Bioaccumulation of Pb, Ni and Cd by Bacillus cereus isolated from Lake Qarun, Egypt, using the spatial technique

Ahmed M. Khalifa1*, Khaled Z. ElBaghdady2, Sameh B. El Kafrawy1, Ahmed M. El-Zeiny3

1 Marine Sciences Department, National Authority for Remote Sensing and Space Sciences (NARSS), Cairo, Egypt.
2 Microbiology Department, Faculty of Science, Ain Shams University.
3 Environmental Studies Department, National Authority for Remote Sensing and Space Sciences (NARSS), Cairo, Egypt.

*Corresponding Author: ahmed.khalifa@narss.sci.eg

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ABSTRACT

The present study aimed to investigate the metal bioaccumulation capability of heavy metal-resistant bacterium isolated from Lake Qarun. Physico-chemical parameters and heavy metals of Lake Qarun water were evaluated using the EUREKA Manta2 device and Atomic Absorption Spectroscopy (ASS), respectively. Geostatistical analyses were used for the spatial representation of the investigated parameters. The bacterial isolate was identified as Bacillus cereus, using the MALDI-TOF MS platform, exhibited notable resistance to higher levels of lead (Pb\(^{2+}\)), cadmium (Cd\(^{2+}\)) and nickel (Ni\(^{2+}\)) at 1700, 175 and 80 mg/l, respectively. Furthermore, this isolate showed a high capacity to remove Pb\(^{2+}\) (59.9%), Ni\(^{2+}\) (83%) and Cd\(^{2+}\) (79.4%) The impact of specific physicochemical factors, including temperature, pH, initial metal concentration, and incubation period, was also evaluated concerning bioaccumulation. The optimal pH for the removal of Pb, Ni and Cd was found to be 7. The bacterium exhibited maximum accumulation capacity after 96 h of incubation with each metal while maintaining a temperature of 37°C. The transmission electron microscope revealed that metals were predominantly observed both extracellularly and intracellularly, concomitant with alterations in cellular morphology. The bioaccumulation capacity (removal efficiency) for each metal by bacteria exhibited a notable swiftness, which presents a potential advantage in the context of treating contaminated sites on a large scale.

INTRODUCTION

Lake Qarun, one of the interior lakes, has significant importance within the geographical context of Egypt and is considered a containment system designed to accommodate the discharge of agricultural wastewater originating from the El-Fayoum region. Qarun Lake suffers from rising salt levels and sewage discharge as a result of a growing population and inadequate infrastructure (Shaaban et al. 2016). Lake Qarun is
widely recognized as one of the oldest lakes in Egypt. In recognition of its ecological significance, it was officially designated as a protected area in the year 1989. In accordance with Decision No. 348 issued by the Prime Minister, Lake Qarun receives agricultural and sewage drainage through a comprehensive system consisting of twelve drains. The majority of the drainage water is directed towards the lake via two primary drains, The Batts and El-Wadi drains and also have minor drains that discharge their drainage water into the lake using hydraulic pumps, although in small quantities (Authman and Abbas, 2007). The presence of heavy metals poses significant challenges to both human health and the environment. This is primarily due to their widespread contamination, limited ability to dissolve in living organisms, and the classification of certain heavy metals as carcinogens and mutagens (Rani and Goel, 2009). The occurrence of heavy metals in the environment can be attributed to both pedogenetic mechanisms, such as the breakdown of parent materials, and anthropogenic factors (D'Amore et al., 2005). Heavy metals and trace elements must be removed from polluted water and soil water and soil must be removed from heavy metals and trace elements to return them to an appropriate state. Furthermore, physicochemical approaches exhibit ineffectiveness or high cost when the heavy metal concentration is extremely low. Instead of physicochemical techniques, biological methods such as biosorption and/or bioaccumulation present a compelling option for the elimination of heavy metals (Kapoor and Viraraghvan 1995). When it comes to cleaning up contaminated areas, bioremediation is often considered one of the best options since it is less dangerous, cleaner, cheaper, and environmentally friendly compared to other methods. The practice of cleaning up polluted areas with the help of living organisms is known as "bioremediation" (Kulshreshtha et al., 2014). Furthermore, researchers have isolated microorganisms resistant to heavy metals from metal-contaminated soils, industrial residues, and the rhizospheres of plants grown on contaminated land (Hrynkiwicz and Baum, 2014; Chaudhary and Shukla, 2019). Bacillus, Arthrobacter, Corynebacterium, Pseudomonas, Enterobacter, etc., were among the isolated bacterial genera (Belogolova et al., 2020). Bacillus cereus, Bacillus subtilis, and Bacillus thuringiensis are the best-described species in terms of their bioremediation capacity. These bacterial species use a variety of bioremediation techniques, including biosorption and Extracellular Polymeric Substances (EPS) mediated mechanisms. Bioaccumulation, bioprecipitation, or biosorption (Wróbel et al., 2023). The ability of living cells to eliminate metal ions from their surroundings is affected by various environmental growth conditions, such as temperature, pH, and biomass concentrations (Abd El-Raheem et al., 2013). The bioaccumulation process includes various mechanisms such as metal binding on intracellular compounds, metal binding proteins, intracellular precipitation, methylation, and other related processes. The uptake process involves the process of adsorption of heavy metal ions onto the bacterial cell wall or cell membrane through interactions with different functional groups and/or transportation into the cell, followed by subsequent
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transformation (Malik, 2004). The utilization of a valuable methodology facilitates the localization and identification of metals that have been deposited within or in the vicinity of microbial cells. The determination of the specific location where metal accumulation occurs is significant as it can provide valuable insights into the underlying biochemical mechanisms that drive this process.

The Geographic Information System (GIS) is a crucial tool for dealing with environmental challenges across diverse disciplines. The implementation of the geostatistical analysis approach in Geographic Information Systems (GIS) applications facilitates the application of interpolation techniques to predict attribute values at unsampled locations based on measurements obtained from locations within the same geographical region (El-Zeiny and Elbeih, 2019). Inverse distance weighting (IDW) and kriging are two well-known methods of interpolation. IDW is preferable when compared to kriging since it is more time-consuming and complicated to apply (Huang et al., 2015).

The objective of this work is to study the capability of a bacterial isolate recovered from Qarun Lake to remove multiple heavy metals from the aquatic environment. Additionally, to investigate the impact of initial metal concentration, temperature, pH and incubation period on metal bioaccumulation.

**MATERIALS AND METHODS**

1. **Study area**

Lake Qarun is a residual body of water that is believed to have originated from the ancient Lake Moreis. Initially, it was a freshwater lake, the lake has an approximate surface area of 243 km$^2$ and an irregular shape measuring approximately 40 km in length and 6.7 km in maximum width, it has a mean depth of 4 meters and a volume of approximately 1 billion cubic meters, due to its location in the arid zone, it is classified as a tropical lake (Shadrin et al., 2016). The geographical coordinates of the lake lie within the longitudes of 30° 24` and 30° 49` E and the latitudes of 29° 24` and 29° 33` N. The lake's latitudes are situated at a depth of 43-44 m below sea level Fig. (1). (Ibrahim and Ramzy, 2013).

2. **Sampling and laboratory analyses**

Water samples were collected from 18 distinct locations within Qarun Lake specified in Fig. (2). The water samples were collected from the subsurface layer using sterilized stopper polyethylene plastic bottles and falcon tubes. Two samples were obtained from each location to conduct bacteriological and physicochemical analyses. The samples were subsequently placed in a cooler box with ice and transported to the laboratory for further examination.
Fig.1. Map of Northern Egypt showing the location of Lake Qarun.

Fig.2. Map of Qarun Lake showing water sampling sites.

3. Physico-Chemical Analyses

In situ measurements were carried out, which include measuring the physicochemical parameters of water samples using the Eureka Manta2 multi probes water quality device (Eureka Environmental Engineering, USA) which is used for measuring many parameters such as temperature and pH, turbidity salinity and dissolved organic matter. Eighteen samples were analyzed for evaluation of heavy metal concentrations in water (Pb, Ni and Cd) using Atomic Absorption
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Spectroscopy (ASS), in the central laboratory (Faculty of science - Ain Shams University).

4. Bacterial Isolation

The samples were collected using a sterile falcon tube from Qarun Lake. Using the spread plate method water is spread over nutrient agar (TM MEDIA, Delhi). The water samples were then disseminated on the agar plates and incubated for 24 hours at 37°C. From each sample bacterial colonies of distinct morphology were purified. The most resistant isolate to multiple heavy metals was chosen for identification procedures.

5. Bacterial characterization

The bacterial isolates with the highest level of resistance were characterized by applying the MALDI -TOF Mass Spectrometry (Biotyper sirrius, BRUKER) device at the Department of Biological Protection, the main laboratory of Chemical Warfare. MALDI -TOF MS platform using the MicroSeq 500 system facilitates the accurate and expeditious characterization of bacterial and fungal microorganisms.

6. Minimum inhibitory concentration (MIC)

The MICs of Pb, Ni and Cd against the bacteria were determined by a streak plate method using nutrient agar plates incorporated with PbNO3, NiSO4.6H2O and CdSO4.8H2O heavy metals salts which were inoculated with varying concentrations of lead (500, 1000, 1500, 1700, and 2000 mg/l), nickel (10, 40, 70, 80, and 90 mg/l) and cadmium (50,100,175, and 200 mg/l) then incubated at 37°C for 24 h, and then tested for their resistance pattern to heavy metals of different concentrations. The lowest concentrations at which the growth of bacteria is inhibited after 24 h are defined as MICs for heavy metals (Schmiatt and Schlegel 1994).

7. Bioaccumulation of heavy metals by Bacteria

The bacterial cells that were harvested from agar plates were cultivated in a nutrient broth that was enriched with varying concentrations of lead (500, 1000, 1500, 1700, and 2000 mg/l), nickel (10, 40, 70, 80, and 90 mg/l and cadmium (50,75,100,150, and 175 mg/l. The incubation process was carried out at a temperature of 30°C, while the shaker incubator (Lab-line 3525) was set at a speed of 150 rpm. After the incubation period, the culture was centrifuged at 6000 rpm for 15 min. Subsequently, the pellet was re-suspended in 1 ml distilled water, then dried overnight in the oven at 50°C and weighed. The atomic absorption spectrophotometer was utilised to quantify the metal concentrations present in the supernatant. The experimental control was comprised of a microbial culture without heavy metals (*Sprocati et al., 2006*). The metal removal capacity percentage was determined utilising the following formula:
The formula for calculating the percentage of metal removal capacity ($\% R$) involves the initial metal concentration ($C_i$) and the final metal concentration ($C_f$) after undergoing either bioaccumulation or biosorption. The quantity of metal that adsorbed to the bacterial biomass was determined using the following equation:

$$\% R = \left( \frac{c_i - c_f}{c_i} \right) \times 100$$

This equation involves the initial metal ion concentration denoted by $C_i$, and the final metal ion concentration denoted by $C_f$, both of which are measured in milligrams per litre. The variable $V$ represents the volume of solution, measured in litres, while $M$ denotes the quantity of biosorbent utilised, measured in grams.

8. Transmission electron microscope

To prepare for transmission electron microscopy (TEM), bacterial cells were harvested via centrifugation of a broth culture sample for a duration of 10 minutes at 4000 revolutions per minute. The cultures were grown on nutrient broth media for a period of 24 h prior to collection. The collected cells were then washed with distilled water, with the supernatant being decanted. The resulting pellet was fixed with a 3% glutaraldehyde solution for a duration of 4 hours at 4°C. The specimens underwent dehydration using a series of ethanol solutions with concentrations ranging from 10 to 90% for a duration of 15 min per dilution, followed by a final immersion in absolute ethanol for a period of 30 min. The specimens underwent a gradual infiltration process using a series of epoxy resin and acetone solutions, culminating in pure resin. Copper grids were utilized for the collection of ultrathin sections. The sections were subjected to a double staining procedure involving uranyl acetate and lead citrate. The stained sections were examined using a JEOL - JEM 1010 transmission electron microscope that was operated at 70 kV. The microscope was situated at The Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University.

9. Spatial and Statistical Analysis

One-way analysis of variance (ANOVA) is used for the comparison of multiple arithmetic means to determine if there are significant differences among parameters at a significance level of $p < 0.05$. To accommodate for all measurements and analyses, a thematic layer was generated. The geostatistical analyses in ArcGIS 10.7.1 were employed to generate spatial distribution maps.
for all parameters, thereby facilitating spatial analyses. This study applied the Inverse Distance Weighting (IDW) method, which is simpler to implement and more accurate in water quality mapping.

RESULTS AND DISCUSSION

1. Estimation of physical and chemical characteristics of Qarun Lake water

The results of the physicochemical analyses of the collected samples are presented in Table 1. The temperature values measured in situ exhibited a range of 19.3 to 22 °C, with a mean value of 20.6 °C. The lake's pH values varied between 7.6 and 8.9, with an average of 8.25. The pH level exhibits a weak alkaline trend that ascends from the eastern region to the western region, with the water in the western portion displaying the highest alkalinity. The analysis using the Inverse Distance Weighting (IDW) method revealed that the results of heavy metals in surface water indicate a gradual increase in the concentrations of the water chemical parameters from the west to the east direction. Pb concentrations ranged from 0.22 to 0.62 mg/l with an average of 0.42 mg/l, while Ni concentrations ranged from 0.05 to 0.31 mg/l with an average of 0.25 mg/l and Cd concentrations ranged from 0.03 to 0.1 mg/l with an average of 0.07 mg/l. Pb, Ni, and Cd levels in the water of Qarun Lake exceed the EPA, (2017) threshold limits for protecting aquatic life. El-Zeiny et al., (2019) analyzed the contamination sources of Lake Qarun, the EPA, (2017) criteria for, lead, and nickel were exceeding the threshold limit, while Goher et al., (2018) declared that cadmium in Qarun Lake water exceeded the guidelines for aquatic life which agree with our results.

Table 1. Range and mean of Lake Qarun water parameters in comparison to aquatic life protection guidelines

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>EPA Threshold limits for Aquatic life protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>19.3 - 22</td>
<td>20.39 ± 0.7</td>
<td>8-28</td>
</tr>
<tr>
<td>pH</td>
<td>7.7 - 8.9</td>
<td>8.5 ± 0.3</td>
<td>6.5-8.5</td>
</tr>
<tr>
<td>Pb (mg/l)</td>
<td>0.22 - 0.62</td>
<td>0.52 ± 0.08</td>
<td>0.21</td>
</tr>
<tr>
<td>Ni (mg/l)</td>
<td>0.05 - 0.31</td>
<td>0.024 ± 0.05</td>
<td>0.074</td>
</tr>
<tr>
<td>Cd (mg/l)</td>
<td>0.03 - 0.1</td>
<td>0.08 ± 0.01</td>
<td>0.033</td>
</tr>
</tbody>
</table>
Fig. 3. Spatial variations of the physicochemical parameters as obtained by IDW technique in GIS.
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**Fig. 4.** Spatial distribution of heavy metals in surface water of Qarun Lake compared to EPA, (2017) threshold limit for aquatic life.
2. Minimum inhibitory concentration (MIC)

The study assessed the cross-resistance of the bacterial isolate to heavy metals, namely lead (Pb), cadmium (Cd) and nickel (Ni), by determining the minimum inhibitory concentration (MIC) of these metals. The agar dilution method was applied to define MIC. *Bacillus cereus* exhibited notable resistance to elevated levels of lead, with a minimum inhibitory concentration (MIC) of 1700 mg/l, in contrast, its resistance to cadmium was observed at a concentration of 175 mg/l and nickel at a concentration of 80 mg/l.

3. Bioaccumulation

Among the isolates that were studied, *Bacillus cereus* isolated from site 13 at the western region of Qarun Lake displayed complete resistance to all three metals across all concentrations. According to Malik (2004), the accumulation of metals within cells can occur due to intracellular processes or metabolic activities. However, these processes are typically gradual and heavily influenced by factors such as nutrient availability and environmental conditions. Additionally, the process of cell surface and cell wall binding is passive in nature and can occur with both viable and non-viable cells. Biosorption exhibits a high rate of speed, typically taking only a few minutes (Pardo et al., 2003). This mechanism of metal removal operates through ion exchange or physical adsorption processes, which involve specific chemical sites on the cell wall that possess a negative charge (Volesky and Holan, 1995). The study of metal uptake by bacteria is of crucial significance in the overall context of biosorption/bioaccumulation application, which provides valuable insights into the efficiency of metal ion removal in the process (Veglió and Beolchini, 1997). The parameter qe is commonly employed as an important factor in the design of equipment, as it represents the maximum accumulation capacity of bacteria.

Table 2. The capacity of bacterial isolate for the accumulation of single heavy metals.

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Initial concentration</th>
<th>After treatment</th>
<th>% Removal</th>
<th>M biomass</th>
<th>qe (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb(mg/l)</td>
<td>1000</td>
<td>501</td>
<td>49.9</td>
<td>0.059</td>
<td>296.02</td>
</tr>
<tr>
<td>Ni (mg/l)</td>
<td>40</td>
<td>23</td>
<td>42.5</td>
<td>0.065</td>
<td>9.15</td>
</tr>
<tr>
<td>Cd(mg/l)</td>
<td>100</td>
<td>55.5</td>
<td>44.5</td>
<td>0.054</td>
<td>28.84</td>
</tr>
</tbody>
</table>
Bioaccumulation of Pb, Ni and Cd by *Bacillus cereus* isolated from Lake Qarun

**Table 3.** The capacity of bacterial isolate for the accumulation of multiple heavy metals

<table>
<thead>
<tr>
<th>The mixture of heavy metals</th>
<th>Pb (mg/l)</th>
<th>Ni (mg/l)</th>
<th>Cd (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial concentration</td>
<td>After treatment</td>
<td>Initial concentration</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>589</td>
<td>40</td>
</tr>
<tr>
<td>% Removal</td>
<td>41.1</td>
<td>32.5</td>
<td>54.25</td>
</tr>
<tr>
<td>M biomass (g)</td>
<td>0.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qe (mg/g)</td>
<td>378.56</td>
<td>11.97</td>
<td>49.97</td>
</tr>
</tbody>
</table>

4. Effect of different conditions on bioaccumulation of heavy metals

4.1. Effect of pH

The pH level of a solution is a crucial factor in the process of metal biosorption/bioaccumulation, as it has a significant impact on both the surface chemistry of bacteria and the solution chemistry of metal ions. The pH values of metal ion solutions were manipulated within the range of 4 to 9 prior to the introduction of biosorbent. *Bacillus cereus* demonstrates a high percentage of removal capacity for Pb (II), Ni (II), and Cd (II) at a pH of 7, with a significant reduction observed at a pH of 9, the findings are consistent with the research conducted by Akhter *et al.*, (2017). The bioaccumulation of Pb, Ni (II), and Cd (II) to the cell surface is favoured by the release of protons from functional groups as pH increases. The limited efficacy observed at pH 9 can be explained by a reduction in the solubility of Pb (II), Ni (II), and Cd (II) due to precipitation, which results in a decrease in the availability of free metal ions for binding with the cell wall (*Lopez et al.*, 2000). The capacity of metal cations to be adsorbed by biosorption increases as the pH of the sorption system increases, although this relationship is not linear. However, it is important to note that the presence of extreme pH values can lead to the precipitation of metal complexes. As the pH level rises, the functional sites present on the microbial surface undergo deprotonation, resulting in the potential for binding cationic metals. This phenomenon leads to a prevalent pattern of heightened accumulation as pH levels rise. Nevertheless, it is observed that in numerous instances, the accumulation begins to decline once more at elevated pH levels. Consequently, when pH levels are low, the cell surface experiences an increase in positive charge, thereby diminishing the affinity between metal ions and functional groups present on the cell wall. On the other hand, an elevated pH level leads to an enhancement in the process of metal biosorption due to an increase in the negative charge
on the cell surface. At elevated pH levels, the metals undergo a transformation into hydroxide complexes.

4.2. Effect of temperature
The bioaccumulation potential of *B. cereus* was evaluated at different temperature ranges, the bioaccumulation capacity of heavy metals exhibited a positive correlation with temperature until the optimal temperature was attained, beyond which it underwent a marked reduction. **Figure (5a)** shows the following trend of Pb (II), Ni (II), and Cd (II) uptake and its percentage removal with the changing of temperature. The maximum capacity for bioaccumulation of lead, nickel, and cadmium was attained across the bacterial isolate at a temperature of 37°C. *B. cereus* exhibits a capacity to withstand temperatures of up to 40°C.

4.3. Effect of incubation time:
The impact of various incubation times on the performance rate of *B. cereus* illustrated in **Fig. 5 (C)** demonstrates a consistent uptake of Pb (II) over the course of 48 h, 72 h, and 96 h, indicating a balanced absorption of the substance. Ni (II) exhibited statistical significance at the time points of 72 h and 96 hours. After 96 hours, there is a significant degree of adsorption of Cd (II). Pb$^{+2}$ removal percentage and uptake improved to 51.8%, after 96 h of incubation, whereas Ni$^{+2}$ removal percentage and uptake improved to 54%, after 96 hours of incubation. Cd$^{+2}$ removal percentage and uptake improved to 51.8%, The reason for the observed trend is the presence of empty sites on the surface of bacteria, which progressively become occupied, resulting in a saturation level and a decline in metal absorption and degradation. A modest increase in the initial period is attributable to the time required for the proliferation of additional microbes in response to the dietary supply.

4.4. Effect of heavy metal concentrations
Bacterial strains that have been subjected to elevated concentrations of Pb (II), Ni (II), and Cd (II) may have acquired biological resilience to their deleterious impacts through the development of diverse resistance mechanisms, such as plasmid-mediated resistance or enzymatic activity at the cellular surface. The observed phenomenon could potentially be associated with functional groups, such as amino, carboxylic, and sulfhydril, present on the surfaces of bacterial cells. These groups may exhibit varying capacities for binding positively charged metal ions, specifically Pb (II), Ni (II), and Cd (II). Bioaccumulation of metals on cells was shown to be reduced at higher medium concentrations with (1700 mg/l Pb), (80 mg/l Ni) & (175 mg/l Cd). The findings are consistent with the research conducted by Murthy et al., (2014).
Bioaccumulation of Pb, Ni and Cd by *Bacillus cereus* isolated from Lake Qarun.
Fig. 5. Effect of (A) temperature, (B) pH, (C) incubation period and heavy metals concentrations; (D) Pb, (E) Ni and (F) Cd on the efficiency of heavy metal removal by *B. cereus*.
5. Transmission electron microscope

Two distinct sets of samples of *B. cereus* were chosen for transmission electron microscopy (TEM) analysis. One set was cultivated in a metal-free broth medium, serving as the control group. The other set was subjected to a 24-hour exposure to 20 mg/l of Cd, 100 mg/l of Pb, and 40 mg/l of Ni. The images (Fig. 6) revealed that metals present within cells manifest as dark entities or spots. Metals were predominantly observed both extracellularly on the cell membrane and intracellularly within the cell, concomitant with alterations in cellular morphology with spore formation. The findings of the study revealed that the morphology of the cell surface exhibited significant alterations after exposure to metals. The predominant cellular distribution of metal ions bound by bacterial cells was observed to be within the confines of the cell membrane. Nevertheless, certain intracellular metal accumulations were also detected within the cytoplasm of the bacterial cells. Following exposure to heavy metals, the manifestation of diminished and malformed cellular membranes was observed in the presence of Pb. Additionally, the excretion of extracellular polymeric substances was detected and this agrees with the findings presented by Odokuma and Emedolu (2005), which demonstrated that *Bacillus sp.* exhibits resistance to the deleterious effects of heavy toxicity metals and the enduring nature of these bacteria in the presence of such metals. The presence of specific heavy metals can potentially be attributed to the spore formation.

6. Bioaccumulation capacity study

The findings depicted in Fig. 5. illustrate the patterns observed in the bioaccumulation capacity of each strain towards the metal ions investigated in the study. As a result, *Bacillus cereus* exhibited resistance to multiple heavy metals. The prospect of an accumulation of lead by *B. cereus* differs as the incubation period, temperature, pH and concentrations change. So, the highest accumulation was 307.29 mg/g after 96 h incubation at 37 °C, pH 7 and the initial concentration was 1000 mg/l of Pb. The accumulation increased as the incubation period and concentrations increased to 1000 mg/l. By studying the accumulation rate of Cd by *B. cereus*, the accumulation increased by increasing of incubation time to 96h, the maximum accumulation capacity attained at 150 mg/l which reach 50.9 mg/g at pH 7 and 37 °C. On the other hand, the accumulation capacity of Ni was increased by increasing the incubation period to 96h at 12.71 mg/g at pH 7 and 35 °C. The statistical analysis of variance for the bioaccumulation of lead (Pb), nickel (Ni) and cadmium (Cd) revealed significance (P>0.05) across all treatments when examining the relationship between time, concentration, pH and temperature. Removal and uptake came out to be 59.9% for Pb2+ at an initial concentration of 500 mg/l whereas, in the case of Ni2+, maximum output was attained with 83 % at an initial concentration of 10 mg/l and Cd 2+ 79.4% at an initial concentration 50 mg/l.
These results are consistent with the screening for multi-metal resistant bacteria performed in previous studies, where similar studies carried out by Costa et al., (2001) showed that Bacillus sp., Bacillus cereus, Bacillus sphaericus, and Bacillus subtilis exhibit bioaccumulation of Cu, Zn, Cd, and Pb. Notably, the most beneficial results were observed with Bacillus subtilis and Bacillus cereus. Naskar and colleagues, (2020) reported an intracellular accumulation of approximately 20% of nickel (II) in the growing cells of Bacillus cereus M116. According to Gadd, (1992), the application of microbes for the extraction and recovery of toxic metals from industrial effluents or polluted sites
may offer a more cost-effective and efficient alternative to physicochemical techniques employed for the removal of heavy metals. The findings are consistent with the research conducted by. Bacteria rely on various mechanisms to exhibit tolerance or resistance towards metal compounds. One mechanism employed by bacteria to prevent intoxication involves the limitation of metal movement across the cell wall. The cellular wall functions as a protective barrier against metal ions. Particularly in Gram-positive bacteria, the binding of metals occurs on the peptidoglycan layer, as well as on the teichoic and teichuronic acids present on the cell wall (Beveridge and Fyfe, 1985). In addition, bacteria have the ability to synthesize extracellular polymers, such as exopolysaccharides, that can effectively bind with metal ions. This mechanism serves to safeguard the entire bacterial cell from potential heavy metal contamination, as supported by previous research (Bruins et al., 2000). Enterococcus and Bacillus species commonly employ a mechanism that entails the utilisation of P-type ATPase pumps to govern the translocation of metal ions across the cellular membrane.

CONCLUSION

In conclusion, the findings of our study indicated that the metal-tolerant strain of B. cereus effectively eliminated various metals, including Pb, Ni, and Cd from aqueous solutions through the utilization of biomass. The bioaccumulation process of heavy metals by the bacterial strain was discovered to be impacted by factors such as the initial metal concentrations, pH levels, temperature and the incubation period. The optimal pH for the maximum bioaccumulation capacity of heavy metals by B. cereus was found to be 7. This occurred within a time frame of 96 h at a temperature of 37 °C. This study provides validation for the utilization of B. cereus biomass as a cost-effective and highly efficient bio-sorbing bio-agent for the successful removal of heavy metals from aqueous environments. The application of geospatial techniques has permitted the conduct of geographic analyses regarding heavy metals in Qarun Lake. These techniques can be employed to predict the levels of metals subsequent to biological treatment, thereby providing a means to assess the effectiveness of bioremediation.

REFERENCES


