ISSN 2449-8955 European Journal of Biological Research *Research Article*

DOI: http://dx.doi.org/10.5281/zenodo.4023161

Heavy metals biosorption by urease producing *Lysinibacillus fusiformis* 5B

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Received: 08 June 2020; **Revised submission:** 10 August 2020; **Accepted:** 27 August 2020

http://www.journals.tmkarpinski.com/index.php/ejbr

ABSTRACT: Biosorption is the ability of biological materials to accumulate heavy metals from wastewater through mediated or physico-chemical pathways of uptake. Urease producing bacteria have been hypothesized to have inherent bioremediation abilities. The aim of this research was to determine the potential of *Lysinibacillus fusiformis* 5B to biosorp Pb, Cr, Cd and Ni. The stock solution of Pb, Cr, Cd and Ni was prepared by dissolving 0.0157 g of $Pb(C_2H_3O_2)_2$, 0.057 g of $K_2Cr_2O_7$, 0.018 g of $CdSO_4$ and 0.026 g of $NiSO_4$ in 100 mL of dH_2O respectively. *Lysinibacillus fusiformis* 5B was screened for the potential to utilise 5 ppm of the heavy metals using agar dilution method. Broth of *L. fusiformis* 5B was inoculated to 10, 15, 20 and 50 ppm of the heavy metals. The rate of biosorption was determined by atomic absorption spectroscopy (AAS) after 0, 7, 14, 21, 28 and 35 days. The biosorption % was determined by Beer Lambart's equation. *Lysinibacillus fusiformis* 5B was able to tolerate 5 ppm concentration of all the heavy metals by showing visible growth on surfaces of nutrient agar Petri plates. Generally, there was an increase in biosorption rate as the days progress. After 35 days of incubation, the highest biosorption rate of 99.96%, 99.97%, and 99.94% were recorded for Pb, Cr, and Cd respectively at 10 ppm and 99.33% of Ni at 15 ppm. The results of this study showed that *L. fusiformis* 5B possess the capacity to biosorp Pb, Cr, Cd and Ni and can be developed as biosorption agent for these heavy metals.

Keywords: *Lysinibacillus fusiformis* 5B; Biosorption; Cadmium; Chromium; Lead; Nickel.

1. INTRODUCTION

Increase in human civilization and active involvement in industrialization have caused improvements in the standard of living of humans across the globe. However, the industrialization has led to the release of harmful chemical substances, which have now become an environmental problem affecting both plants, animals and man [1]. Some of these harmful substances enter the environment as solid, liquid and gaseous wastes [2] from anthropogenic activities, which have greatly contributed to the abundant presence of heavy metals in the environment [2-5].

Aside anthropogenic activities, heavy metals can also be released naturally into the environment *via* volcanic eruption and the weathering of metal-bearing rock particles [2, 6-7]. These heavy metals have been

reported to deter physiological functions of biological systems [1]. Metals whose densities are greater than 5 g/cm^3 are categorized as heavy metals. These heavy metals have atomic number greater than 20 and are poisonous and toxic at low concentrations [3]. Some heavy metals (i.e. Cu, K, Cr, Fe, Zn, Ni, Na, Mg and Mn) serve as essential micronutrient required by biological systems in small concentration to stabilize molecules through interactions that are electrostatic [3, 8-9]. They are also required for various redox processes and the regulation of osmotic pressure as well as a component required for a functional enzyme system [8]. Whereas other heavy metals (i.e. Al, Pb, Cd, Au, Hg, Ag) are non-essential and have no biological function. They are toxic and a potential threat to all biological system [8-11].

Human exposure to these heavy metals above the World Health Organization safe level published by Olawale [12] and Calderón *et al*. [2] could lead to adverse health effects such as; nausea, skin allergy, fluorosis of skin, indigestion, diarrhea as well as cancer. Other health impacts include neuronal damage, chronic asthma, kidney and liver damage, cardiovascular disorders, teratogenicity, mutations, congenital disorders, impairment of sensory nerves, chronic anemia, memory loss, depression, autoimmune diseases, mood swings, anxiety, drowsiness, hair loss, fatigue, blindness, brain damage and insomnia [4, 8, 12-14].

These among many more health disorders associated with heavy metal exposure have caused scientists and research bodies all over the world to search for novel technologies to alleviate this environmental threat. Conventional methods involving physical or chemical processes have been and are still in use in alleviating heavy metal polluted environment [12]. Some of these conventional methods include; membrane filtration, chemical precipitation, floatation, electrodialysis, reverse osmosis, solvent extraction, photocatalysis, ion exchange, electrochemical treatment, microfiltration, ultrafiltration and nanofiltration [11, 13-15], seem effective but cause other environmental problems such as destruction of soil structure, generation of secondary pollutants, which are resistant to other cleaning treatments, expensive to install as well as time consuming [16-18]. These limitations have made researchers to exploit biological means.

The use of biological materials in remediating an environment polluted by heavy metals is known as bioremediation [11, 19]. Bioremediation is inexpensive and above all, environmentally friendly. It could either be *ex-situ* or *in-situ* through biomineralization, bioaccumulation, bioleaching, biotransformation or biosorption of the heavy metals [1, 5, 13, 20-21].

Biosorption have emerged as the most promising technology among other bioremediation techniques, which involves a passive uptake mechanism and is most times reversible and is not dependent on metabolism of the biological material but rather the surfaces of the materials, which acts in the sorbent of heavy metals [1, 2, 12, 20]. Biosorption could either be carried out by sorbent materials from biological means, which could be from plants and most times from microbial source [1, 11, 23]. The use of microorganisms in biosorption of heavy metals have received great attention due to fact that they can be sourced cheaply, with high effective adsorption capacity, are reusable, and could utilize both living and dead cells since it only involves the cell wall of the microorganisms [3, 11].

Many factors influence the capacity of microorganisms to adsorb metals from the environment, which include; microbial status (age of cell), properties of the metal ions (valence, radius among others), biosorption conditions (i.e. temperature, pH, contact time, concentration of microbial biomass and metals, presence of other ions, micronutrition and metal ions availability) and culture conditions (composition of growth media, nutrition supply and carbon source) [11, 19]. Among microorganisms (fungi, yeast, algae and bacteria) [24] involved in biosorption, though fungi are effective but bacteria have emerged as the most promising with fast growing rate and a wide range of binding sites. Bacteria have over time evolved and created specific genes

that help them to survive in environment contaminated with heavy metals. Most used bacteria genera in alleviating environmental pollution include; *Bacillus* and *Pseudomonas* owing to their high binding affinity for heavy metals [4]. The functional groups present on bacteria surfaces such as sulfonate, carboxyl, hydroxyl, phosphonate, amide and sulfonate are mainly utilized in active metal uptake from the environment [1]. The presence of these functional groups on bacterial surfaces makes it possible for binding of metals through ionic binding of metal cations involving electrostatic forces. Structure of bacteria cell has made it possible to be distinguished as either Gram positive or Gram negative. Cell wall of Gram positive bacteria is thicker than that of the Gram negative bacteria because of the thick peptidoglycan and presence of teichuronic and teichoic acids [25]. This is however opposite of Gram negative bacteria, which have a thin peptidoglycan without teichuronic and teichoic acids. This characteristic has made Gram positive bacteria to be most efficient in adsorption of heavy metals [1].

Lysinibacillus fusiformis is Gram positive, rod-shaped, spore forming, non-motile bacteria belonging to the Bacillaceae family. It is referred to as *Lysinibacillus* due to the presence of Asp-Lys type of peptidoglycan in the cell wall. Varying bacterial species have been engineered such that their enzymes play an essential role in biomineralization of heavy metals [26]. This is true about ureolytic bacteria such as *L. fusiformis* capable of producing urease, which hydrolyses urea to give ammonia and carbon dioxide [27]. Ureolytic bacteria are capable of forming bonds with heavy metals to form minerals as an important path during biogeochemical cycles of elements [28]. In these biogeochemical cycles, calcium carbonate produced during hydrolysis of urea causes the precipitation of soluble heavy metals in what is known as microbial induced minerals precipitation (MIMP). During MIMP, bacterial cells can hold heavy metals and radionuclides within their cells by adsorption and/or coprecipitation of the heavy metals in lattice of calcite [29]. *Lysinibacillus fusiformis* have been previously reported by varying literatures on their prowess potential in the sequestration, intracellular transformation, precipitation and volatilization of chromate [30], boron [31], mercuric chloride [32], cadmium and copper [23], magnesium and calcium ions [33]. As such, the aim of this research was to biosorp lead, chromium, cadmium and nickel by urease producing *L. fusiformis* 5B.

2. MATERIALS AND METHODS

2.1. Sample collection

The bacterial strain *L. fusiformis* 5B used in this study was collected from the laboratory of Department of Microbiology, Federal University of Technology Minna, Nigeria. The isolate was reported to have the ability to produce urease. The isolate was subcultured into newly prepared nutrient agar (NA) so as to get a fresh culture for the study. The purity of the strain was confirmed by Gram staining and viewing under the microscope at ×100 objectives lenses.

2.2. Preparation of heavy metal solutions

The stock solution of nickel sulfate and cadmium sulfate was prepared by dissolving 0.026 g and 0.018 g respectively into 100 mL of distilled water. Whereas 0.057 g of potassium dichromate and 0.0157 g of lead acetate was measured and dissolved into 100 mL of distilled water to get their respective stock solutions. Agitation was carried out on the stock solutions for 15 minutes and allowed to stand for 24 h to ensure complete dissolution of metal salts. Atomic Absorption Spectrophotometry (AAS) was used to measure the initial concentration of metal solutions (Ni, Cd, Pb and Cr). The pH of heavy metal solutions was also adjusted using sodium hydroxide (NaOH) and hydrochloric acid (HCl) to a pH of 7 [6].

2.3. Heavy metals tolerance of *Lysinibacillus fusiformis* 5B

Heavy metal tolerance by *L. fusiformis* 5B was ascertained using agar dilution method. Concentration of 5 ppm of the heavy metals (lead, chromium, nickel and cadmium) was prepared and incorporated into nutrient agar before sterilizing using the autoclave at 121°C for 15 minutes. The modified media were all allowed to cool down to 40°C before dispensing into their respective well labelled Petri dishes and allowed to solidify. From a 24 h culture broth of *L. fusiformis* 5B, a sterile swab stick was used to aseptically inoculate into the different heavy metal Petri plates by swabbing gently on the surfaces of the media. The culture plates were then incubated at 37°C for 24 h in an inverted position. Development of bacterial colonies indicates the ability of the isolate to tolerate the heavy metal while absence of visible colonies indicates that the test organisms were unable to tolerate the heavy metals [18].

2.4. Biosorption of heavy metals

The heavy metal nutrient broth culture medium was prepared into different concentrations (10, 15, 20 and 50 ppm) using the prepared stock solutions. The culture broth containing the varying concentration of heavy metals was then sterilized at 121°C for 15 minutes, after which the culture broth was allowed to cool before inoculating 5 mL of 24 h old culture, where cells of *L. fusiformis* 5B have attained 1.5 $\times 10^6$ cfu/mL with the exception of the blank, which was used as control. The heavy metal culture broths were incubated aerobically in an incubator with shaker at 37°C for 35 days.

2.5. Wet digestion for the determination of Total Cd, Pb, Ni and Cr using Atomic Absorption Spectroscopy

The 0.2-0.5 grams of sample was weighed into a 100 mL volumetric flask, 30 mL of wet digestion acid (650 mL of nitric acid in 1 L beaker, 80 mL of perchloric acid and 20 mL of sulfuric acid) and stirred to mix. Sample was placed on a fume cupboard and digested until sample reduce to 20 mL. The heating was continued until white fumes of nitric acid disappeared and sample reduced to 10 mL. The sample was transferred quantitatively to a 50 mL volumetric flask and made to mark with dH_2O. It was then shaken vigorously and filtered through a Whatman 0.45 µm filter paper. A 1 mL of the clear digest was pipetted into another 50 mL volumetric flask and made to mark with dH_2O. Samples were read using AAS (AA WIN 500 PG instrument) at 7 days interval starting with zero reading (day 1) using wavelengths 359.4 nm, 326.1 nm, 283.3 nm and 231.1 nm for chromium, cadmium, lead and nickel respectively. The percentage of biosorption was determined by measuring the amount of heavy metal removed from the medium through estimation of the residual metal concentration using AAS. Beer Lambert's law (Equation 1) was used to achieve the percentage biosorption [6].

% Biosorption = [(Initial metal concentration – final metal concentration) / Initial metal concentration] x 100 Equation 1

2.6. Data analysis

Statistical package for social science (SPSS 24) utilizing one-way analysis of variance (ANOVA) was used to analyze the data generated from this study.

3. RESULTS

3.1. Heavy metal tolerance of *Lysinibacillus fusiformis* 5B

Lysinibacillus fusiformis 5B was able to tolerate concentration of 5 ppm of all heavy metals (Ni, Cd, Cr

and Pb) by showing visible growth of abundant colonies on surfaces of heavy metals nutrient agar Petri plates (Figure 1).

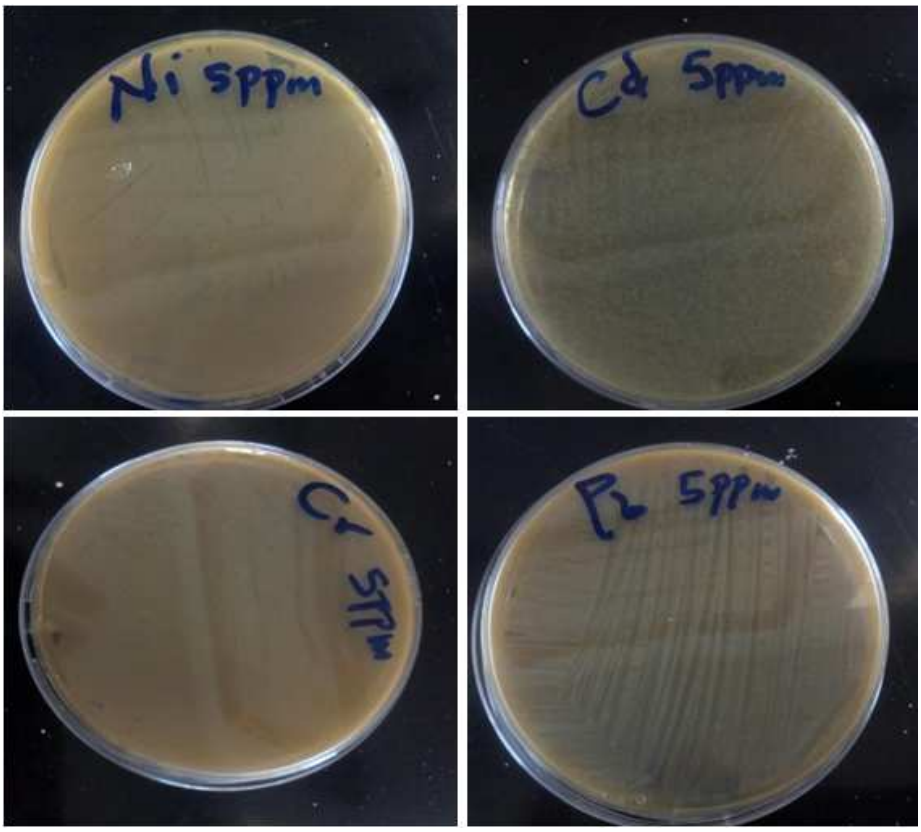

Figure 1. Growth of *Lysinibacillus fusiformis* 5B in 5 ppm of heavy metals.

3.2. Biosorption of lead by *Lysinibacillus fusiformis* 5B

The result obtained from the biosorption of lead by *L. fusiformis* 5B at different concentrations and at different time intervals is shown in Table 1. A general increase in the absorption of lead was observed across all concentration observed (5, 15, 20 and 50 ppm). Within the first seven days of incubation, the highest rate (70.44 %) of biosorption was recorded in 20 ppm and the least (40.06 %) was recorded for 50 ppm. After the 35 days of incubation, the highest biosorption (99.96%) of lead was recorded in 10 ppm and the least (86.61 %) was recorded for 50 ppm.

Table 1. Biosorption percentage of lead by *L. fusiformis* 5B.

	Lead concentration (%)			
Days	**10**	**15**	**20**	**50**
7	58.01 ± 0.01^{d}	52.36 ± 0.36^{d}	70.44 ± 0.44^{e}	40.06 ± 0.06^{e}
14	76.54 ± 0.54^{c}	69.87 ± 0.87^{c}	74.44 ± 0.44^{d}	50.11 ± 0.11^{d}
21	97.40 ± 0.40^{b}	94.68 ± 0.68^{b}	78.11 ± 0.11^{c}	57.29 ± 0.29^{c}
28	99.80 ± 0.80^{a}	99.41 ± 0.41^{a}	88.14 ± 0.14^{b}	70.14 ± 1.14^{b}
35	99.96 ± 0.96^{a}	99.89 ± 0.89^{a}	99.24 ± 0.24^{a}	86.61 ± 0.61^{a}

Values are x̄±SEM of duplicate values. x̄ with dissimilar letter(s)s are not significantly different from each other according to Duncan Multiple Range Test (DMRT).

3.3. Biosorption of Chromium by *Lysinibacillus fusiformis* 5B

The biosorption of chromium by *L. fusiformis* 5B at different concentration and at different time intervals is presented in Table 2. After seven (7) days of incubation, a high rate of biosorption was recorded across all concentration with the highest (75.23%) recorded at 20 ppm and the lowest (55.69%) being 50 ppm. This high amount of biosorption of chromium was also recorded after fourteen (14) days. This however declined after 28 days of incubation. At the end of 35 days, biosorption of chromium was highest (99.97%) at 10 ppm and lowest (91.26%) at 50 ppm.

Table 2. Result showing biosorption percentage of chromium by *L. fusiformis* 5B.

	Chromium concentration (%)			
Days	**10**	**15**	**20**	**50**
7	67.33 ± 0.33^{c}	64.58 ± 0.58^{d}	75.23 ± 0.23^{c}	55.69 ± 0.69^{e}
14	95.95 ± 0.95^{b}	83.33 ± 0.33^{c}	89.01 ± 0.01^{b}	66.62 ± 0.62^{d}
21	99.87 ± 0.87^{a}	97.29 ± 0.29^{b}	98.96 ± 0.96^{a}	70.47 ± 0.47^{c}
28	99.92 ± 0.92^{a}	99.34 ± 0.34^{a}	99.86 ± 0.86^{a}	86.72 ± 0.72^{b}
35	99.97 ± 0.97^{a}	99.86 ± 0.86^{a}	99.93 ± 0.93^{a}	91.26 ± 0.26^{a}

Values are x̄±SEM of duplicate values. x̄ with dissimilar letter(s) are not significantly different from each other according to Duncan Multiple Range Test (DMRT).

3.4. Biosorption of nickel by *Lysinibacillus fusiformis* 5B

Biosorption of nickel by *L. fusiformis* 5B at different interval and concentration is represented in Table 3. Biosorption of nickel was recorded across all concentration and was highest (46.81%) at 20 ppm and lowest (20.99%) at 15 ppm. After 35 days of incubation, the biosorption was recorded highest (98.13%) at concentration of 10 ppm and the lowest (84.2 %) at 50 ppm.

Table 3. Result showing biosorption percentage of nickel by *L. fusiformis* 5B.

	Nickel concentration (%)			
Days	**10**	**15**	**20**	**50**
7	37.91 ± 0.91^{d}	20.99 ± 0.99^{d}	46.81 ± 0.81^{d}	24.60 ± 0.60^{e}
14	66.27 ± 0.27^{c}	54.72 ± 0.72^{c}	64.14 ± 0.14^{c}	38.40 ± 0.40^{d}
21	90.47 ± 0.27^{b}	78.27 ± 0.27^{b}	82.49 ± 0.49^{b}	44.90 ± 0.90^{c}
28	92.89 ± 0.96^{b}	96.96 ± 0.96^{a}	80.49 ± 0.49^{b}	64.90 ± 0.90^{b}
35	98.13 ± 0.33^{a}	99.33 ± 0.33^{a}	91.70± 0.07^{a}	84.24 ± 0.22^{a}

Values are x̄±SEM of duplicate values. x̄ with dissimilar letter(s) are not significantly different from each other according to Duncan Multiple Range Test (DMRT).

3.5. Biosorption of cadmium by *Lysinibacillus fusiformis* 5B

The biosorption of cadmium by *L. fusiformis* 5B at different concentration and interval is recorded in Table 4. The highest biosorption rate (99.94%) recorded after 35 days of incubation was observed at 10 ppm whereas the lowest (97.23%) was recorded at 50 ppm. However, after day 7 of incubation, biosorption rate was highest (60.03%) and the lowest (44.31%) was recorded at concentration of 10 ppm.

Table 4. Result showing biosorption percentage of cadmium by *L. fusiformis* 5B.

	Cadmium concentration (%)			
Days	**10**	**15**	**20**	**50**
7	44.31 ± 0.31^{c}	53.59 ± 0.59^{e}	54.43 ± 0.43^{e}	60.03 ± 0.03^{e}
14	83.54 ± 0.54^{b}	79.73 ± 0.73^{d}	68.49 ± 0.43^{d}	63.56 ± 0.56^{d}
21	99.53 ± 0.53^{a}	86.94 ± 0.94^{c}	76.29 ± 0.29^{c}	65.29 ± 0.29^{c}
28	99.74 ± 0.74^{a}	94.42 ± 0.42^{b}	91.89 ± 0.89^{b}	82.16 ± 0.16^{b}
35	99.94 ± 0.94^{a}	98.79 ± 0.79^{a}	99.77 ± 0.77^{a}	97.23 ± 0.23^{a}

Values are $\bar{x}$±SEM of duplicate values. $\bar{x}$ with dissimilar letter(s) are not significantly different from each other according to Duncan Multiple Range Test (DMRT).

4. DISCUSSION

Bacterial cells have been reported in the past to possess inherent ability to survive in an environment polluted by varying contaminants such as petroleum and heavy metals [33]. Their survival have been attributed to their ability to respond adequately to stress from the environment through production of extracellular substances such as enzymes, fatty acids as well as polysaccharides making researchers to search for such microorganisms in an environment filled with heavy metal contaminants [34], among which bacteria genera such as *Bacillus, Micrococcus, Streptomyces, Pseudomonas* and *Lysinibacillus* have shown great potentials [24]. In this study, heavy metal tolerance was exhibited by *L. fusiformis* 5B against 5 ppm concentration of tested heavy metal salts. This was ascertained by the presence of abundant growth on the surfaces of cultured nutrient agar. This is however possible, owing to the components of the cell wall of *Lysinibacillus* species, which contains thick peptidoglycan, teichuronic and teichoic acid bonded by Asp-Lys [1].

Lysinibacillus species also have a mechanism that helps them actively pump out toxic substances from their cells in what is known as efflux pumps. Extracellular and intracellular sequestration of metal ions as well as reduction in membrane permeability are also strategies used by Gram positive bacteria to resist entry of toxic metal substances into their cells [31, 35]. He *et al.* [29] reported *Lysinibacillus fusiformis* ZC1 to be highly resistant to chromium. *L. fusiformis* ZC1 showed highest resistance reported so far for chromium as it recorded minimum inhibitory concentration of 60 mM. Likewise studies by Mathivanan *et al*. [23], which reported high heavy metal tolerance of *L. fusiformis* KMNTT-10 to lead (II) up to a concentration of 500 ppm.

Biosorption of heavy metal carried out by *L. fusiformis* 5B in this study was observed across all concentrations (i.e. 10, 15, 20 and 50 ppm). After 7 days of incubation, the result obtained showed high rate (> 40%) of biosorption of heavy metals (Cd, Cr and Pb) across the concentration considered with the exception of nickel (Ni), which showed as low as 20.99% (15 ppm) and the highest at day 7 being 46.81% (20 ppm). This could be as a result of varying degree of toxicity of different heavy metal. In the biosorption of heavy metals by bacteria cells, the amount of time in which the bacterial cells are in contact with the heavy metal play a key role in biosorption. This was observed in this study, as the longer time the cells of *L. fusiformis* 5B were in contact with the heavy metal solution, the more the cells adsorb the heavy metal onto their cells. *L. fusiformis* 5B recorded low biosorption of Ni after the seventh day and a high rate of biosorption (>50%) across all concentration (10, 15 and 20 ppm) with the exception of 50 ppm. This shows that nickel may be more toxic to *L. fusiformis* 5B or the affinity of the functional groups present on the cell wall of *L. fusiformis* 5B was less compared to other heavy metals [24].

A high biosorption rate (> 55%) of chromium was recorded at day 7. This shows that the functional groups present on cell surface of *L. fusiformis* 5B have high affinity for ions of chromium present in the solution, which is in line with the observation made by He *et al*. [29] using *L. fusiformis* ZC1. It is important to note that biosorption of heavy metal reduces with increase in the concentration of the heavy metals (Cr, Ni and Pb). This is evident in this study, as at day 21, a high rate of biosorption (>90%) was recorded across all the heavy metals at concentration of 10 ppm.

However, in the case of biosorption of cadmium in this study is not in correspondence with the general notion that the higher the heavy metal concentration the lower the biosorption as the result obtained for cadmium at day 7 of incubation of *L. fusiformis* 5B showed lowest biosorption (44.31%) at 10 ppm whereas the highest biosorption (60.03%) was recorded at 50 ppm. This could be related to the affinity the functional groups present on the surface of *L. fusiformis* 5B have on the metal ions since they all have binding sites, which could either be inhibited or enhanced at varying concentration of the heavy metal. This study observed little percent increase in the biosorption of metals by *L. fusiformis* 5B towards the latter stages of incubation. This could be accounted for as a result of aging in bacterial cells typical of a batch culture having no renewal of nutrients or bacterial cells [6].

5. CONCLUSIONS

Lysinibacillus fusiformis 5B was observed to have the capacity to biosorp cadmium, chromium, lead and nickel with increasing capacity as the days of incubation progressed. Thus, this urease producing bacterium can be explored to biosorp environments contaminated with these heavy metals and thereby help to reclaim these environments of heavy metals toxicity.

Authors' Contributions: AMJ carried out the research in the laboratory, OAO designed and supervised the research, JGY isolated the organisms and wrote the article, AH characterized and identified the test organism, ECE co-supervised the research and corrected the manuscript. The final manuscript has been read and approved by all authors.

Conflict of Interest: The author has no conflict of interest to declare.

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