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Biosorption of Heavy Metals by *Oscillatoria* Species

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Authors' contributions

This work was carried out in collaboration among all authors. Author MLR designed the study, performed the experiments, wrote the protocol, and wrote the first draft of the manuscript. Author HYI performed the statistical analysis, wrote the manuscript and managed the literature searches. Author MS performed the experiments and the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Several anthropogenic activities have led to serious health and environmental problems as a result of releasing different contaminants in to the ecosystem including heavy metals. This called for search of possible methods that could be used to ameliorate the environment and biosorption was found to be promising.
Aim: The potential of *Oscillatoria* sp. was investigated with a view to determining its suitability in the biosorption of Cr^{2+} and Pb^{2+}.
Place and duration of Study: The study was conducted at the Research Laboratory, Department of Microbiology, Usmanu Danfodiyo University Sokoto, between January and July, 2016.
Methodology: Samples of the algal species were collected from an irrigation site at Kwalkwalawa area of Usmanu Danfodiyo University Sokoto. The samples were dried and powdered for biosorption studies. Biosorbents were prepared and used for sorption of heavy metals at different time and substrate concentration.

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Results: It was observed that *Oscillatoria* sp. biomass could adsorb appreciable amounts of the metals in a dose and contact time-dependent manner. At lower biomass doses, lower rates were recorded with a mean of 0.002 mg/g and increased to 0.1mg/g when 2 g and 5 g biomass were used respectively in both cases of the metals uptake. Based on contact time, Cr^{2+} uptake was initially slow with mean value of 0.002 mg/g for the first 50 minutes and rapidly increased to optimum at 60 minutes of contact time. For Pb^{2+} however, uptake was rapid with peak value of 0.1mg/g for the first 20 minutes. The uptake drastically decreased at 35 and 50 minutes and equilibrium was attained at 60 minutes of contact time.

Conclusion: *Oscillatoria* sp. has the potentials of Cr^{2+} and Pb^{2+} uptake and thus suitable for biosorption of low heavy metals concentrations.

Keywords: Oscillatoria sp; heavy Metal; biosorption; biomass; chromium; lead.

1. INTRODUCTION

Heavy metals are metals with relatively high densities, high atomic weight or high atomic numbers. Heavy metals are found naturally on the Earth without trepidation. The persistence and non-biodegradability of heavy metals remain one of the major threats to living organisms and ecosystems. They impose serious health risks when present in living tissues and subsequent accumulation throughout the food chain [1]. The increase in abundance of heavy metals in the ecosystem results from anthropogenic and geogenic activities [2]. Increase in small-scale industrial activities especially metal finishing, alloy making, electroplating, mining and battery manufacturing has led to many-fold increase in the release of heavy metals [3] due mainly to carelessness and low returns from investing in the area of effluent treatment. Presence of these metals in living systems leads to serious problems in different living organisms. In humans, accumulation of some toxic metals like Mercury, Nickel, Copper, Cadmium, Chromium and Zinc has several bad consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and a wide range of other kinds of illnesses [4]. In plants also, retention of such metals in the plants' bodies lead to malfunction and ultimate decrease in productivity [5]. In aquatic ecosystem, the effect is believed to be deleterious to various fauna and flora.

Lead (Pb) is the most abundant of the heavy metals in the Earth's crust. It has been used since prehistoric times, and has become widely distributed and mobilized in the environment [6]. At high levels of human exposure, there is damage to almost all organs and organ systems, most importantly the central nervous system, kidneys and blood; culminating in death at excessive levels. At low levels, haeme synthesis and other biochemical processes are affected, psychological and neuro-behavioural functions are impaired in addition to other effects [6,7]. Similarly, chromium (Cr) levels in the environment are on the increase day-by-day as a result of continuous effluent discharge from industries like chrome leather, tanning, metallurgy, chrome plating, textiles, ceramics, photography and photoengraving [8]. Its health implication in humans includes lung cancer, chromate ulcer, nasal septum perforations and damage to kidney [9].

However, several methods have been devised for the treatment and removal of heavy metals in contaminated sites. Conventional physico-chemical methods for heavy metal removal such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation and sorption are economically expensive and have disadvantages that are linked to incomplete metal removal, expensiveness and eco-friendliness [10,11]. Bioremediation has emerged as the most desirable technology which uses living organisms for removal of environmental pollutants or detoxification to make them harmless. A lot of studies have explored the applicability of microorganisms in ameliorating heavy metal contamination and were found to be promising [12,13,14]. This led to the development of some biotechnological processes for remediation of polluted environments tagged as bioaccumulation and biosorption [15]. These technologies are believed to be more effective than the conventional processes that are used for the removal of heavy metals from industrial wastewaters. Advancement in these technologies has led to use of living or dead organic matter as bio-adsorbents for removal of pollutants including heavy metals [16].

Studies have shown that members of the group algae are very important in sorption of heavy metals [3,5,17]. The algae have many features that make them ideal for the selective removal and reducing the concentration of heavy metals, which include high biosorption capacity, high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin production and its potential for genetic manipulation [18]. Effectiveness of some species of the genera *Phormidium, Scenedesmus, Euglina, Chlorella, Spirogyra, Spirullina, Cladophora* and *Oedogonium* as reported by several investigators ranged between 31% and 99% heavy metal removal [5]. The present study intended to explore the potentials of *Oscillatoria* sp. for biosorption of Cr and Pb, with a view to studying some parameters that might influence the sorption process. *Oscillatoria* sp. is one of the most common algae considered as bioindicators of eutrophication and readily available in tropical regions.

2. MATERIALS AND METHODS

2.1 Sample Collection

Algal samples were collected from an irrigation farm site at Kwalkwalawa, along Usmanu Danfodiyo University, Sokoto using a sieve and a clean transparent rubber bucket which was immediately transported to the laboratory for identification. The metal salts and the reagents used were of analytical grade and they were obtained from Fisher Scientific (Loughborough Leics, England). $Pb(NO_3)_2$ (331.21 g/mol, 99% purity) and $CrCl_3$. $6H_2O$ (266.5 g/mol, 99% purity) were used as sources of heavy metals for Pb and Cr respectively.

2.2 Sample Identification

The samples were identified to generic level using standard phycological procedures. An aliquot of the algal sample was transferred to a clean glass slide. A drop of water was added to the slide and was covered with clean cover slip and observed under microscope using×10 objective lens. The structures observed were compared with those in Phycological Atlas [19].

2.3 Preparation of Biosorbent

The biomass of the blue-green algae (*Oscillatoria* sp.) used in the present study was washed with sterilized distilled water several times to remove dirt particles. The washing process was continued till the wash water contains no dirt. The washed *Oscillatoria* sp. was air-dried in a room for 7 days. The dried *Oscillatoria* sp. was then cut into small pieces and powdered using domestic mixer (blender). The powdered material was passed through a domestic (100 mm in diameter) sieve then directly used as biosorbent without any pre-treatment [20].

2.4 Preparation of Stock Solutions

Stock solutions of the metal concentration 1000 mg/L were prepared by dissolving 1.6 g of $Pb(NO_3)_2$ and 5.1 g of $CrCl_3$. $6H_2O$ in 1000 ml of distilled water. Working solutions of different concentrations of the metal stock solutions varying between 10 and 40 mg/L were prepared. The pH of the solutions was adjusted with 0.1 M H_2SO_4 and 0.1 M NaOH. Blank experiments were conducted to ensure that no biosorption was taking place on the walls of the apparatus used. All glass wares were washed with 0.1 M H_2SO_4 before and after each experiment to avoid binding of the metal [21,22].

2.5 Effect of Biosorbents Dose

Effect of biomass on biosorption was investigated based on the methods of [23]. The biosorbents dosages used were 2,5,8 and 10 g. Using uniform initial concentration of metal ions (100 mg/L) at 25°C and pH of 7, it was agitated on a flask shaker (Gallenkamp, England) at a speed of 6m/s for 3 hours. The biomass was separated from the residual sorbate solutions by centrifuging for 10 minutes using an Mpw12 centrifuging machine. Residual metal ions of both metals were analysed using a Bulk Scientific Atomic Absorption Spectrophotometer AAS (Model 210 VGP) after filtering with Whattman filter paper (110 mm diameter×100 circles).

2.6 Effect of Contact Time on Biosorption

The effect of contact time on biosorption was obtained by varying the time in the range of 5, 15,20,35,50 up to 60 minutes. The experiment was carried out using the best performing-quantity of biosorbents obtained from the biosorbent dose experiment; uniform initial concentration of metal ions (100 mg/L) and optimum pH. It was agitated on a flask shaker (Gallenkamp, Englang) at a speed of 6m/s for the different time range mentioned above. The biomasses were separated from the residual sorbate solutions by centrifuging for 10 minutes using an Mpw 12 centrifuging machine. Residual metal ions of both metals were analyzed using a Bulk Scientific Atomic Absorption Spectrophotometer AAS (Model 210 VGP) after filtering with Whattman filter paper (110 mm diameter x 100circles) [23].

3. RESULTS

Table 1 shows the characteristics of the alga used in this study. The morphology of this species indicated that it was a filamentous, dark green, single-celled and slimy alga, typical of *Oscillatoria* sp.

Chromium (Cr^{2+}) uptake by *Oscillatoria* sp. at a temperature of 25°C and pH of 7 is shown in Fig. 1a. Cr^{2+} uptake initially increased with increase in biomass dosage from 0.002 to 0.1 mg/g for biomass of 2 and 5 g respectively. However, at biomass concentration of 8 g, the uptake decreased to 0.002 mg/g of the solution. It also showed that equilibrium was reached at 10g of the biomass dose. Lead (Pb^{2+}) uptake by *Oscillatoria* sp. at a temperature of 25°C and a pH of 7 is shown in Fig. 1b. Pb^{2+} sorption initially increased with increase in biomass dosage from 0.002 to 0.01 mg/g for biomass of 2 and 5 g respectively. However, equilibrium was attained at 8 g (0.1 mg/g). Fig. 1b also showed a decrease in Pb^{2+} uptake at 10 g (0.02 mg/g) of the biomass dose.

Chromium (Cr^{2+}) uptake by *Oscillatoria* sp. at a temperature of 25°C and a pH of 7 obtained by varying the contact time is shown in Fig. 2a. Cr^{2+} uptake was initially slow with constant values of 0.002mg/g for the first 50 minutes. Cr^{2+} uptake rapidly increased and reached optimum at 60 minutes of contact time. Lead (Pb^{2+}) uptake by *Oscillatoria* sp. at a temperature of 25°C and a pH of 7 obtained by varying the contact time is shown in Fig. 2b. Lead (Pb^{2+}) uptake was rapid with peak values of 0.1 mg/g for the first 20 minutes. The uptake drastically decreased at 35 and 50 minutes of contact time and equilibrium was fully attained at 60 minutes of contact time.

4. DISCUSSION

Biosorption is considered one of the most important techniques for removal of heavy metals in solution. In this study, dried biomasses of Oscillatoria sp. were used to investigate its suitability in Cr^{2+} and Pb^{2+} biosorption. The organism was collected and identified based on morphological characteristics as observed under microscope. Previous studies have used both living and dead cells of *Oscillatoria* sp. in biosorption of different metals. Brahmbhatt et al. [24] exposed exponentially growing *Oscillatoria* sp. to various concentrations of Cr^{2+} and Pb^{2+} and observed appreciable accumulation of both metals from solution. Azizi et al. [1] observed that dried biomass of *Oscillatoria* sp. showed a higher biosorption capacity than the living cells. It is believed that nonliving microbial biomass may display a higher affinity for metal ions compared with viable biomass probably due to the absence of competing protons produced during metabolism. Recovery of metals and regeneration of biosorbent is complicated for living cells. Living cells are likely to be more sensitive to metal ion concentration and adverse operating conditions of pH and temperature; in addition to constant nutrient supply required for sustaining living cells. As a result, the dried, non-living, or pre-treated microbial biomass seems to be a preferred alternative to the use of living cells in industrial applications for the removal of heavy metal ions from waste waters [25].

Results from this study showed that the biosorption of Cr^{2+} and Pb^{2+} were influenced by the biomass dosage. At lower doses, the metal sorption was low and increased with increase in biomass. This might be due to the fact that increases in the biomass led to increase in binding sites for metal attachment. This agreed with the findings of [1] who observed increase in biomass concentration caused an increase in Cd (II) biosorption capacity. However, further increase in biomass above 5g/L led to drastic decrease in biosorption capacity especially for Pb^{2+}. Ahuja et al. [26] observed that at higher biomass concentrations, aggregates are formed which can reduce the active biosorption sites thus, less sites are available for metal binding. According to Abbas et al. [27], biosorption depend greatly on biomass concentration and at low concentrations, adsorption sites took up the available metal more quickly, whereas, at higher concentrations, metal ions need to diffuse to the biomass surface by intra-particle diffusion and greatly hydrolyzed ions will diffuse at a slower rates. However, it is well established that the extent of metal binding depends on metal chemistry, nature of binding and metal affinity for binding sites on the adsorbent [25]. Studies by Hussain et al. [28] revealed that the percentage of heavy metals removal does not alter greatly if the concentration increases from 10 to 50 mg/l, and thus support our findings.

Table 1. Morphological characteristic of the alga

Sample	Observation/colour	Texture/identified organism
Algae	Filament not a spiral A single spiral cell dark green	Slimy/thread-like *Oscillatoria*

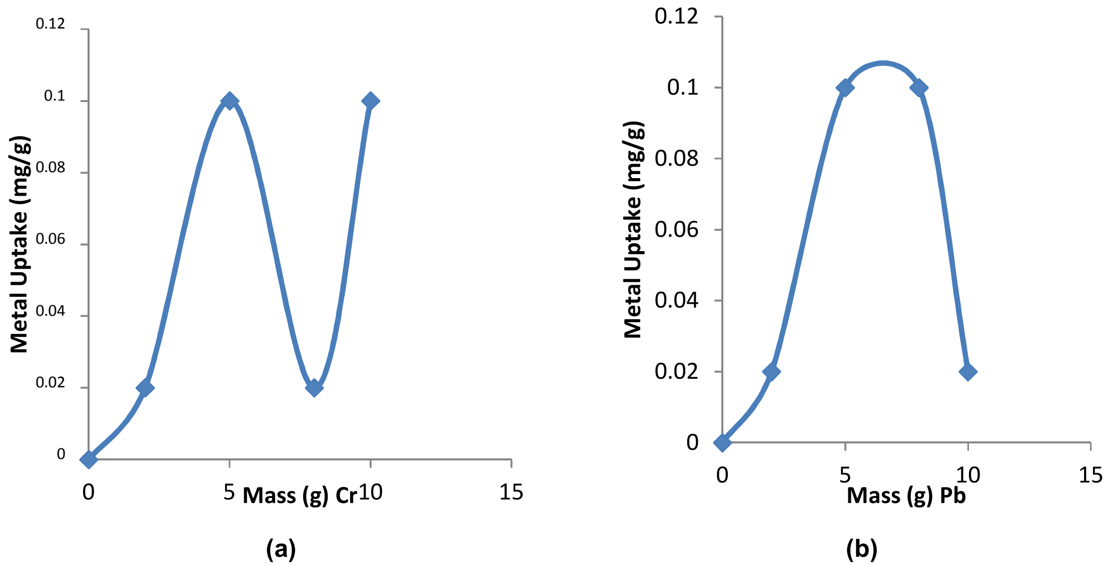

Fig. 1. Dose-dependent biosorption of a). Chromium and b). lead

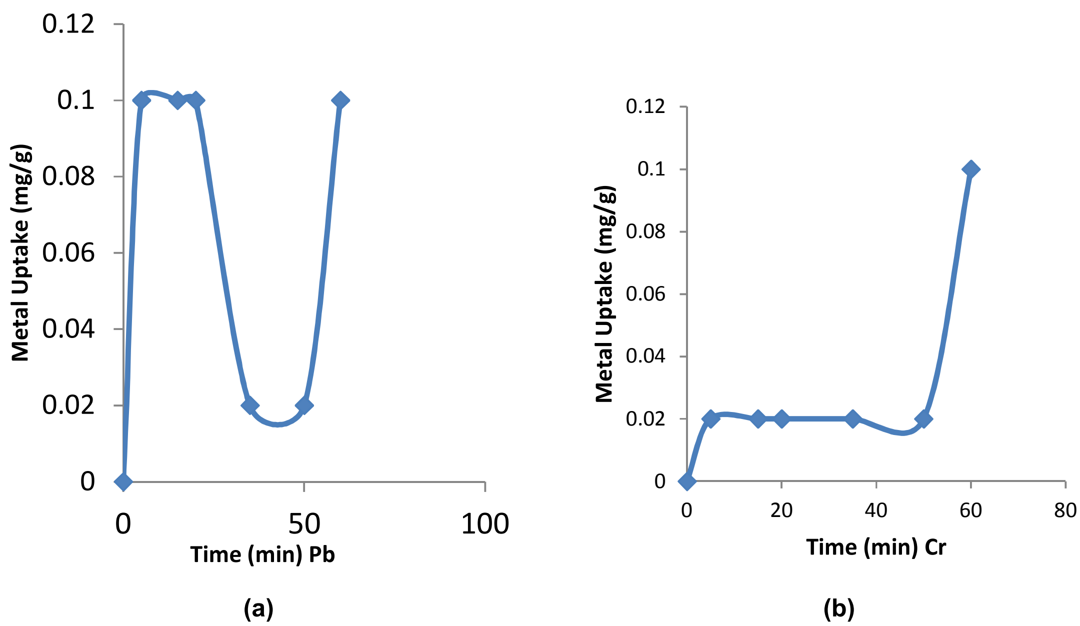

Fig. 2. Effect of time on biosorption of (a). Chromium and (b). Lead

The effect of contact time on biosorption of the metals was investigated. Fig. 2 showed that sorption of both of the metals was increased in the first 5 minutes followed by a constant activity for the next 40 to 50 minutes before a rapid increase was observed within the next 10 minutes. This may be due to the fact that a large number of vacant surface sites were available for adsorption during the initial stage. Near the steady phase, the remaining vacant surface sites were difficult to be occupied due to repulsive forces between the solute molecules on the solid and bulk phase. In biosorption studies conducted by [29,30] similar observations were made using *Spirogyra* and *Oscillatoria* respectively. According to some researchers, adsorption of heavy metals could be divided into two stages: a fast initial phase followed by a much slower biosorption phase. The fast (initial) metal biosorption phase was attributed to the surface binding between the negatively charged cell surface ligands and metal cations and the following slower sorption rate was attributed to the interior penetration of the metals which slows further uptake as shown in similar researches [1,31]. However, in this study, a second fast rate was observed and might be as a result of subsequent increase in vacant surface sites after initial penetration. At 60 minutes period, the biosorption reached its peak and some studies have suggested that, beyond that period no much effect is observed. Peng et al. [3] observed that about 90% of the total adsorbed Cu (II) or about 80% of the total adsorbed Zn (II) was removed from solution within 30–40 minutes contact time. The amount of biosorption increased with time at slower rates after the rapid biosorption period, and no further significant increase was observed beyond 1 h. This is in accordance with the study of [32] who observed that adsorption equilibrium of Cr could be reached in about 60 min period using biomass of *Spirulina* sp. Ibrahim et al. [33] have also made similar observations using a marine alga - *Ulva lactuca*.

It can be deduced from this experiment that Cr^{2+} and Pb^{2+} can best be adsorb between 20 to 60 minutes of exposure using 5 g *Oscillatoria* biomass at 25ºC and pH 7. Several parameters such as contact time, algal dose, effect of pH, and initial concentration of metal ions have been shown to significantly affect the uptake of various metals in biosorption process. A study by [34] has listed biosorbent size and agitation speed among other factors that influence heavy metal biosorption.

Generally, results in this study showed a low biosorption capacity by the algal species when compared with some similar investigations. This might be a function of pH at which the experiment was carried out. In biosorption process, pH of the medium is believed to affects two aspects: metal ion solubility and biosorbent total charge, since protons can be adsorbed or released [35]. More so, [31] observed that in untreated algal biomass, there is presence of alkali and alkaline earth metals such as K^+, Na^+, Ca_2^+, and Mg_2^+ which comes from the algal growth medium like sea- and freshwater. As a result, pH increases and releases light metal ions when algae biomass reacts with heavy metal-bearing solutions. This could have been the main reason of low biosorption capacity observed in this study.

5. CONCLUSION

In this study it is evident that dried biomass of *Oscillatoria* sp. have the potentials of adsorbing Cr^{2+} and Pb^{2+} in solutions. Biomass dosage and contact time influenced the biosorption process; and optimum adsorption was obtained at 60 min using 5 g biomass. Effect of other physicochemical properties need to be investigated in order to reap the full potentials of the organism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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