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Biosorption of Lead and Copper by Epiphytic Rhizobacterial Species Isolated from *Lepironia articulata* and *Scirpus grossus*

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ABSTRACT

In this study, biosorption potential of nine epiphytic bacteria isolated from the rhizosphere of *Lepironia articulata* and *Scirpus grossus* were assessed. Identification of the isolated epiphytic rhizobacteria using 16S rRNA analysis showed species belonging to the four genera of *Bacillus*, *Enterobacter*, *Aeromonas*, and *Chromobacterium*. Batch biosorption studies were carried out to assess the capacity of the isolated bacteria to act as Pb and Cu biosorbents. Different initial concentrations of the two heavy metals (50, 100, 200, 300, and 400 ppm) were used to determine the ability of the biosorbent to reach a tolerance level and then calculate the percentage of biosorption with respect to 0.1 g dry weight. Initial concentration of Pb and Cu exposed showed that the isolated bacteria have high tolerance up to 400 ppm. Bacteria prefer Pb ions over Cu, which is indicated by higher removal of Pb in all tested reactors. *Bacillus* sp. (coded Sc1) showed the highest biosorption capacity with 100% Pb and 97% Cu removal.

Keywords: biosorption, epiphytic bacteria, *Lepironia articulata*, *Scirpus grossus*, Pb, Cu

INTRODUCTION

Biosorption is a technology used to remove contaminants from many environmental media, especially from the aquatic environment (de Freitas et al. 2019). Biosorption technology is highly related to the removal of inorganic pollutants, while biodegradation is mostly related to the organic pollutants (Imron et al. 2019a, 2020). The ability of a certain type of biomass to effectively bind and absorb metal ions is directly linked to its efficacy in removing inorganic pollutants, namely metal ions, from aqueous solutions (Imron et al. 2021). These interaction mechanisms were used in the engineering sector to either treat or remediate contaminated environment (Redha 2020; Ayele and Godeto 2021).

The process of metal ion biosorption onto microbial biomass involves a synergistic interplay of physical adsorption, complexation, ion exchange, and precipitation mechanisms (Ayele and Godeto 2021). Microorganisms, encompassing both viable and non-viable forms, have the potential to serve as biosorbents in the context of heavy metal remediation (Arliyani et al., 2023). The main advantages of the biosorption technology lie in its ability to effectively reduce heavy metal ion concentrations to very low levels and its cost-effectiveness, as the biosorbent used is quite affordable (Purwanti et al., 2023). Additionally, biosorbent generates minimum residue that poses minor environmental risks (Kanamarlapudi et al. 2018). The Malaysian Ministry of Environment has designated heavy metal pollution, such as copper and lead, as a priority pollutant (Ahmed et al. 2020; Shahbudin and Kamal 2021).

Both living and non-living biosorbents may be generated from appropriate biomass and employed for efficient heavy metal removal and recovery from wastewater, with bacteria being the most often used microorganism in biosorption technology (Chellaiah 2018; Okoro et al. 2022). Bacteria can serve as a supplementary agent in the process of phytoremediation for the treatment of heavy metal-contaminated media in wastewater treatment (Ismail et al. 2020; Kurniawan et al. 2022c). The presence of bacteria in the rhizosphere of plants has been seen to contribute to the enhancement of plant development and its ability to withstand the exposure to pollutants, particularly the epiphytic rhizobacteria (Jo atilde o et al. 2016). Epiphytic bacteria have the capacity to enhance plant growth and development in adverse environmental circumstances through many mechanisms (Zolti et al. 2019). Epiphytic bacteria can assist their host plants in overcoming the toxicity effects of pollution, particularly metal ions (Purwanti et al. 2020; Raklami et al. 2021; Said et al. 2021; Kurniawan et al. 2022c).

Plant growth-promoting activity has been demonstrated in epiphytic bacteria, which may be attributed to the generation of phytohormones (Bahadur et al. 2017; Pattnaik et al. 2020). Enzymes play a role in the metabolism of growth regulators, which has direct implications for waste management and pollution prevention employing phytoremediation methods (Zhang et al. 2014; Zhimiao et al. 2019). The effectiveness of pairing heavy metal-resistant epiphytic bacteria with plants for increased pollutant remediation has been demonstrated in the removal of hazardous metals from metal-contaminated sites (Ma et al. 2019; He et al. 2020). However, the assessment of the standalone potential of epiphytic rhizobacterial species to remove heavy metals from aqueous solutions is still limited. The objective of this work was to assess the standalone efficacy of epiphytic rhizobacterial species derived from *Lepironia articulata* and *Scirpus grossus*, which are indigenous Malaysian plants, with the intention of utilizing them in potential bioaugmentation strategies for the phytoremediation of the wastewater contaminated with Pb and Cu.

MATERIALS AND METHODS

Isolation of epiphytic bacteria

Collection of plant samples

To obtain the epiphytic rhizobacterial species in the root system of *S. grossus* and *L. articulata*, plant samples were collected from the reed bed area and Lake Chini in Pahang, Malaysia. The samples were collected in sterile zip-lock plastic bags (6 bags for each plant), stored in an icebox, and immediately transferred to the laboratory at Universiti Kebangsaan Malaysia for isolation and further analysis of epiphytic rhizobacteria. The samples were stored for analysis within six hours of collection from the reed bed or lake (Al-Ajalin et al. 2022).

Incubation and isolation of the epiphytic bacteria

The plant samples that were gathered, namely those that were in a healthy condition, were partitioned into two distinct groups. Each group was thereafter placed in individual sterile zip-lock plastic bags for storage purposes. The plant root specimen underwent a thorough rinsing process using distilled water in order to effectively remove any silt that was adhered to the roots. Subsequently, the roots were rinsed with sterile distilled water to facilitate the isolation of epiphytic rhizobacteria (Titah et al. 2018). The root specimen for each plant was dissected into smaller fragments. A quantity of 5 grams of the sample was taken from each bag and introduced into 250 milliliters of sterilized nutrient broth. This process was carried out using an analytical balance (XT 220A, Malaysia). The mixture was then placed in a shaker incubator (model SI-100D, Germany) and incubated for 24 hours at a temperature of 37°C and a speed of 150 revolutions per minute. A volume of 1 ml was extracted from the incubated nutrient broth and subsequently mixed with 9 ml of sterilized normal saline solution containing 0.85% sodium chloride. This process led to the creation of a dilution series reaching a concentration of 10^6. A volume of 100 μL of the respective dilutions was inoculated onto a nutrient agar medium and incubated at 37°C for 24 hours (Imron et al. 2019b). All different colonies grown were isolated for identification.

Measurement of bacteria dry weight

There are several methods for determining microbial growth. These are based on various parameters of the cells, such as measuring the dry weight of the cell mass, which is a simple method for measuring cell growth (Jafari et al. 2015). A fresh sterile culture of bacteria was established on a medium containing nutrient. Subsequently, three distinct colonies of each bacterium were suspended in 250 ml of nutrient-rich liquid medium that had been sterilized using autoclaving. The suspension was then subjected to incubation at a temperature of 37°C and a rotational speed of 150 rpm in a shaker incubator (model SI-100D, Malaysia). The dry weight was measured at three specific time points: 0, 18, and 24 hours. At each time point, the optical density (OD) at a wavelength of 660 nm was determined using a UV spectrophotometer (Genesys 10, USA). The culture sample from the nutrient solution was subjected to centrifugation using an Eppendorf 5804 centrifuge from Germany. The resulting pellets of microbial cells were collected on a weighed filter paper, specifically a Whatman GF/C glass fiber filter with a diameter of 47 mm and a nominal pore size of 1.2 µm. The dry weight of the filter paper, along with the microbial cell pellets, was then measured, as described in the study by Alessandrello and Vullo (2018).

Investigation of biosorption capability

Stock solutions of lead ($PbCl_2$) and copper ($CuCl_2.2H_2O$) were prepared with a concentration of 1000 ppm. The pH of both stock solutions was determined (lead: 4.9-5.2 and copper: 4.6-4.8), and different concentrations (50, 100, 200, 300 and 400 ppm) were prepared for the following experiments. Fresh bacteria obtained from nutrient agar cultures were then inoculated in nutrient broth and incubated at 37°C. Incubation was maintained at 37°C for 24 hours on a 150 rpm shaker (Kurniawan et al. 2021). Bacteria were then harvested for 15 minutes at 4000 rpm using a centrifuge (Eppendorf 5804, Germany) (Kurniawan et al. 2022a). The harvested bacteria were inoculated with different concentrations of heavy metals at 37°C and 150 rpm for 1, 2, and 4 hours, and the pH of the copper and lead binding solutions was adjusted to pH 6 with 0.1 N NaOH or 0.1 N HCl for the experiments (Kurniawan et al. 2022b). To guarantee that no heavy metals stuck to the surface of the bacteria or equipment, the bacteria were centrifuged at 4000 rpm for 15 minutes and rinsed with a 0.01 M ammonium acetate solution as a chelating agent. All biosorption calculations were carried out based on 0.1 g dry weight. To disintegrate the cells and clear the solution, the collected biomass was decomposed with 1:1:3 nitric acid: hydrogen peroxide: distilled water and kept at room temperature for 24 hours before analysis (Mohammed et al. 2017).

Identification of the isolated bacteria with 16S rRNA

The utilization of 16S rRNA gene sequences for the examination of bacterial phylogeny and taxonomy is very prevalent due to several factors. The steps of identification of bacteria consisting of gram staining, DNA extraction, PCR ribotyping, and sequencing were conducted based on authors' previous research (Muhamad et al. 2021; Kurniawan et al. 2021; Al-Ajalin et al. 2022).

Statistical analysis

Minitab v12 was used to conduct statistical analysis for this study following the steps by Imron et al. (2021). All collected data was checked for normality, homogeneity, and independence and found to meet the three criteria for further processing using ANOVA. The correlation between employed parameters (adsorbed concentration and retention time) and response (decrease/increase in biosorption capacity) was then examined using one-way ANOVA. For all significant correlation, Tukey HSD analysis was then performed as Post-Hoc analysis to obtain the differences between responses. The conclusion for each statistical analysis in this study was established by considering the p-value within the 95% confidence interval ($\alpha = 0.05$). A p-value < 0.05 was used as the criterion to identify the presence of significant differences between the replies (Kurniawan and Imron 2019a, b).

RESULTS AND DISCUSSION

A total of nine bacterial strains were isolated from the root systems of *Lepironia articulata* and *Scirpus grossus* and tested for highest tolerance to each heavy metal. Five strains were derived from

L. articulata and another four strains from *S. grossus*. The isolated bacteria were named as follows:

- Isolated epiphytic rhizobacteria from the root system of *L. articulata* were referred to as Lap(1+2)1, Lap(1+2)2, Lap1, Lap2, and Lap2.
- Isolated epiphytic rhizobacteria from the root system of *S. grossus* were referred to as (Sc(1+2)1, Sc(1+2)2), Sc1, and Sc2.

These isolated bacteria were tested for their ability to play a role in treatment as biosorption to be used in a green technology prior to the use of the l6S rRNA identification procedure. Batch adsorption experiments were performed to determine the ability of the isolated bacteria to act as biosorbents for Pb and Cu.

Biosorption of Pb and Cu by Lap(1+2)1

Figure 1 summarizes the average concentration and percentage of biosorption of Pb and Cu by Lap(1+2)1 within four hours and over three time periods (1, 2, and 4 hours) at different initial concentrations starting at 50 ppm and ending at 400 ppm. The results for the five different concentrations of heavy metals used (Pb and Cu) showed that the biosorption capacity of Lap(1+2)1 is different for Pb and Cu. According to these results, Lap(1+2)1 shows greater bioaccumulation of Pb than Cu, reaching 100% within one hour. Cu bioaccumulation with an initial concentration of 50 ppm reached a maximum of 36% within four hours. The epiphytic bacteria used could tolerate the increase of both heavy metals up to 400 ppm, but the percentage of bioaccumulation still decreases despite an increase in the initial heavy metal concentrations. These epiphytic bacteria absorb more Pb than Cu and might be employed as a microbe to remediate lead as well as copper-polluted areas.

The study examined the changes in biosorption concentration throughout a 4-hour retention period for various heavy metal concentrations. To determine the significance of these variations, a multiple comparison of the mean was conducted, comparing the differences between 1 hour and 2 hours, as well as between 2 hours and 4 hours. A significance level of $p < 0.05$ was utilized. A statistically significant disparity was seen in the retention times across all concentrations employed. Significant variations were not observed just at the initial concentration of 50 ppm, suggesting that equilibrium might be achieved during the first hour. The heavy metal tolerance of two separate strains of Bacillus sp. was examined in a batch approach by Çolak et al. (2011). The study examined operational parameters, including the starting metal concentration, exposure period, and adsorbent dosage. In comparison to the control strain, it was shown that both isolates exhibited a high degree of resistance towards copper and lead. Rodríguez-Tirado et al. (2012) conducted a study to investigate the biosorption capacities of Bacillus thioparans, a kind of subtropical estuarine bacterium, in relation to Cu and Pb. The researchers performed batch experiments under different laboratory conditions to assess the biosorption capabilities of the bacteria. The findings indicate that the bacteria have potential

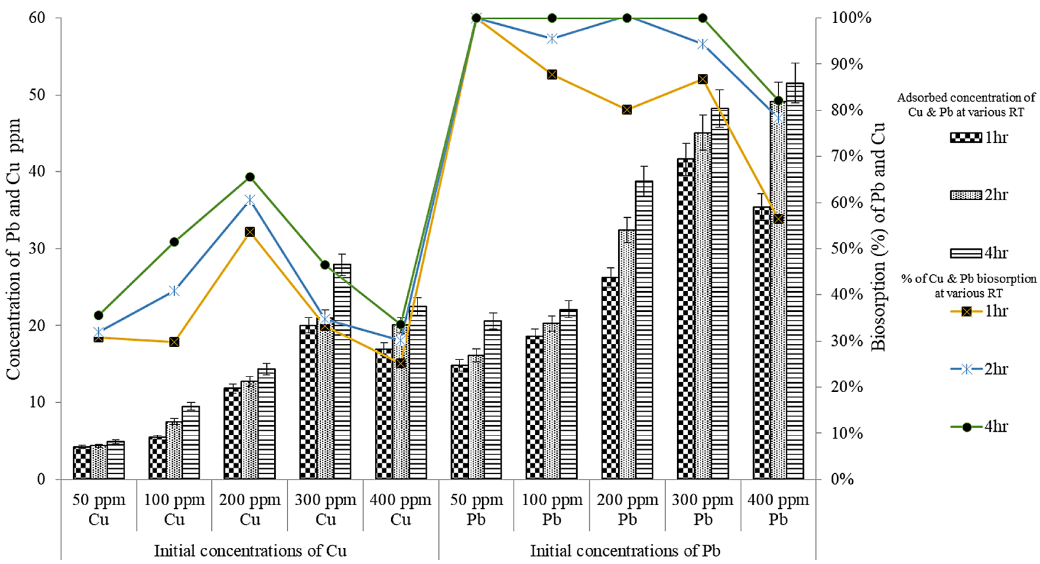


Figure 1. Biosorption of Pb and Cu by Lap(1+2)1

as an effective biosorbent for these metals, with Pb demonstrating superior biosorption capabilities compared to Cu across all conditions.

Biosorption of Pb and Cu by Lap (1+2)2

The results of biosorption of Pb and Cu by Lap (1+2)2 are shown in Figure 2, which presents the biosorption concentrations over a 4-hour residence time period and the percentage of biosorption of Pb and Cu at 0.1 g dry weight. Lap (1+2)2 was analyzed for biosorption and maximum bioaccumulation of Pb and Cu. In this case, the highest bioaccumulation for Pb was observed at an initial concentration of 50 ppm, reaching 100% within the first hour. The bioaccumulation of Cu was 31% in the first hour and reached 52% after four hours. After increasing the initial concentration to 400 ppm, the bioaccumulation of Pb decreased to 60%, and the bioaccumulation of Cu was only 16% at this concentration. This indicates that the isolated epiphytic bacteria Lap (1+2)2 are better than Cu at biosorption of Pb.

The variation of adsorption capacity of Lap (1+2)2 with biosorption time for different concentrations of Pb and Cu at different initial concentrations (50-400 ppm) was verified by multiple comparison of the mean significant difference at the 0.05 level ($p \leq 0.05$) for 1 h versus 2 h and 2 h versus 4 h. The biosorption of both heavy metals at 50 ppm showed no significance in the last period of adsorption time. Pb at 100 ppm showed no significant differences for all adsorption periods, indicating that equilibrium was reached within the first hour. Cu, on the other hand, showed no significant difference in the second period of adsorption time at the last two initial concentrations (300 and 400 ppm). However, the epiphytic bacteria showed very good abilities in biosorption of Pb and Cu, with Pb being far superior to Cu.

Enterobacter sp., which is commonly present in the rhizosphere, on root surfaces, and in roots, can directly and/or indirectly improve the length or quality of plant development (Abedinzadeh et al. 2019). Zaki and Farag (2010) isolated three distinct bacterial strains: *Chryseobacterium* sp., *Enterobacter* sp., and *Stenotrophomonas* sp. The three bacteria were employed to biosorb copper (II) from an aqueous solution at varying starting copper (II) concentrations of 25-250 mg/L at a constant temperature of 30°C. The highest copper (II) biosorption efficiency was obtained using Enterobacter sp., which reached 71%. *Pseudomonas aeruginosa* was also shown to remove 100% of Cu in the pH range 7.00-7.72 (Pérez Silva et al. 2009). Oves et al., (2013) investigated the metal-absorbing capabilities of bacterial epiphytic bacteria isolated from the rhizosphere of cauliflower cultivated in the soils constantly flooded with industrial effluent. Maximum lead and copper biosorption were found at a low starting metal concentration of 25 mg/L, with 90.6% and 91.8%, respectively, achieved. Biosorption was reported to be 82.7% at an initial concentration of 150 mg/L.

Biosorption of Pb and Cu by Lap1

Figure 3 shows the change in adsorption capacity and bioaccumulation by Lap1 at a

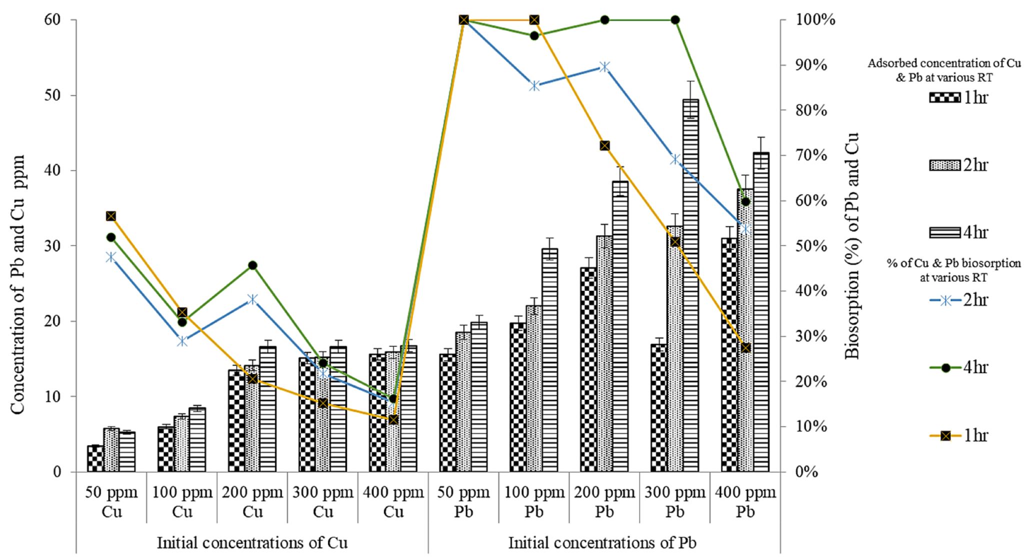


Figure 2. Biosorption of Pb and Cu by Lap (1+2)2

biosorption time of four hours at different concentrations of Pb and Cu (50-400) ppm, where the bioaccumulation capacity was calculated at 0.1 g dry weight. The amount of Pb and Cu biosorbed by Lap1 almost increases with time and eventually reaches a constant value beyond which nothing is removed from solution. At this point, the amount of heavy metals desorbed by the adsorbent is in dynamic equilibrium with the amount of lead biosorbed by the bacteria. The adsorption capacity increases along with the initial Pb and Cu concentrations from 50 to 400 ppm, making Lap1 an efficient biosorbent for Pb and Cu in aqueous solution as the process gradually reaches equilibrium. The biosorption of Pb ions by Lap1 increases with time; however, the ability to bioaccumulate decreases with increasing concentration. This is evident from the results, as the biosorption of Pb was 100% at 50 ppm and then decreased to 47% at 400 ppm. The biosorption of Cu started at 83% and ended at 12% when the initial concentration reached 400 ppm.

All data were analyzed for multiple comparison of the mean significant difference at the 0.05 level ($p \leq 0.05$) for different biosorption periods at the following intervals: 0 h to 1 h, 0 h to 2 h, and 0 h to 4 h. In other words, 1 h versus 2 h and 2 h versus 4 h. The differences in mean metal concentrations between biosorption periods for all initial concentrations are summarized as follows. For Cu biosorption, all results were significant except at 100 ppm in the second and final periods (1 h vs. 4 h), which showed no significant differences. No significant differences were also observed at 400 ppm as well as between the first and second periods. The Pb biosorption showed no significant differences at 50 ppm between the first and second periods. There were also no significant differences for all biosorption periods at 100 ppm. The experimental results show that strain Lap1, isolated from the root system of *L. articulata*, has an excellent ability to remove significant amounts of both metals from aqueous solutions.

The efficacy of *Aeromonas hydrophila* in the biosorption of lead from aqueous solutions has been demonstrated in a study conducted by Hasan et al. (2010). The study examined the influence of process variables, such as the starting concentration of Pb(II) and the dose of biomass, on the absorption of lead. The results of ANOVA indicated a curvilinear relationship between the concentration and absorption of Pb(II). According to the optimization plots, the maximum predicted absorption of Pb(II) was found to be 122.18 mg/L at a temperature of 20°C. This absorption was seen while using a biomass dose of 1.0 g dry weight and an initial Pb(II) concentration of 259 mg/L. The biomass of *A. hydrophila*, both in its free and immobilized forms, was employed for the purpose of removing Pb(II) from an aqueous solution. The findings of the sorption experiments indicated that the biomass had a sorption capacity of 163.9 mg/L and 138.88 mg/L for the free and immobilized forms, respectively.

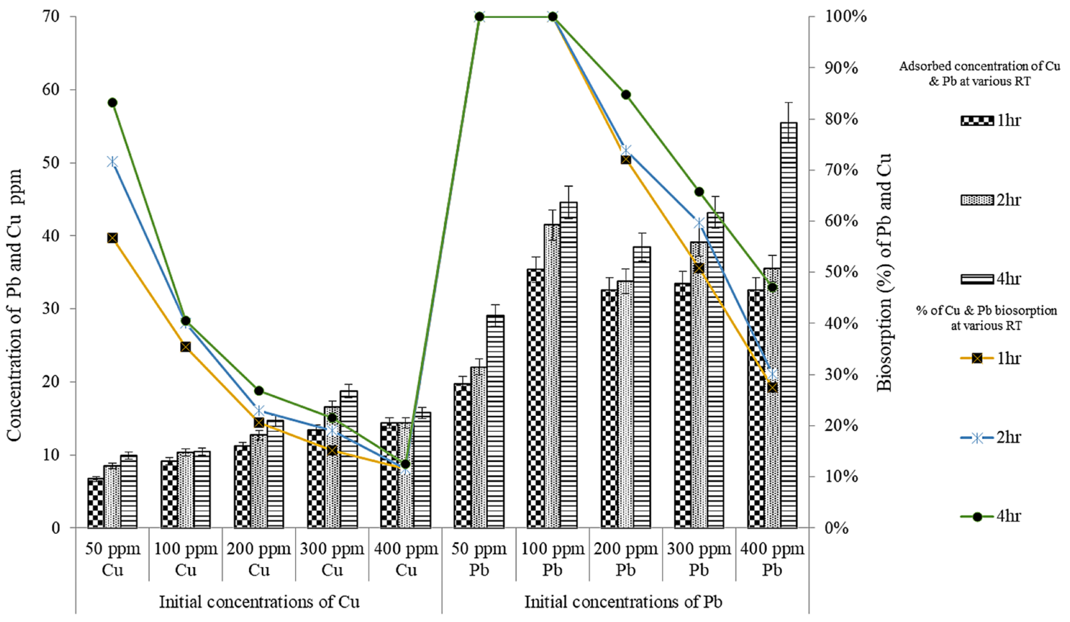


Figure 3. Biosorption of Pb and Cu by Lap1

In a study conducted by Odokuma (2009), the author investigated the bioconcentration capabilities of *Bacillus* sp., *Pseudomonas* sp., and *Aeromonas* sp. in relation to several heavy metals (namely Fe, Zn, Cu, Cd, Pb, and Ni) that are linked with crude oil. The findings of the study indicated that the absorption of metals was most pronounced in the Bacillus culture that was 24 hours old, and this uptake dropped dramatically as the culture age increased from 24 to 96 hours. The absorption of different metals by *Pseudomonas* sp. and *Aeromonas* sp. exhibited a notable enhancement as the culture age rose. The most favorable uptake of the corresponding metals was achieved when the cultures reached a maturity of 72 hours, for instance, *Aeromonas* sp. with a dry weight of 64 mg/L. The research revealed that the bacterial isolates had a higher capacity for heavy metal bioaccumulation when there was an increase in biomass and when the culture age was suitable.

Biosorption of Pb and Cu by Lap2

The case of sorption by Lap2 considered here involves the biosorption of Pb and Cu from aqueous solutions at different initial concentrations, starting at 50 ppm and ending at 400 ppm over a biosorption period of four hours. The results are presented in Figure 4, as biosorption concentrations in the biomass used and as bioaccumulation calculated in 0.1 g dry weight. The biosorption of the heavy metals Pb and Cu by Lap2 feathers was investigated at different initial concentrations between 50 and 400 ppm. The percentage bioaccumulation of Pb and Cu increased along with initial concentration up to 300 ppm, at which the bioaccumulation of Pb was 100% and that of Cu was 57%. With further increases in concentration, however, the maximum bioaccumulation of the metals decreased; the percentage of bioaccumulation of Pb and Cu at 400 ppm by Lap2 was 69% and 28%, respectively. The biosorption capacity of Pb was very high as 100% bioaccumulation could be achieved within the first hour of biosorption at an initial concentration of 200 ppm. The highest bioaccumulation of Cu was reached with 38% within the first hour at an initial concentration of 200 ppm.

The present study aimed to evaluate the bioaccumulation potential of Lap2 in relation to heavy metals, namely Pb and Cu, in aqueous solutions. This assessment involved comparing the mean significant difference at a significance threshold of $p < 0.05$ over several time periods (1, 2, and 4 hours) for each initial concentration utilized. Time and concentration have a significant impact on heavy metal bioaccumulation; they are therefore an important prerequisite for adsorption. Time indicates contact time, while concentration indicates accumulation ability as a biosorbent. The statistical test showed that there was a significant difference between the Pb biosorption results at the last three initial concentrations (200, 300 and 400 ppm), but not at 100 ppm. The results for Cu indicate that the first two initial concentrations

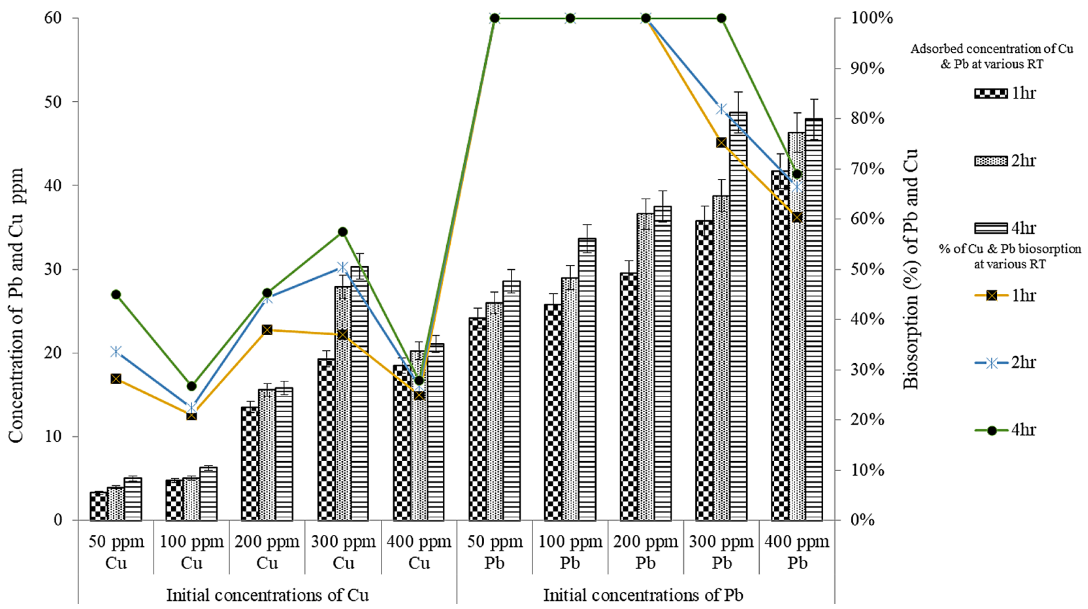


Figure 4. Biosorption of Pb and Cu by Lap2

show a significant difference between the second and third periods of biosorption time. At 200 ppm, the differences were only significant between the first and second periods, while the remaining initial concentrations showed significant differences between all periods. Thus, Lap2 has a high ability to remove significant amounts of both metals from aqueous solutions.

This study aimed to research the process of bioleaching metals from electronic wastes, employing cyanogenic bacterial strains (*Chromobacterium* sp. and *Pseudomonas* sp.). As a single and mixed culture, *Chromobacterium* sp. was used as biosorption agent for the heavy metals from electronic waste. It was discovered that a single culture removed 79% of copper (Cu), whereas a mixed culture removed 83% of Cu (Pradhan and Kumar 2012). In a prior study conducted by Webb (1998), batch tests were conducted to investigate the time dependency of optimum binding and metal binding capabilities for copper(II) and lead(II), as well as the desorption process of the bound metal. The temporal analysis revealed that the cyanobacterium demonstrates rapid binding. The results of the capacity studies demonstrated that the examined cyanobacterial strain had the ability to adsorb 11.3 mg of copper (II) per gram of biomass and 30.4 mg of lead (II) per gram of biomass. Furthermore, the recovery rate for both copper (II) and lead (II) metal ions exceeded 98%. The experimental findings indicated that the immobilized biomass effectively bound an average of 143 mg/L copper (II) and 1456 mg/L lead (II). Furthermore, treatment with 0.2 M HCl resulted in near-complete recovery of both copper (II) and lead (II). According to Buhari et al. (2023), Cyanobacteria have the potential to serve as a viable resource for implementing an innovative method inside biosystems, aimed at the remediation of pollutants from solution. Additionally, this technique can facilitate the accessibility of these pollutants to the industrial sector, while ensuring the utilization of an ecologically sustainable biofiltration system.

Biosorption of Pb and Cu by Lap3

Lap3 was used for the biosorption of aqueous Pb and Cu solutions with different initial concentrations ranging from 50 to 400 ppm within four hours at three biosorption times (1, 2 and 4 hours). The bioaccumulation calculated at 0.1 g dry weight is shown in Figure 5. Lap3 showed very high Pb biosorption capacity and achieved 100% bioaccumulation within the first hour of biosorption at initial concentrations of 50 and 100 ppm. Thereafter, biosorption decreased with increasing initial concentration down to 400 ppm, and bioaccumulation decreased to 56% throughout the biosorption period. The accumulation of Cu was only 53% at an initial concentration of 50 ppm and dropped to 28% at an initial concentration of 400 ppm. To test the effects of Lap3 on the biosorption of Pb and Cu from aqueous solutions,

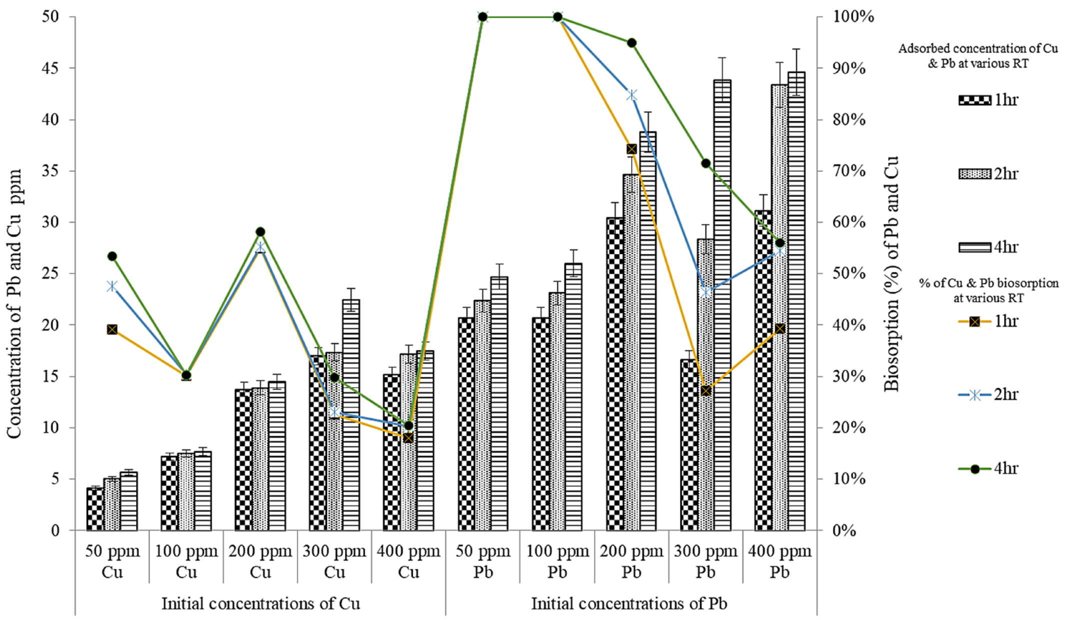


Figure 5. Biosorption of Pb and Cu by Lap3

the results were analyzed by comparing the mean significant difference at the 0.05 level ($p \leq 0.05$) at five different initial concentrations (50, 100, 200, 300, and 400 ppm) and at three different biosorption times (1, 2, and 4 hours). In this way, each initial concentration was tested for significance based on 1 h versus 2 h and 2 h versus 4 h. The statistical test showed that there was a significant difference between the results obtained for the biosorption of Cu at all initial concentrations for the biosorption times considered. Also, the results obtained for the biosorption of Pb showed the same result, except that the initial concentration of 50 ppm did not show any significant difference when compared between 1 hour and 2 hours within the biosorption time. Thus, Lap3 showed a very good ability to absorb Pb and Cu, but the biosorption of Pb was still better than that of Cu.

In their study, Shin et al. (2012) successfully isolated and identified a strain of lead-resistant *Bacillus* sp. from the root system of *Alnus firma*, a plant known for its ability to hyperaccumulate metals. The results of the study revealed that the epiphytic bacteria, when present in isolation, demonstrated the ability to mitigate the harmful effects of heavy metals on plant growth, specifically in the hyperaccumulator species *A. firma*. Additionally, these bacteria were shown to enhance the accumulation of Pb in the plant. Previous research findings have shown a correlation between *Bacillus* sp. and the predominant occurrence of Pb and Cu accumulation (Costa and Duta, 2001). *Micrococcus luteus* DE2008 was obtained from a consortia of *Microcoleus* sp. and shown the ability to take up Pb and Cu. The findings of this study indicate that the examined microorganism has a higher ability for absorbing Pb(II) compared to Cu(II). This is supported by the specific elimination capacities observed, which were 408 mg/g for Cu and 1965 mg/g for Pb. Consequently, it may be considered a microorganism with the ability to remediate Pb- and Cu-contaminated settings (Puyen et al., 2012).

Biosorption of Pb and Cu by Sc(1+2)1

To evaluate the biosorption capacity of Sc(1+2)1, experimental studies were conducted with heavy metals (Pb and Cu) in aqueous solutions at different initial concentrations, ranging from 50 to 400 ppm. The bioaccumulation capacity was calculated at 0.1 g dry weight and the results are shown in Figure 6. This series of experiments was performed to evaluate the efficiency of Sc(1+2)1 in the biosorption of Pb and Cu. It was found that the bioaccumulation of Pb at an initial concentration of 50 ppm could reach 100% within the first hour of the biosorption time. Thereafter, it decreased with increasing initial concentration up to 400 ppm, with 51% accumulation in the first hour and 96% over the entire four-hour period. For Cu accumulation with an initial concentration of 50 ppm, the initial accumulation within the first hour of the biosorption time was 36%, which

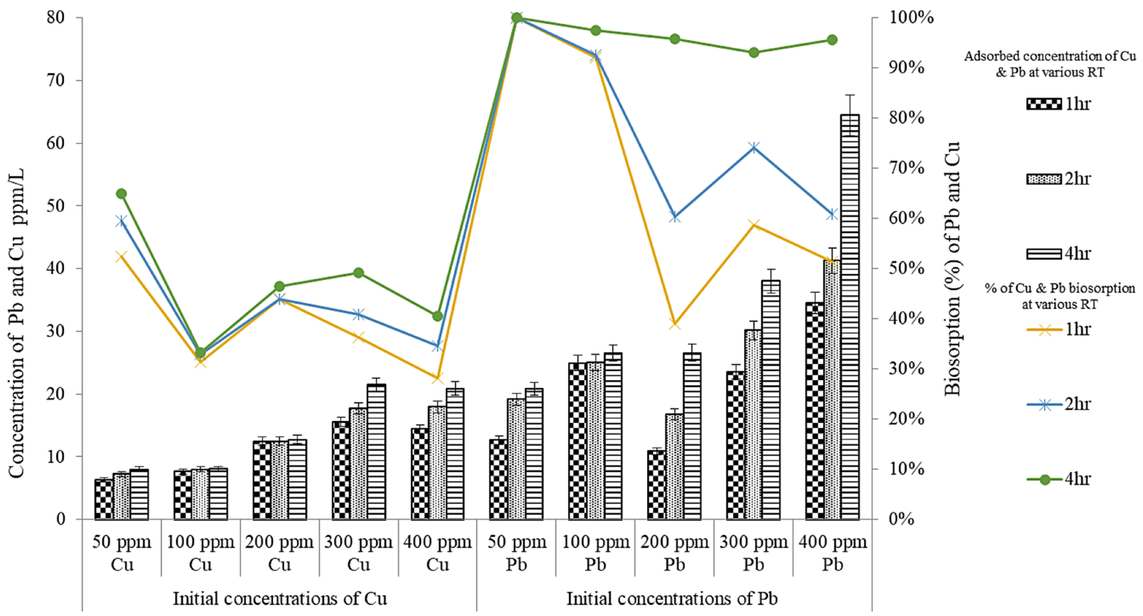


Figure 6. Biosorption of Pb and Cu by Sc(1+2)1

decreased to 14% at an initial bioaccumulation of 400 ppm and reached 20% during the four-hour biosorption time.

Sc(1+2)1 acts as a biosorbent for Pb and Cu from aqueous solution at different initial concentrations, starting at 50 ppm and ending at 400 ppm, with a biosorption time of four hours. The results were analyzed by comparing the mean significant difference at the 0.05 level ($p \leq 0.05$). The parameters to be compared are initial concentration and biosorption time (1, 2 and 4 hours contact time). As for Cu biosorption for the initial concentrations (50, 300 and 400 ppm), there was a significant difference at all biosorption contact times, while the 100 ppm showed a significant difference only between the second and third intervals (2 and 4 hours), and the 100 ppm showed no significant difference between all contact times. However, the process of Pb biosorption indicated that the last three initial concentrations were significantly different in all comparisons and the first initial concentration (50 ppm) showed the significance between the first and second interval (1-2 h), while the 100 ppm showed a significant analysis between the second and third interval (2-4 h). Here, all experiments were performed to investigate the ability of Sc(1+2) to absorb Pb and Cu, clearly indicating that the isolated bacteria were more capable of bioaccumulating Pb than Cu, but that they could still be a good biosorbent for both heavy metals.

El-Deeb (2009) investigated the involvement of epiphytic bacteria (*Enterobacter* sp.) in resistance and accumulation of metals. The experimental results demonstrated that the isolated epiphytic *Enterobacter* sp. was not only heavy metal resistant, but also accumulated significant quantities of heavy metals from the growing medium. On the basis of cell dry weight, the sequence of biosorbed metals by the parent strain and its healed derived strain was discovered to be as follows: $Pb^{2+} > Zn^{2+} > Cd^{2+}$. The exopolysaccharide synthesized by the isolated *Enterobacter* sp. exhibited remarkable Cu (20%) biosorption capabilities, indicating its strong heavy metal chelation characteristics. Nevertheless, the augmentation of the initial copper concentration did not exert a substantial impact on the biosorption process. The biosorption efficiency remained rather stable, hovering around 18-20% (equivalent to 3.11-6.60 mg/g biomass) as reported by Iyer et al. (2005).

Enterobacter sp. was also isolated from a local industrial wastewater treatment facility in Taiwan and utilized to absorb lead (Pb) and copper (Cu) from aqueous solutions (Lu et al. 2006). The results demonstrate that *Enterobacter* sp. can take up more than 50 mg of Pb and 32.5 mg of Cu per gram of dry cell, with a Pb and Cu ion recovery of more than 90%. The capacity of these bacteria to take up metals via biosorption or bioaccumulation has piqued the interest of researchers, since it has the potential to be a successful and cost-effective method of heavy metal cleanup.

Biosorption of Pb and Cu by Sc(1+2)2

The biosorption of Cu and Pb from aqueous solutions in batch biosorption studies was performed to explore the ability and efficiency of the isolated epiphytic bacteria Sc(1+2)2 in heavy metal biosorption. Figure 7 summarizes the calculated percentages of bioaccumulation at 0.1 g dry weight for all data. It was shown that the amount of Pb accumulated in the first hour of biosorption at an initial concentration of 50 ppm accounts for 100% of Pb biosorption. Thereafter, the initial concentration gradually increased until it reached 400 ppm, at which time the percent accumulation decreased to 58%. 76% of Pb accumulation was detected after four hours of biosorption contact time. Cu bioaccumulation reached 36% in the first hour at an initial concentration of 50 ppm, and when the initial concentration was increased to 400 ppm, 14% was accumulated in the first hour, and at the end of the biosorption time, the accumulation was only 20%. The current investigation involved the use of Sc(1+2)2 for the biosorption of Pb and Cu from an aqueous solution. The obtained experimental data was subjected to statistical analysis to determine its significance, specifically by comparing the mean significant difference at a significance threshold of 0.05. The primary emphasis of the study was to compare the initial concentrations of individuals (ranging from 50 to 400 ppm) throughout successive biosorption periods. This comparison aimed to determine the impact of the starting concentration and the duration of biosorption contact on the effectiveness of biosorption.

The Cu biosorption processes showed significant differences in all mean comparison analyses for all intervals, except that the initial concentration of 50 ppm showed significant differences only between the first and second intervals. The biosorption of Pb showed significant differences for all comparison analyses used, except for the initial concentration of 50 ppm, where a

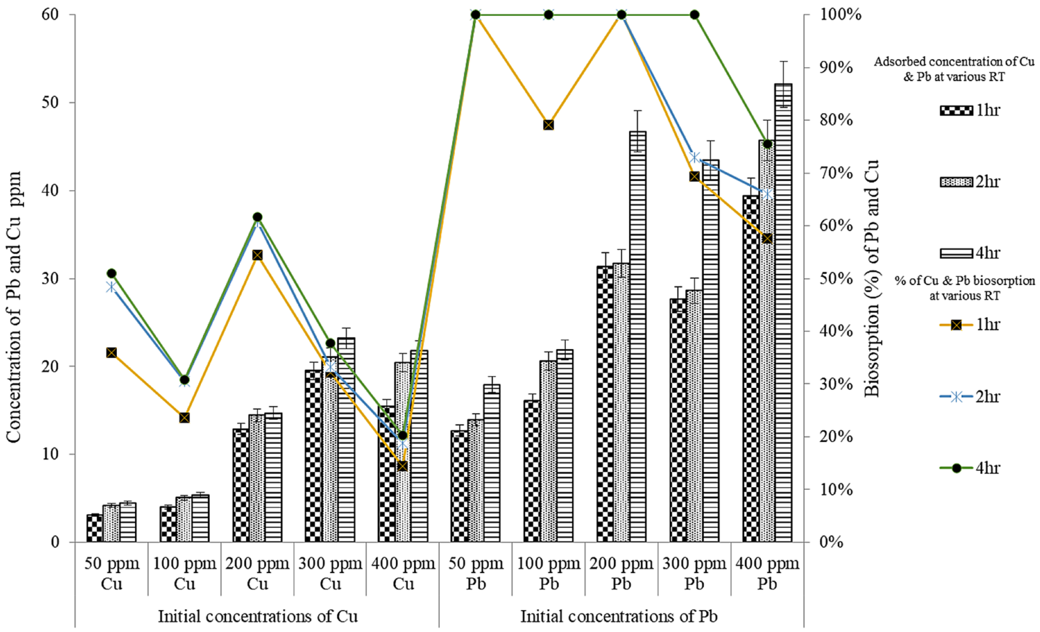


Figure 7. Biosorption of Pb and Cu by Sc(1+2)2

significant difference was found between the second and third intervals, and at 100 ppm, a significant analysis was performed only between the first and second intervals. The reported values for both heavy metals showed that the biosorption of Pb was significantly more efficient than the biosorption of Cu. However, the epiphytic bacteria Sc(1+2)2 indicated higher biosorption capacity and efficiency for both metals.

The earlier study conducted by Pan et al. (2007) investigated the biosorption capacity of *Bacillus* sp. by batch adsorption experiments. The objective was to assess the biosorption potential of biomass for Cu(II) and Pb(II) ions. According to experimental findings from systems with varying biomass percentages, it was shown that the maximum adsorption capacity for heavy metals, namely Cu(II) and Pb(II), was determined to be 50.32 mg/g and 36.71 mg/g, respectively. The study conducted by Nourbakhsh (2002) examined the biosorption of Pb (II) and Cu(II) on *Bacillus* sp. in a batch stirred system, under optimal circumstances and with a maximum starting concentration of 150 mg/L. The *Bacillus* sp. strains that were examined exhibited a lead adsorption capability of 100% and a Cu adsorption capacity of 47%. The results of this investigation demonstrate a favorable outcome and hold potential significance for the field of industrial wastewater treatment. The biosorption of Pb and Cu in aqueous systems was investigated in both individual and combined solutions. The findings of the study indicate that there was an increase in metal absorption by both the cellular component and the total microbial system when the initial lead concentration was raised from 10 to 20 ppm. The statistical analysis of the data indicated that the efficiency of metal absorption by the separated components was higher than that of the unseparated system, with a confidence level of 90%. According to Pradhan and Levine (1995), microorganisms exhibited a preference for Pb over Cu within the concentration range that was investigated in the bimetallic system.

Biosorption of Pb and Cu by Sc1

The total biosorption capacity of Sc1 for Pb and Cu exposed to five different initial concentrations ranging from 50 to 400 ppm at three intervals within four hours was calculated for 0.1 g dry weight and shown in Figure 8. From the results for Sc1, the total percentage of biosorption capacity for Pb at initial concentrations of 50 and 100 ppm showed an accumulation of 100% in the first hour of biosorption. The same percentage was reached for 200 ppm after four hours of biosorption time, and the percentage of bioaccumulation for Pb reached 91% at 400 ppm, which is still considered a very high percentage of bioaccumulation. The highest percentage of accumulation for Cu within the first hour of biosorption time was 83% at 50 ppm, after which the percentage decreases with increasing initial concentration, reaching 18% at an initial concentration of 400 ppm. The accumulation over a 4 hour biosorption time period was 21% for 400 ppm, while the total accumulation of Cu at an initial concentration of 50 ppm and 4 hour biosorption contact time was 97%.

In this study, Sc1 was tested for its efficiency as a biosorbent. The results were analyzed for viability by comparing the mean significant difference at the 0.05 level ($p \leq 0.05$). A change in the range of initial concentrations from 50 to 400 ppm was considered in the analysis of significant differences by comparing the average accumulation within different intervals of biosorption contact time (1, 2, and 4 hours) and comparing with the next biosorption contact time for each interval. The process for the second initial concentration of Cu and the last two showed significant differences in all comparison intervals for these initial concentrations, while the first and initial concentrations of 50 and 200 ppm did not show significant differences in all intervals. The biosorption of Pb showed significant differences in all comparisons between the intervals for the last three initial concentrations, with 50 ppm showing a significant difference between the first and second intervals, while 100 ppm showed significant differences between the second and third intervals of the biosorption time. The results of total biosorption capacity of Sc1 for heavy metals (Pb and Cu) show that these bacteria are good biosorbent for both heavy metals. However, the biosorption for Pb is better than for Cu. Sixty bacterial strains were previously isolated from the industrial wastewater collected in the Peenya industrial area of India, and all were identified as *Bacillus cereus* and selected for further study to investigate the biosorption of lead ions from solutions. The lead biosorption potential of all six isolates was assessed at different Pb concentrations, namely 100, 200, 300, 400, and 500 mg/L. All of the samples exhibited a significant biosorption capacity for Pb, with certain cases achieving a maximum removal efficiency of 79.0%. The present results highlight the biosorption capacity of *Bacillus cereus*, which could be utilized in the bioremediation of lead (Murthy et al. 2012). In a separate study, a batch system was employed to examine the biosorption of Pb(II) and Cu(II) ions from contaminated water by a bacterial strain (*Bacillus* sp.) that was isolated from soil contaminated with heavy metals. The removal of Pb and Cu was effectively achieved at values of 100 mg/L. According to Gong and Li (2011), the findings of the study indicated that the *Bacillus* sp. strain exhibited significant efficacy as a biosorbent in the removal of Pb(II) and Cu(II) ions from contaminated water.

Biosorption of Pb and Cu by Sc2

Figure 9 displays the outcomes of batch biosorption studies, whereby the biosorbent epiphytic bacteria Sc2 were employed to investigate the bioaccumulation of heavy metals (Pb and Cu) from aqueous solutions. The experimental

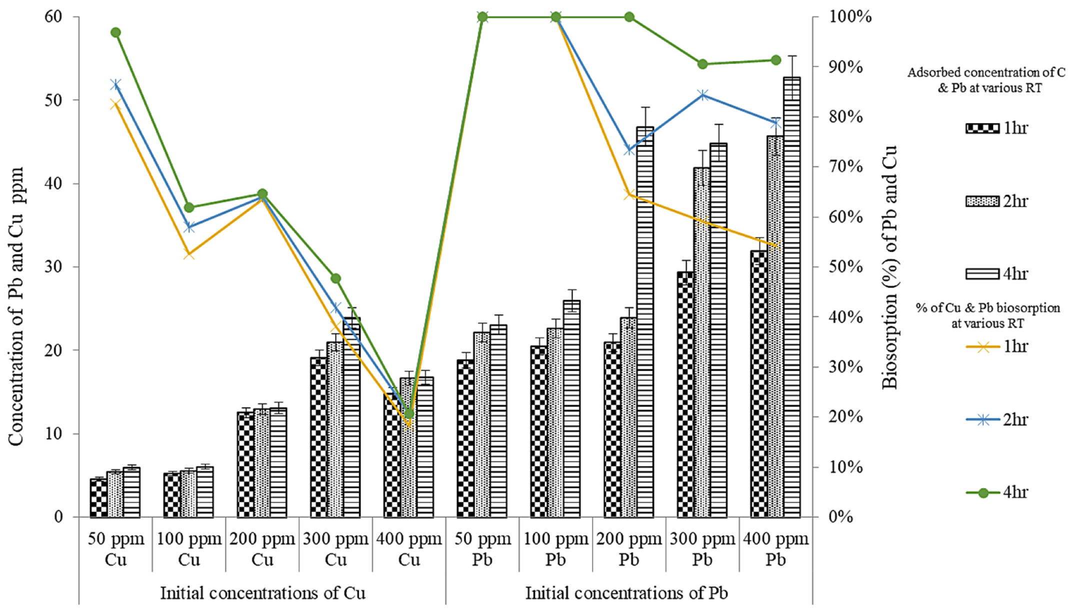


Figure 8. Biosorption of Pb and Cu by Sc1

findings illustrate the biosorption of two distinct heavy metals at varying beginning concentrations, ranging from 50 ppm to 400 ppm, across a four-hour period. This duration was separated into three periods to assess the efficacy of biosorption at a constant dry weight of 0.1 g. This study investigated the influence of contact duration and starting metal concentration on the degree of enrichment, expressed as a percentage. The findings indicated that both factors had an impact on the biosorption ability of the candidate bacterium Sc2. Specifically, the accumulation of Pb was shown to be very high, reaching 100% during the initial hour of biosorption contact time at the two starting concentrations of 50 and 100 ppm. The biosorption efficiency first declined to 49% at a concentration of 400 ppm within the initial hour of biosorption duration, thereafter peaking at a maximum biosorption rate of 84% after 4 hours of biosorption contact time.

The percentage of Cu biosorption was observed to be 41% when the initial concentration was 50 ppm during the first hour of the biosorption contact time. However, this percentage decreased to 19% when the initial concentration was increased to 400 ppm. After a 4-hour biosorption contact time, the bioaccumulation of Cu from the initial concentration was found to be 27%. The biosorption capacity is significantly influenced by the initial concentration and contact time of biosorption. Therefore, the effectiveness of using epiphytic bacteria Sc2 in the biosorption process of heavy metals Pb and Cu from aqueous solutions was evaluated through batch biosorption experiments. The efficiency of this process was assessed by comparing the mean significant difference at a significance level of 0.05. The starting concentrations ranged from 50 to 400 ppm, and three biosorption intervals were examined with a contact period of four hours. The biosorption of both heavy metals exhibited significant results across all beginning concentrations within the comparative biosorption intervals, with the exception of the initial concentration of 200 ppm for Cu and 50 ppm for Pb. Both intervals of biosorption exhibited a notable disparity between the initial and subsequent observations. On the basis of the empirical findings including various starting concentrations of both heavy metals, it can be inferred that Sc2 has promising potential as a biosorbent for both Pb and Cu. Notably, Sc2 demonstrates a significantly better biosorption capacity for Pb in comparison to Cu.

Ölmezoğlu et al. (2012) considered two isolated bacteria from a region where metal industries are located and subjected them to biosorption of copper. Maximum bio removal efficiency of 82% and 75% were obtained at initial concentrations of 20 mg/L, respectively, while a bio removal of 31.7% was found when the initial concentration was raised to 100 mg/L. Various bacterial strains employed in batch studies for copper (Cu) biosorption demonstrate that *Bacillus* sp. biosorption capability was most efficient at concentrations ranging from 25 to 200 mg/L. The results

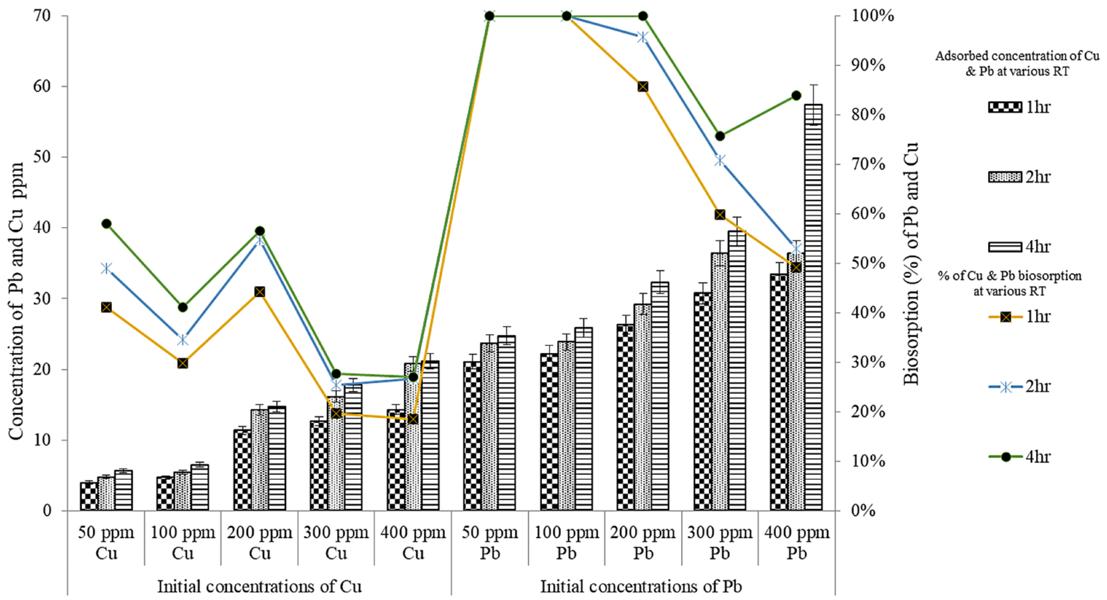


Figure 9. Biosorption of Pb and Cu by Sc2

reveal that these bacterial strains are excellent at removing Cu (II) ions. These bacteria may be utilized in large-scale systems, since they are both cost-effective and efficient at eliminating harmful metal ions (Sethuraman and Kumar 2011). Raja and Omine (2012) isolated and characterized four potential strains of accumulation bacteria selected for their high heavy metal resistance. Identification of these bacteria revealed that one of the isolates was *Lysinibacillus sp*. and the other three were *Bacillus* sp. The strains showed resistance to several heavy metals such as Cu and Pb. The selected strains were found to accumulate the metals efficiently and ranged from 2.3 to 4.7 mg/L for both heavy metals after 24 hours of biosorption time. The research found that the bacteria used acted as potent bioaccumulators and that the removal properties can be useful in bioremediation.

Identification of isolating epiphytic bacteria using 16S

The profile of the identified bacteria isolated from the root systems of *S. grossus* and *L. articulata* plants is summarized in Table 1, and the efficacy of these identified strains as bioaccumulators was discussed and analyzed as well as shown in Table 2. Although the 16S rRNA gene sequence data from a single strain with a nearest neighbor that has a similarity value of < 97% has been shown to represent a new species, the significance of similarity values of > 97% is not as clear, and no "thresholds" have been defined (Janda & Abbott 2007).

Numerous epiphytic bacteria have been identified in various plants and rhizospheres. These bacteria encompass a range of genera, including Achromobacter, Acetobacter, Anabaena, Azoarcos, Arthrobacter, Azotobacter, Azospirillum, Burkholderia, Bacillus, Clostridium, Enterobacter, Frankia, Flavobacterium, Hydrogenophaga, Kluyvera, Microcoleus, Pseudomonas, Phyllobacterium, Staphylococcus, Serratia, Streptomyces, and Vibrio. Additionally, the well-established legume symbiont Rhizobium is also among these bacteria (Bashan et al., 2008). Several studies have shown that Bacillus sp., a species found in isolated environments, has significant resistance

Table 1. Profile of isolated bacteria from root systems of *S. grossus* and *L. articulata*

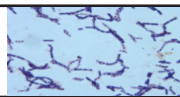
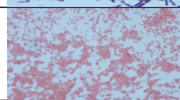
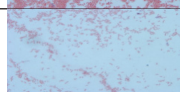
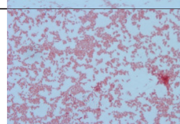
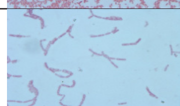
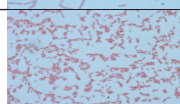
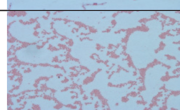
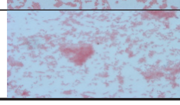
Bacteria code	Shape	Arrangement	Gram stain	Image	16S rRNA Name	Similarity
Lap(1+2)1	Rod	Chain spore forming	Positive		*Bacillus sp*.	98%
Lap(1+2)2	Rod	Mono	Negative		*Enterobacter sp*.	98%
Lap1	Rod	Mono	Negative		*Aeromonas sp*.	98%
Lap2	Rod	Mono	Negative		*Chromobacterium sp*.	98%
Lap3	Rod	Chain spore forming	Negative		*Bacillus sp*.	98%
Sc(1+2)1	Rod	Mono	Negative		*Enterobacter sp*.	98%
Sc(1+2)2	Rod	Chain spore forming	Negative		*Bacillus sp*.	97%
Sc1	Rod	Mono	Negative		*Bacillus sp*.	98%
Sc2	Rod	Mon	Negative		*Bacillus sp*.	100%

Table 2. Effectiveness of isolated bacteria from root systems of *S. grossus* and *L. articulata* for biosorption processes

Bacteria code	Identified species	Biosorption capacity	
		Pb	Cu
Lap(1+2)1	*Bacillus sp.*	100%	66%
Lap(1+2)2	*Enterobacter sp.*	100%	52%
Lap1	*Aeromonas sp.*	100%	83%
Lap2	*Chromobacterium sp.*	100%	57%
Lap3	*Bacillus sp.*	100%	53%
Sc(1+2)1	*Enterobacter sp.*	100%	65%
Sc(1+2)2	*Bacillus sp.*	100%	62%
Sc1	*Bacillus sp.*	100%	97%
Sc2	*Bacillus sp.*	100%	58%

to metal exposure and has the ability to conduct biosorption of heavy metals, such as Pb and Cu (Tunali et al., 2006; Çolak et al., 2011; Carvalho et al., 2013; Nayak et al., 2020). Enterobacter species have been found to exhibit significant resistance to metal exposure and demonstrate effective biosorption of lead (Lu et al., 2006; Carvalho et al., 2013; Chitraprabha and Sathyavathi, 2018). Pradhan and Kumar (2012) revealed that the Chromobacterium sp. species has bioleaching capabilities, whereas Mgbemena et al. (2012) found that the Aeromonas sp. species demonstrates strong resistance to metal exposure.

CONCLUSIONS

Identification of the nine isolated epiphytic bacteria from the root systems of *L. articulata* and *S. grossus* with 16S rRNA showed that these bacteria belong to four genera: *Bacillus, Enterobacter, Aeromonas* and *Chromobacterium*. These isolated bacteria showed high biosorption capacity for the tested metal ions. The isolated epiphytic bacteria showed a good biosorbent potential for Pb and Cu, showing excellent ability to remove significant amounts of both metals, removing Pb up to 100% while Cu ranging from 52% to 97%. The bacteria seem to prefer Pb as compared to Cu, with the highest biosorption capacity showed by bacteria coded Sc1 (*Bacillus* sp.) with 100% removal of Pb and 97% removal of Cu.

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