remarkable biosorption capacity. The microalgal isolates were considerably efficient in the biosorption of Cu.

The results of this study are in agreement with those reported by the following authors: Inthorn, Sidtitoon, Silapanuntakul, and Incharoensakdi (2002) stated that Chlorella vulgaris var. vulgaris has the highest Cd, Hg, and Pb removal efficiency at 89%, 94%, and 88%, respectively. Also, the authors specified that C. vulgaris had the highest maximum adsorption capacity of 127 mg Pb g-1 dry weight at a minimum concentration of 130 mg L-1 [31]. The work of Abu Al-Rub, El-Naas, Ashour, and Al-Marzouqi (2006) showed that C. vulgaris were also effective in removing Cu ions from single, binary, and ternary metal aqueous solutions [32]. The study of Al-Qunaibit (2009) showed the performance of C. vulgaris in terms of binding divalent Cd, Cu, and Pb ions from aqueous solutions [33]. In addition, Manuel and Neyra-Tanabe (2010) observed that Chlorella sp. had the highest maximum uptake capacity of Hg at 40% [14]. Salam et al. (2014) which showed that a mixed culture of Navicula exigua removed variable amounts of Cu and Ni from artificial wastewaters at 37% and 96%, respectively [34]. Cherifi, Sbihi, Bertrand, and Cherifi (2017) reported the ability of N. subminuscula for the

removal of hexavalent chromium (Cr6+) up to 10 mg L-1 from tannery effluents and wastewaters. Their research also showed that N. subminuscula has the ability to sorb Cd, Cu, and Zn at a concentration of 100-130 mg L-1 from aqueous solutions [35].

Copper has no effect on other cell functions such as photosynthesis, respiration, ATP production, electron transport and membrane ultrastructure, although it inhibits cell division of microalgae [36]. However, as reported by Kebeish, El-Ayouty, and Husain (2014), there is a significant increase in carotenoids, intracellular proline contents, and activity of antioxidative enzymes such as catalase, peroxidase, polyphenol oxidase, and superoxide dismutase in C. vulgaris following Cu exposure. Consequently, C. vulgaris showed diverse response to Cu stress on biochemical, molecular, and physiological levels [37]. As studied by Rueter (2003) in diatoms (Class Bacillariophyceae), silicic acid uptake was found to be the function of the cupric ion activity in the growth medium. Accordingly, intracellular soluble pools of silicic acid are a good indicator for the relative spontaneity of metal uptake [38].

Lead affects photosynthesis by interacting with the essential metals integrated in photosynthetic apparatus or in metalloenzymes necessary for photosynthesis [39]. However, studies of its effect on algal photophysiology, including the functional organization of photosystems, are limited.

Microalgae have relatively high biosorption capacities arising from the intrinsic composition of their cell walls which contain negatively charged functional groups and sorption of heavy metals involves the exchange of metal ions with surface-bound cations [23]. For this reason, Dwivedi (2012) believed that during biosorption, the competition for the functional groups between metal ions and other ions played an important role. Therefore, the presence of various functional groups such as amine, carboxyl, and hydroxyl groups had enabled the freshwater microalgae to remove heavy metals from wastewater. Considering its mechanism, biosorption is still affected by several physical factors [9]. As reported by Stirk and Staden (2001), the size of the sorbent particles affects the initial rapid metal ion biosorption. Their study has shown that the larger the surface area of the sorbent material, the more rapid the biosorption of heavy metals [40]. Thus, Chlorella, whose wider cell surface, has a greater biosorption capacity than Navicula.

Considering the role of microalgae in aquatic ecosystems as primary producers of biomass for higher forms of animals or the basis of the food chain in any ecosystem, this may bring out issues on bioaccumulation of copper and lead on sources of human consumption such as fishes and shellfishes. It is then recommended that further study be conducted to design a technology that allows the application of microalgae-mediated bioremediation without biomagnification on other organisms. As a final point, the use of algal biomass for heavy metal removal can offset the cost of eutrophication.

To maximize algae biosorption sustainability, nutrients must be recycled. Improved strains, as well as downstream efficiency, are integral aspects of the algae bioremediation strategy.

The adsorbed metal is removed by desorption process and the biosorbent can be reused for further treatments. Algae will be harvested and the oil will be extracted, the remaining biomass will either be recycled for nutrients through anaerobic digestion or similar means, producing methane gas and exogenously produced fertilizers, or used for high-value co-products, ranging from industrial enzymes, and nutraceuticals. Some of these nutrients can be recycled through wastewater, while others will be lost due to runoff. Algal-based bioremediation could become more attractive if the biomass cultivated in bioremediation ponds could be used as a feedstock for the production of bioproducts. The integrated cultivation of microalgae overcomes constraints to the production of biomass by using non-arable land, non-potable water, and carbon emissions to support productivity. Integrated algal systems can be used for wastewater treatment and bioremediation to capture inorganic nutrients from industrial wastewaters.

With the foregoing results, it is highly recommended that these microalgae species be used in the in situ biosorption of heavy metal polluted effluents toward improvement of the associated economic framework. Further studies are necessary to ascertain results with other initial algal densities and higher efficiencies in the

biosorption process of the microalgae. Later on, concrete applications of such techniques at a larger scale would be beneficial for the bioremediation of wastewaters since there is a lack of industrial wastewater treatment.

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