Check for updates

ASCE

# Bioremediation of Copper with Endophytic Bacteria *Bacillus sp.* and *Streptomyces griseus*

Hülya Böke Özkoç, Ph.D.[1]; Mirac Tansu Aliustaoğlu[2]; and İlknur Şentürk, Ph.D.[3]

**Abstract:** The present study investigated the copper tolerance and bioremediation potential of endophytic bacteria because endophytic bacteria are the most common bacterial strains associated with heavy metal bioremediation. The acute toxic effects of copper on living organisms were determined using two endophytic bacterial species, *Bacillus sp.* and *Streptomyces griseus* (*S. griseus*). After 4 days of acute toxicity test, changes in metal and bacteria concentrations in water, inhibition (%), bioaccumulation rate, and bioconcentration factors were evaluated. According to the evaluations, cell weights decreased, and inhibition rate (%) increased with increasing metal concentration after a certain level (10 mg/L Cu). With increasing metal concentrations from 5 to 25 mg/L, biosorption efficiency decreased from 35.94% to 20.73% for *S. griseus* and from 56.36% to 34.47% for *Bacillus sp.* The bioaccumulation quantities increased with increasing metal concentrations for both species. Based on the present findings, it is concluded that *Bacillus sp.* and *S. griseus* are suitable candidates for the bioremediation of copper ions from contaminated environments. These endophytic bacteria use hyperaccumulating plants for more effective bioremediation of heavy metals. **DOI: 10.1061/JOEEDU.EEENG-7397.** 

**Author keywords:** Bioaccumulation; Biosorption; Copper; Endophytic bacteria; Metal tolerance; Nature-based solutions.

## Introduction

Heavy metals are encountered naturally in trace quantities in various ecosystems. However, metal electroplating and fertilizer industries, mining facilities, battery manufacturing, paper mills, and pesticide production facilities induce heavy metal growth in ecosystems (Rajkumar et al. 2009). Metals are highly toxic to animals and aquatic biota due to their persistence and bioaccumulation. Negative impacts of metals on flora and fauna have primarily been observed in recent years (Şentürk et al. 2023). Heavy metals may pass into human bodies through the food chain (Abo-Alkasem et al. 2023). Some heavy metals are carcinogenic and mutagenic even at low concentrations. Therefore, heavy metals should be removed from receiving bodies or treated before discharge through proper technology (Sagadevan et al. 2022; Jeyakumar et al. 2022).

Copper (Cu) is used in large quantities, especially in metal and metal-related industries. Cu, an essential micronutrient for living cells, can be absorbed acutely or chronically, depending on its amount, exposure, and timing. However, copper is highly toxic at high concentrations, although it is a trace element at minimum concentrations, on soil and water resources (Manohari and Yogalakshmi 2016; Maltsev et al. 2023). Therefore, copper-containing wastewater effluents should pass through proper treatment processes before being discharged into the receiving bodies (Liu et al. 2023; Orozco et al. 2023).

Heavy metals are removed from wastewater through various treatment processes such as filtration, membrane technologies, electrochemical treatment, chemical precipitation, oxidation/reduction, reverse osmosis, and ion exchange (Rajeshkumar et al. 2012; Abo-Alkasem et al. 2023). However, the most of these methods are costly, labor-intensive, inefficient, and generate large quantities of secondary pollutants (sludge) (Chen et al. 2005; Guo et al. 2010; Gupta et al. 2023). These traditional physical and chemical techniques eventually lose applicability because they produce significant amounts of chemical waste (Goutam et al. 2021). Therefore, in situ sustainable techniques should be developed and implemented to ameliorate heavy metal contaminated areas (Mahar et al. 2016). In situ techniques such as bioremediation, phytoremediation, biotransformation, nonhost inoculation, and other methods are used to treat heavy metals in the environment (Abo-Alkasem et al. 2023).

Bioremediation technology, especially among these methods, is a promising technology that uses living green plants or microorganisms to remove pollutants from soils, surface water, and groundwater. Also, bioremediation technology has great application potential due to its environmental protection, safety, cost-effectiveness, and no secondary pollution (Wu et al. 2021; Sui et al. 2021; Priya et al. 2022; Liu et al. 2023; Priya and Tamilselvi 2023). Bioremediation typically achieves through two main mechanisms: bioaccumulation and biosorption (Bharagava and Chowdhary 2019). Bioaccumulation is a bioremediation technique in which the metabolic activity of living organisms is used for metal removal from wastewaters (Davis et al. 2003). Biosorption can be defined passive uptake by living organisms (Demarco et al. 2023). Biosorption and bioaccumulation are suitable for in situ applications (Timková et al. 2018).

Sustainable systems using microorganisms, plants, or their products are used as alternative solutions to reduce metal-induced risks and environmental pollution (Dixit et al. 2015; Timková et al. 2018). Microorganisms can also detoxify metals by bioreduction, biosorption, bioleaching, and biomineralization (Wang et al. 2021). Bacteria, fungi, or algaelike microorganisms are used to reduce the

[1]Associate Professor, Engineering Faculty, Dept. of Environmental Engineering, Ondokuz Mayıs Univ., Samsun 55139, Turkey. ORCID: https://orcid.org/0000-0002-8775-837X. Email: hbokeozkoc@gmail.com; hozkoc@omu.edu.tr

[2]Engineering Faculty, Dept. of Environmental Engineering, Ondokuz Mayıs Univ., Samsun 55139, Turkey. Email: miractansu@gmail.com

[3]Associate Professor, Engineering Faculty, Dept. of Environmental Engineering, Sivas Cumhuriyet Univ., Sivas 58140, Turkey (corresponding author). ORCID: https://orcid.org/0000-0002-8217-2281. Email: ilknurg.senturk@gmail.com; ilknursenturk@cumhuriyet.edu.tr

Note. This manuscript was submitted on April 5, 2023; approved on July 11, 2023; published online on September 6, 2023. Discussion period open until February 6, 2024; separate discussions must be submitted for individual papers. This paper is part of the ***Journal of Environmental Engineering***, © ASCE, ISSN 0733-9372.

negative effects of heavy metals on soil and water resources (Bharagava and Saxena 2020; Volaric et al. 2021). Bacteria have been the primary focus of recent studies conducted for the bioremediation of heavy metals (Govarthanan et al. 2016; Jeyakumar et al. 2022; Aiswarya Sudheer and Chattopadhyay 2023; Singh et al. 2023; Vulpe et al. 2023). Bacterial bioremediation is an effective and reliable technique to degrade, detoxify, mineralize, transform, or reduce the concentration of heavy metals. Recently, a group of microorganisms known as endophytes (endophytic bacteria) have gained attention for their potency to remove or immobilize heavy metals (Priya and Tamilselvi 2023).

Endophytes, microorganisms that live in the host plant's tissues, have historically been investigated for their ability to promote plant development, engage in biocontrol, and produce bioactive substances. There is much space for research and advancement in using endophytes, a relatively new practice. Therefore, endophytes are intriguing microorganisms in our quest to develop novel tools for the bioremediation of contaminants (Bharagava and Chowdhary 2019; Sharma and Kumar 2021). Endophytic bacteria colonize the lower epidermal layer of plant tissues. The endophytic bacteria protect plant cells from heavy metal stress conditions by altering their phytoutilization, reducing or eliminating phytotoxicity (Abo-Alkasem et al. 2023).

Additionally, endophytic bacteria isolated from accumulator plants actively contribute to reducing heavy metal stress and enhancing the rate at which those metals are absorbed by plants (Wang et al. 2020). Burges et al. (2016) found that *Festuca rubra* reduced the bioavailability of Cd and Zn in rhizosphere soil by 19% and 22% after inoculation with endophytes. Endophytes have characteristics that encourage plant growth, which increase plant biomass and phytoextraction amounts of Cd and Zn (Yao et al. 2022). Previous studies (Doty et al. 2007; Fan and Song 2014) demonstrated that natural or engineered endophytes enhanced the performance of bioremediation processes. So far, organic pollutant bioremediation has been given more attention than heavy metals, probably due to their biodegradable nature.

Nevertheless, recent studies showed that many endophytes are metal resistant (Manohari and Yogalakshmi 2016; Nguyen and Phan 2023). *Paenibacillus sp.* RM, endophytic bacteria isolated from the roots of *Tridax procumbens* displayed exceptional resistance to Cu, Zn, As, and Pb. Batch experiments employed in bioremediation investigations revealed that the isolate *Paenibacillus sp*. RM removed the highest amounts of Cu (59.4%), followed by Zn (51.4%) (Govarthanan et al. 2016). Endophytic strain *B. cereus* isolated from *Vigna radiata* showed maximum 57.2% biosorption efficiency for Ni (Kashyap et al. 2022).

Endophytic bacteria may have a more significant potential to reduce the negative effects of heavy metals because plants that do not have a chance to relocate can survive without being affected by accumulated toxic substances. In this respect, the potentials of endophytic bacteria, which are the elements of this relationship established in different plants, especially plants living in natural environments, will be necessary. Therefore, this study evaluated the copper bioremediation potential of bacterial endophytes isolated from plants and provided data for copper tolerance of *Bacillus sp.* and *S. griseus* endophytic strains isolated from wild plants.

## Materials and Methods

### Reagents and Growing Medium

Analytical-grade reagents were used in the present experiments. The required amount of $CuSO_4 \cdot 5H_2O$ was dissolved in distilled water to prepare 1,000 mg/L copper stock solution. The stock solution was then subjected to serial dilutions to prepare the copper solution of different concentrations. All glassware was cleaned with nitric acid and rinsed several times with ultrapure water (Milli-Q system, Millipore Bay City, New Zealand) before the experiments.

### Bacteria Cultures

In this study, endophytic bacterial strains *Bacillus sp.* and *S. griseus* were obtained as a pure culture from the culture collection of the Biology Department, Faculty of Sciences, Ondokuz Mayıs University. These bacterial cultures were isolated from orchid plants and molecularly characterized by Altinkaynak and Ozkoc (2020). Nutrient agar media was autoclaved at 121°C for 15 min, and then it was transferred into plates and used for culturing endophytic bacteria. The plates inoculated with endophytic bacteria were incubated in a climate room at 25°C for 7 days to allow bacterial growth (Albert et al. 2018). Malt extract broth (MEB) agar medium (100 mL) was taken for the liquid culture, placed into 250-mL Erlenmeyer flasks, and then autoclaved at 121°C for 15 min.

After exponential growth, bacteria cells in the solid growth medium were cultivated into a broth medium. The experimental flasks were incubated with shaking on a rotatory shaker at 150 revolutions/min (rpm) for 72 h under continuous cool-white fluorescent lighting of 3,500–4,000 lux and 16/8 h light/dark cycles at 25°C (Ali˙ustaoğlu 2020). The bioremediation rate of living metal-resistant bacteria should be strongly depend on the population of cells at optimal growth conditions (Guo et al. 2010; Ali˙ustaoğlu 2020). Therefore, an incubation period of 72 h was applied, and the desired organism count of $10^4$ cells/mL was reached after 72 h.

### Effects of Heavy Metals on Medium pH

The pH values of the liquid growth medium (1) before inoculation ($pH_o$), (2) after 72 h of incubation ($pH_1$), and (3) after 96 h of metal exposure ($pH_2$) were measured with the use of a pH meter (Ohaus Stater 3100 pH meter, Parsippany, New Jersey). After 72 h of incubation, the growing bacteria were exposed to different copper concentrations for 96 h.

### Bioremediation of Copper with Bacillus sp. and S. griseus

*Bacillus sp.* and *S. griseus* were inoculated into 250-mL Erlenmeyer flasks containing 100 mL of MEB agar medium and incubated in a shaking incubator at 150 rpm for 72 h at 25°C. At the stage of the late exponential phase, every one of the copper solutions (5, 10, 20, and 25 mg/L) was inoculated separately into the culture flasks and incubated in an incubator shaker at 150 rpm at 25°C for 96 h to determine the appropriate range of toxicity for *Bacillus sp.* and *S. griseus* (Rand and Petrocelli 1985; USEPA 1994, 2002). All copper concentrations were added to MEB agar after autoclaving. The growth of the bacteria without copper was considered the control for this experiment.

Toxicity tests were carried out under the test conditions applied for the growth of bacteria. At the end of 96 h, the bacterial cells were separated from the liquid growth medium by filtering through a Whatman glass microfiber filter (Grade GF/C) (Darmstadt, Germany). The resultant filtrate was rinsed through 50-mL ultrapure water, and the bacteria cells were dried at 40°C for about 48 h until a constant mass. The toxic effect of Cu at different concentrations was evaluated by measuring the cell weight as dried mass. Experiments were conducted in triplicate.

Three new fractions were obtained at the end of the experiments, as specified by Albert et al. (2018): Fraction A corresponds to

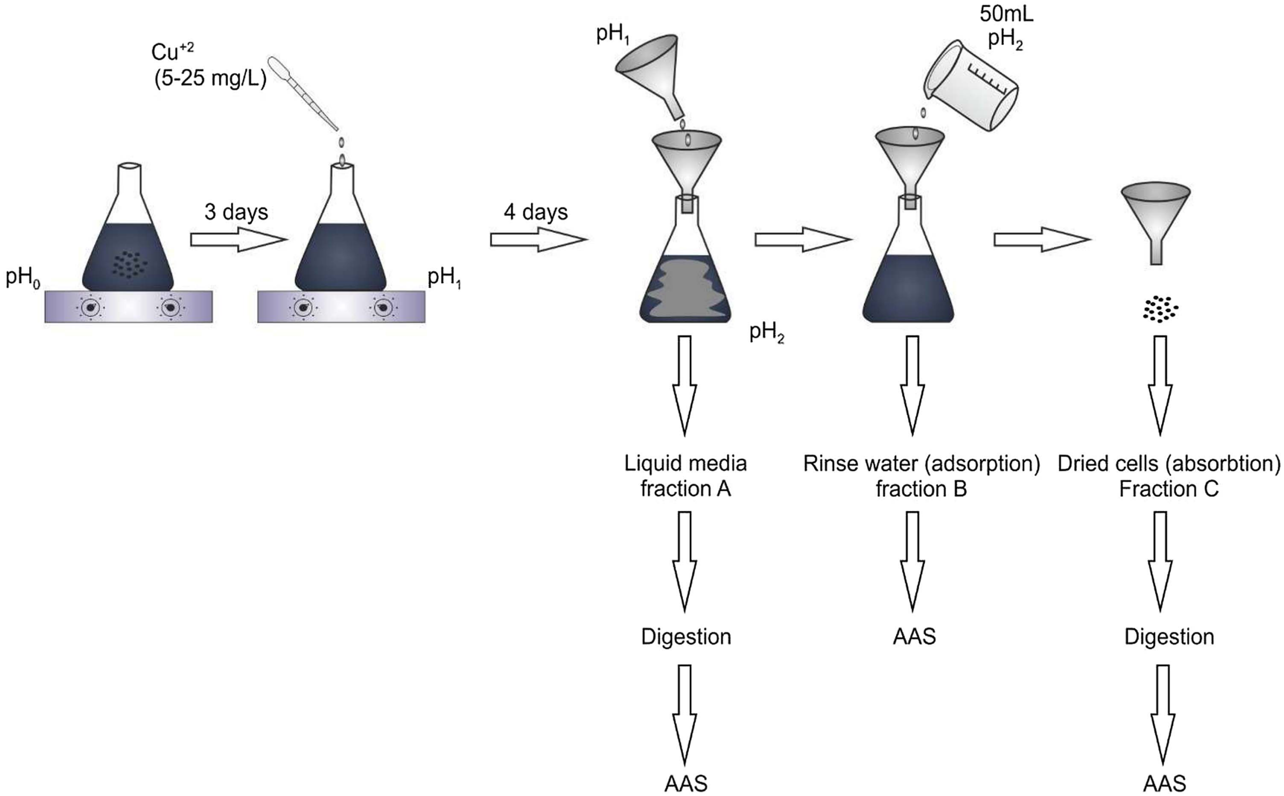


**Fig. 1.** Experimental system designed according to Albert et al. (2018).

liquid media, Fraction B corresponds to rinsing water, and Fraction C corresponds to dried bacteria cells (Fig. 1). Fractions A and C were mineralized before relevant analyses. A 5-mL aliquot of Fraction A and the entire Fraction C were placed separately into a Teflon polytetrafluorethylene beaker and digested in aqua regia [10 mL $HNO_3$ (65% w/w), 10 mL HF (40% w/w), 5 mL $HClO_4$ (60% w/w) and 10 mL HCl, 1 M] (APHA 1995). After digestion, the samples were diluted to 25 mL with distilled water and filtered through a Whatman GF/C glass fiber filter for analysis using a UNICAM 929 AA spectrometer (UNICAM, Cambridge, UK).

The amount of metal absorption (*%ab*) and metal adsorption (*%ad*) was calculated using Eqs. (1) and (2), respectively (Albert et al. 2018)

$$\%ab = Q_{\text{abs}}/Q_o \tag{1}$$

$$\%ad = Q_{\text{ads}}/Q_o \tag{2}$$

where $Q_{\text{abs}}$ = Fraction C (amount of metal absorbed inside the bacteria); $Q_o$ = initial amount of metal in the culture media; and $Q_{\text{ads}}$ = Fraction B (amount of metal adsorbed on the bacteria cell wall).

The growth rate ($\mu$) was calculated using Eq. (3) (Atay and Özkoc 2010):

$$\mu = \frac{\ln X_L - \ln X_0}{t_L} \tag{3}$$

where $X_L$ = cell density measured at the end of $t_L$ time; $X_o$ = initial cell density; and $t_L$ = time between the $L$th measurement and the beginning of the test (h).

The $\mu$ values calculated for each test concentration were used to determine the percent inhibition at different metal concentrations (Eq. 4)

$$I\mu i = \frac{\mu_c - \mu_i}{\mu_c} \times 100 \tag{4}$$

where $\mu_c$ = mean growth rate for control; and $\mu_i$ = mean growth rate for the test concentration $i$ (Atay and Özkoc 2010).

*Bacillus sp.* and *S. griseus* were inoculated into broth medium containing different concentrations of $CuSO_4 \cdot 5H_2O$, and the growth inhibiting copper concentration was measured. During the acute toxicity test lasting 96 h, changes in metal and bacteria concentrations in water, cell density, bioaccumulation (BA) rate, and bioconcentration factor (BCF) were evaluated. In addition, the metal removal efficiency of the organisms was determined by measuring the remaining Cu concentrations in water.

BA was used to describe the uptake of chemicals from the water. The BA values were determined through addition of adsorption and absorption [Eq. (5)]. BCF, representing the distribution of heavy metals between organisms and water and the ability of bacteria to accumulate trace metals, was calculated using Eq. (6) (Ibrahim et al. 2021; Şentürk et al. 2023)

$$\text{BA} = Q_{\text{ads}} + Q_{\text{abs}}\ (\mu\text{g/g DW}) \tag{5}$$

$$\text{BCF} = \frac{C_{\text{organism}}}{C_{\text{water}}}\ (\text{L/kg DW}) \tag{6}$$

where $C_{\text{organism}}$ = total metal concentration in bacteria ($\mu$g metal/g dry weight of bacteria); $C_{\text{water}}$ = total metal concentration in water (mg metal/L); and DW = dry weight.

## Results and Discussion

In this study, two endophytic bacteria (*Bacillus sp.* and *Streptomyces griseus*) were assessed for their copper metal bioremediation potential (Ali˙ustaoğlu 2020).

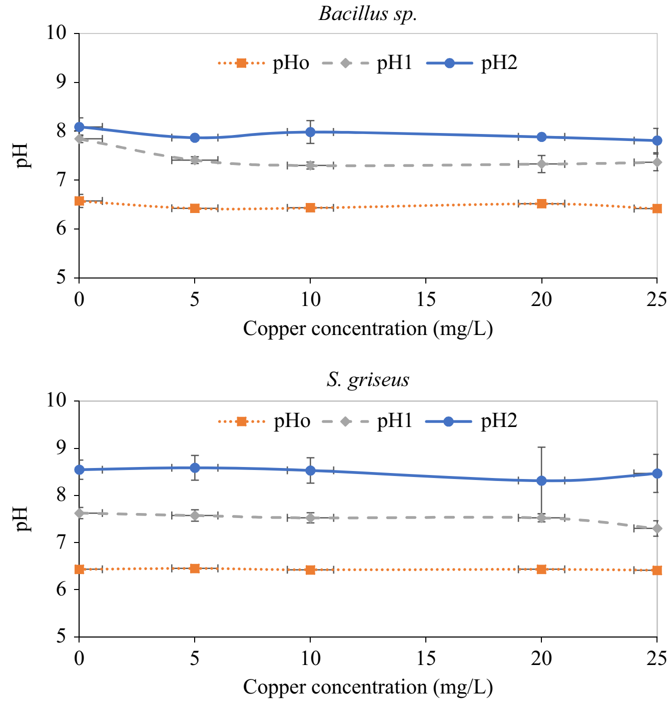


**Fig. 2.** Change in pH during the study ($n = 3$, mean value ±SD).

### Change of Medium pH

The pH changes in the initial culture medium ($pH_o$), after the cultivation of endophytic bacteria into the culture medium ($pH_1$) and after 96 h of exposure to metal concentrations ($pH_2$) are presented in Fig. 2. The initial average pH of the medium was 6.32, the average pH values reached 7.50 when heavy metal was added, and increased to 8.0–8.40 after 96 h of exposure. It was observed that the pH increased as the exposure time increased for each metal concentration.

Solution pH values have a great effect on the solubility of heavy metals. The increases in pH values when copper was added to the medium indicate that biological activities affected the pH of the medium (Fig. 2). Black et al. (2014) conducted bacteria biosorption tests with lead and copper at neutral pH (7.0–7.5) and indicated increased pH values after bacterial cultivation and exposure. It was stated that the pH value affected the solubility of metal ions and the total charge of the biosorbent because protons can be adsorbed or released (Esposito et al. 2002; Yadav et al. 2018). In another study, it was stated that the deprotonation of functional groups occurred on increasing pH, and these acted as negatively charged particles that began to attract positively charged metal ions (Yadav et al. 2018). These studies support our results on the increase in pH after metal exposure for both endophytic bacteria.

### Toxic Effects of Copper on Bacillus sp. and S. griseus Activity

Experiments were carried out using various copper concentrations (5–25 mg/L) on *Bacillus sp.* and *S. griseus* to specify the toxic doses for the growth experiment. Following the metal exposure, the liquid medium was filtered through filter paper, and the cell culture remaining on the filter paper was dried at 103°C. The cellular concentrations of both species were determined as DW (g). The growth responses of *Bacillus sp.* and *S. griseus* at different initial Cu concentrations in liquid cultures are illustrated in Fig. 3.

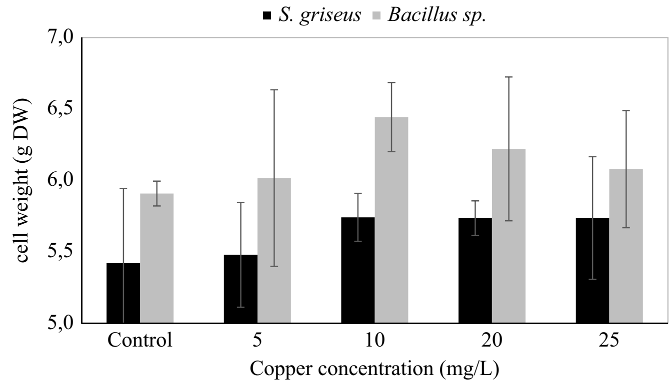


**Fig. 3.** Changes in *Bacillus sp.* and *S. griseus* cell weight at the end of 96 h exposure to different concentrations of copper for each medium ($n = 3$, mean value ±SD).

Following 96-h of exposure of bacteria to Cu, the cell weight values were essentially stable with no significant upward trend. This phenomenon showed that the biomass of *S. griseus* and *Bacillus sp.* did not increase significantly.

Bacteria cells exhibited better growth at all metal concentrations than control treatments (0 mg/L Cu). Cell growth was not affected negatively at 5 and 10 mg/L Cu metal concentrations. However, bacterial growth decreased for both species at Cu concentrations above 10 mg/L. Although an essential nutrient, copper can be toxic to plants and bacteria at high concentrations (Fathollahi et al. 2021). High doses negatively influence the metabolic activities of endophytic bacterial strains and reduce cell weight. However, a more substantial weight decrease was not observed in this study. Manohari and Yogalakshmi (2016) investigated copper tolerance and bioremediation potential in endophytic bacteria isolated from *Vigna unguiculata* root nodules. Their study used four endophytes belonging to the genera *Bacillus* and *Arthrobacter*, and Manohari and Yogalakshmi (2016) observed a decrease in bacterial growth at higher Cu concentrations. It is only partially correct to compare the findings of our study with those of previous studies. Because biotic and abiotic stressors may restrict the bacterial metal detoxification rates and bioavailability of metals in the bacterial system (Govarthanan et al. 2016).

Survival and growth are directly related to the resistance of bacteria to heavy metals (Li and Ramakrishna 2011). Therefore, the inhibition effects of the copper solution at different copper concentrations on selected bacteria have been evaluated, and the results are presented in Fig. 4. According to this, the inhibition rate also increased as the metal concentrations increased. In the present study, the inhibition rates increased from 1.59% to 7.11% for *S. griseus* and from 1.34% to 2.72% for *Bacillus sp.* with increasing Cu concentrations from 5 to 25 mg/L. Do et al. (2022) reported that the inhibition ratio at 1 mg/L Cu was 2.22%, which increased gradually with increasing Cu concentration. The toxic substance's highest inhibition (29.37%) was observed at 10 mg/L Cu. It was understood that increased metal concentrations led to cell growth inhibition. Our experiments' results agree with the findings of Do et al. (2022).

The results showed that metals exert their toxic effects on microorganisms through various mechanisms. Metal-tolerant bacteria could survive in these habitats and be isolated and selected with potential for use in the bioremediation of contaminated sites. A similar study was conducted by de Abreu et al. (2014) to evaluate the effects of cadmium and copper exposure on *Chlorella vulgaris*.

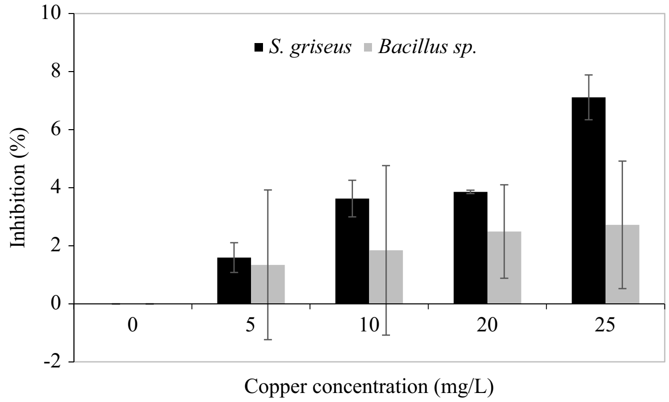


**Fig. 4.** Percentage inhibition of *Bacillus sp.* and *S. griseus* at 96 h versus copper concentration ($n = 3$, mean value $\pm$SD).

de Abreu et al. (2014) indicated that 1 mg/L Cu was determined as the half-maximal effective concentration ($EC_{50}$), for *C. vulgaris*, and inhibition effects on algae culture were encountered even at relatively low concentrations.

### *Biosorption of Copper by Endophytic Microorganisms*

The metal biosorption capacity, the sum of the percentage of the metal absorbed and adsorbed, was studied during the growth cycle of *Bacillus sp.* and *S. griseus* cells. *Bacillus sp.* and *S. griseus* cells accumulated copper in the cell's interior (absorption) and bound it to the cellular surface (adsorption) (Albert et al. 2018). The growth phase is a biotic variable that can affect metal biosorption by bacterial population. The biosorption percentages measured at the end of the 96-h periods under different metal concentrations are given in Table 1.

Considering our results for *Bacillus sp.*, the greatest biosorption rate (56.36%) was seen at an initial concentration of 5 mg/L, and absorption was more dominant (42.36%) under these conditions. Although the biomass concentration reached the maximum level when the Cu concentration increased to 10 mg/L (Fig. 3), an 8% decrease (from 56.36% to 48.18%) was seen in biosorption. As the initial metal concentrations increased, 34.47% biosorption capacity and 22.73% absorption capacity were observed at a 25-mg/L Cu concentration; however, a slight increase was observed in biomass concentration compared with the control.

At the end of the exposure period for *S. griseus*, the greatest biosorption (46.65%) was obtained from a 10-mg/L initial concentration, and the absorption capacity was measured as 33.40%. The biosorption capacity decreased to 20.73% when the metal concentration increased to 25 mg/L. Endophytic *Bacillus sp.* was more resistant than *S. griseus* at low concentrations. Burbank (2022) has also reported that various bacterial strains may have varying tolerance to copper.

According to Majhi et al. (2023), copper removal efficiency significantly decreased at higher Cu concentrations (100 mg/L), although the highest removal percentage was achieved at lower Cu concentrations (20 mg/L). Cu ions can successfully bind with the most significant number of available active sites at lower concentrations, increasing the sorption potential of the sorbent. However, when the concentration rises, all available adsorption sites become saturated. Additionally, high metal concentration becomes toxic to bacteria, which lowers their metabolic activity by preventing active cell growth (Majhi et al. 2023).

It was seen that the absorption potentials were higher when the adsorption and absorption potentials of endophytic bacteria were evaluated separately. These data may indicate that endophytic bacteria were not much affected by Cu metal. This may be because microorganisms convert the metals they interact with into less toxic substances (Darwesh et al. 2021). Copper uptake with endophytic bacteria such as *Bacillus thuringiensis* GDB-1, *Pseudomonas sp.* Lk9, and *Pseudomonas koreensis* AGB-1 studied by different researchers (Babu et al. 2013, 2015; Chen et al. 2014). In another study, Peng et al. (2019) studied the copper biosorption efficiency of *Ochrobactrum* MT180101 and reported the biosorption efficiency as 85.5% at a 2-mg/L copper concentration. All these studies also reported that biosorption efficiency decreased with increasing ionic copper concentration exceeding the threshold that bacteria can tolerate.

In this study, endophytic *Bacillus sp.* was more resistant than *S. griseus* at low concentrations. It was determined that they were not resistant at increasing metal concentrations. The organism continually absorbs the metal into the cell (deposits it for later use and excretes the rest) as exposure time and metal concentration increase (Özkoc and Taylan 2010). Burbank (2022) also reported that various bacterial strains may have varied tolerance to copper.

The present findings revealed that biosorption might vary based on the organisms used. Although the biosorption rate of the endophytic bacteria was lower than that of the organisms mentioned in the literature, these biosorption rates can be increased when endophytic bacteria are evaluated together with plants, especially hyperaccumulating plants (Franco-Franklin et al. 2021). The endophytic bacteria could increase the host plant's resistance to multimetal contamination. Plant and endophytic bacteria interactions may be more effective because endophytic bacteria neutralize toxic metals in the environment. In this sense, it was decided that hyperaccumulator plants may provide more effective results. Hyperaccumulators can accumulate large quantities of heavy metals and may offer a specialized environment for bacterial endophytes (Rajkumar et al. 2009).

**Table 1.** Biosorption (%) of copper ions from endophytic culture media by *Bacillus sp.* and *S. griseus*

| Metal biosorption capacity | Concentration (mg/L) 5 | 10 | 20 | 25 |
|---|---|---|---|---|
| | *S. griseus* | | | |
| %ab | 25.10 | 33.40 | 24.42 | 11.26 |
| %ad | 10.84 | 13.25 | 12.01 | 9.47 |
| %Biosorption | 35.94 | 46.65 | 36.43 | 20.73 |
| | *Bacillus sp.* | | | |
| %ab | 42.36 | 35.21 | 25.98 | 22.73 |
| %ad | 14.00 | 12.97 | 13.84 | 11.74 |
| %Biosorption | 56.36 | 48.18 | 39.82 | 34.47 |

### *Risk Assessment for Endophytic Microorganisms*

BA refers to the uptake of contaminant concentrations from the ambient medium (Mello et al. 2020). The BCF indicates a substance's ecotoxicity, and these values are useful in interpreting the distribution rates of chemicals in the ecosystem. Therefore, BCF is a key parameter for risk assessment and modeling studies (Atay et al. 2013; Rubio-Santiago et al. 2023).

BA and BCF values after 96 h exposure to different metal concentrations are presented in Fig. 5. BA is generally used to evaluate metal tolerance and the potential use of bacteria in bioremediation (Ribeiro et al. 2020). This parameter indicates microorganisms' heavy metal accumulation capacity (Aladesanmi et al. 2019).



J. Environ. Eng., 2023, 149(11): 04023073

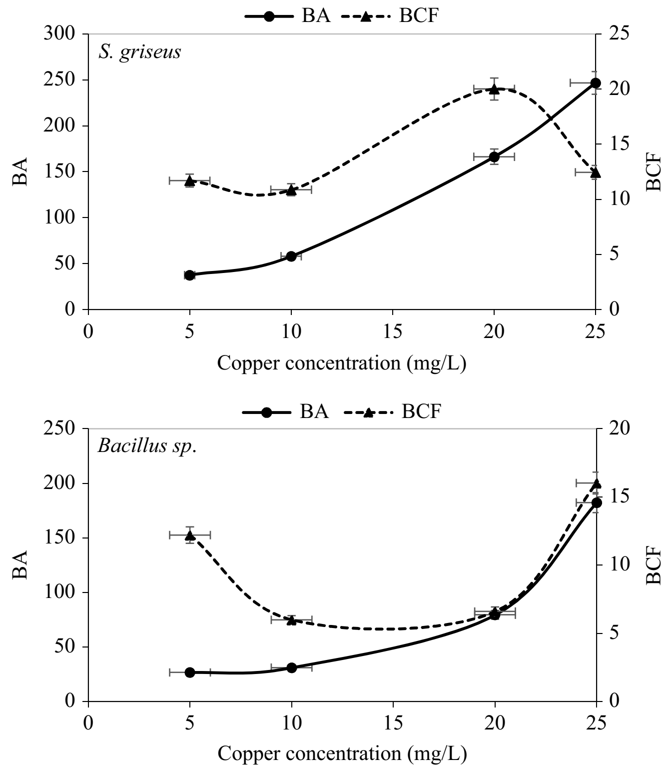


**Fig. 5.** BA ($\mu$g metal/g dry weight of cell) and BCF [$\mu$g metal/g dry weight of cell $(\text{mg metal/L})^{-1}$] values at the end of 96 h.

The bioaccumulation effect of *S. griseus* and *Bacillus sp.* on metal uptake was assessed through BA values. The present BA values increased with increasing Cu concentrations. After the increase of metal concentration from 5 to 25 mg/L, metal bioaccumulation increased from 37.47 to 246.8 $\mu$g/g DW for *S. griseus* and from 26.63 to 182.3 $\mu$g/g DW for *Bacillus sp.*

The bioaccumulation of metals is determined mainly by metabolic activity, biochemical characteristics, genetic adaptation capacity of microorganisms, and prevailing environmental conditions (Varghese 2012). Microorganisms continually absorb the metal into the cell (deposit it for later use and excrete the rest) with increases in exposure time and metal concentration. Therefore, the BA value also increases, as reported by Özkoc and Taylan (2010). As can be inferred from Fig. 5, the present BA values increased in both bacteria species with increasing metal concentrations. BCF values exhibited a different trend in each organism. For *S. griseus*, a decrease was seen at 25 mg/L Cu concentration compared with the initial concentration (from 20 to 12.45 L/kg DW), whereas an increase was seen in *Bacillus sp.* (from 6.6 to 16.02 L/kg DW). Metal concentrations influence the metal binding capacity of bacteria. Microorganisms better accumulate essential metals at low concentrations to meet metabolic requirements (Muyssen and Janssen 2002; Parven et al. 2022).

## Conclusion

The present experiments were conducted to compare the bioremediation potential of *Bacillus sp*. and *S. griseus* exposed to various copper concentrations. During experiments, pH increased after metal exposure to endophytic bacteria. Cell growth was not affected negatively at 5- and 10-mg/L Cu metal concentrations. However, bacterial growth decreased for both species at Cu concentrations above 10 mg/L. The inhibition rate also increased as the metal concentrations increased. The highest biosorption rate under operating conditions was obtained as 56.36% at a 5-mg/L concentration for *Bacillus sp.* and 46.65% at a 10-mg/L concentration for *S. griseus*. It was seen that the selected endophytic species removed copper from water with 50% efficiency up to a 10-mg/L copper concentration.

BA values increased with increasing Cu concentrations. After the increase of metal concentration from 5 to 25 mg/L, metal bioaccumulation increased from 37.47 to 246.8 $\mu$g/g DW for *S. griseus* and from 26.63 to 182.3 $\mu$g/g DW for *Bacillus sp*. BCF values exhibited a different trend in each organism. For *S. griseus*, a decrease was seen at a 25-mg/L Cu concentration compared with the preceding concentration (from 20 to 12.45 L/kg DW), whereas an increase was seen in *Bacillus sp.* (from 6.6 to 16.02 L/kg DW).

These findings suggest that endophytic *Bacillus sp.* and *S. griseus* could be potential candidates for bioremediation of copper ions from heavy metal–contaminated environments. These endophytic bacteria can cultivate hyper accumulating plants in polluted areas. In this way, heavy metals can be effectively removed from the environment and converted into less harmful and even useful products because endophytic organisms have transformative potential. However, the isolated strains can also be used as a consortium for large-scale treatment of industrial effluents in the future.

## Data Availability Statement

All data, models, and code generated or used during the study appear in the published article.

## Acknowledgments

This work was supported by Ondokuz Mayıs University Research projects PYO.MUH.1904.18.027 directed by Associate Professor Dr. Hülya Böke Özkoç.

## References

Abo-Alkasem, M. I., N. H. Hassan, and M. M. Abo Elsoud. 2023. "Microbial bioremediation as a tool for the removal of heavy metals." *Bull. Natl. Res. Cent.* 47 (1): 31. https://doi.org/10.1186/s42269-023-01006-z.

Aiswarya Sudheer, C. K., and I. Chattopadhyay. 2023. "Endophytic bacteria for drug discovery and bioremediation of heavy metals." Chap. 8 in *Endophytic association: What, why and how*, edited by M. Shah and D. Deka, 159–181. Cambridge, MA: Academic Press.

Aladesanmi, O. T., J. G. Oroboade, C. P. Osisiogu, and A. O. Osewole. 2019. "Bioaccumulation factor of selected heavy metals in *Zea mays*." *J. Health Pollut.* 9 (24): 191207. https://doi.org/10.5696/2156-9614-9.24.191207.

Albert, Q., L. Leleyter, M. Lemoine, N. Heutte, J. P. Rioult, L. Sage, F. Baraud, and D. Garon. 2018. "Comparison of tolerance and biosorption of three trace metals (Cd, Cu, Pb) by the soil fungus *Absidia cylindrospora*." *Chemosphere* 196 (Apr): 386–392. https://doi.org/10.1016/j.chemosphere.2017.12.156.

Ali˙ustaoğlu, M. T. 2020. "Evaluation of heavy metal toxicity with bacterial struts insulated from soil." Master's thesis, Institute of Graduate Studies, Environmental Engineering Dept., Ondokuz Mayıs Univ.

Altinkaynak, H., and I. Ozkoc. 2020. "Isolation and molecular characterization of plant growth promoting bacteria from the rhizosphere of orchids in Turkey." *Rhizosphere* 16 (Dec): 100280. https://doi.org/10.1016/j.rhisph.2020.100280.

APHA (American Public Health Association). 1995. *Standard methods for the examination of water and wastewater.* Washington, DC: APHA.

Atay, Ş., and H. B. Özkoç. 2010. "Effect of sediment on the bioavailability and toxicity of copper and zinc to a green alga." *Fresenius Environ. Bull.* 19 (12a): 3018–3027.

Atay, Ş.H. B. Özkoç, and V. R. Uslu. 2013. "Sediment toxicity for evaluation of sediment quality guidelines and bioavailability of copper and zinc." *Fresenius Environ. Bull.* 22 (4a): 1031–1042.

Babu, A. G., J.-D. Kim, and B.-T. Oh. 2013. "Enhancement of heavy metal phytoremediation by *Alnus firma* with endophytic *Bacillus thuringiensis* GDB-1." *J. Hazard. Mater.* 250–251 (Apr): 477–483. https://doi.org/10.1016/j.jhazmat.2013.02.014.

Babu, A. G., P. J. Shea, D. Sudhakar, I. B. Jung, and B. T. Oh. 2015. "Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal(loid)-contaminated mining site soil." *J. Environ. Manage.* 151 (Mar): 160–166. https://doi.org/10.1016/j.jenvman.2014.12.045.

Bharagava, R. N., and P. Chowdhary. 2019. *Emerging and eco-friendly approaches for waste management*. Singapore: Springer.

Bharagava, R. N., and G. Saxena. 2020. *Bioremediation of industrial waste for environmental safety: Volume II: Biological agents and methods for industrial waste management*. Singapore: Springer.

Black, R., M. Sartaj, A. Mohammadian, and H. A. M. Qiblawey. 2014. "Biosorption of Pb and Cu using fixed and suspended bacteria." *J. Environ. Chem. Eng.* 2 (3): 1663–1671. https://doi.org/10.1016/j.jece.2014.05.023.

Burbank, L. P. 2022. "Threat of *Xylella fastidiosa* and options for mitigation in infected plants." *CABI Rev.* 17 (Jun): 1–12. https://doi.org/10.1079/cabireviews202217021.

Burges, A., L. Epelde, G. Benito, U. Artetxe, J. M. Becerril, and C. Garbisu. 2016. "Enhancement of ecosystem services during endophyte-assisted aided phytostabilization of metal contaminated mine soil." *Sci. Total Environ.* 562 (Aug): 480–492. https://doi.org/10.1016/j.scitotenv.2016.04.080.

Chen, L., S. Luo, X. Li, Y. Wan, J. Chen, and C. Liu. 2014. "Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas sp*. Lk9 on soil heavy metals uptake." *Soil Biol. Biochem.* 68 (Jan): 300–308. https://doi.org/10.1016/j.soilbio.2013.10.021.

Chen, X. C., Y. P. Wang, Q. Lin, J. Y. Shi, W. X. Wu, and Y. X. Chen. 2005. "Biosorption of copper(II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZ1." *Colloids Surf., B* 46 (2): 101–107. https://doi.org/10.1016/j.colsurfb.2005.10.003.

Darwesh, O. M., M. F. Eida, and I. A. Matter. 2021. "Environmental nanobiotechnology: Microbial-mediated nanoparticles for sustainable environment." In *Microbial nanobiotechnology*. Edited by A. Lateef. Singapore: Springer.

Davis, T. A., B. Volesky, and A. Mucci. 2003. "A review of the biochemistry of heavy metal biosorption by brown algae." *Water Res.* 37 (18): 4311–4330. https://doi.org/10.1016/S0043-1354(03)00293-8.

de Abreu, F. C., P. N. da Costa, A. M. Brondi, E. J. Pilau, F. C. Gozzo, M. N. Eberlin, M. G. Trevisan, and J. S. Garcia. 2014. "Effects of cadmium and copper biosorption on *Chlorella vulgaris*." *Bull. Environ. Contam. Toxicol.* 93 (4): 405–409. https://doi.org/10.1007/s00128-014-1363-x.

Demarco, C. F., M. S. Quadro, F. Selau Carlos, S. Pieniz, L. B. G. A. Morselli, and R. Andreazza. 2023. "Bioremediation of aquatic environments contaminated with heavy metals: A review of mechanisms, solutions and perspectives." *Sustainability* 15 (2): 1411. https://doi.org/10.3390/su15021411.

Dixit, R., et al. 2015. "Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes." *Sustainability* 7 (2): 2189–2212. https://doi.org/10.3390/su7022189.

Do, M. H., H. H. Ngo, W. Guo, S. W. Chang, D. D. Nguyen, A. Pandey, P. Sharma, S. Varjani, T. A. H. Nguyen, and N. B. Hoang. 2022. "A dual chamber microbial fuel cell based biosensor for monitoring copper and arsenic in municipal wastewater." *Sci. Total Environ.* 811 (Mar): 152261. https://doi.org/10.1016/j.scitotenv.2021.152261.

Doty, S. L., et al. 2007. "Enhanced phytoremediation of volatile environmental pollutants with transgenic trees." *Proc. Natl. Acad. Sci. USA* 104 (43): 16816–16821. https://doi.org/10.1073/pnas.0703276104.

Esposito, A., F. Pagnanelli, and F. Vegliò. 2002. "pH-related equilibria models for biosorption in single metal systems." *Chem. Eng. Sci.* 57 (3): 307–313. https://doi.org/10.1016/S0009-2509(01)00399-2.

Fan, X., and F. Song. 2014. "Bioremediation of atrazine: Recent advances and promises." *J. Soils Sediments* 14 (10): 1727–1737. https://doi.org/10.1007/s11368-014-0921-5.

Fathollahi, A., S. J. Coupe, A. H. El-Sheikh, and E. O. Nnadi. 2021. "Cu(II) biosorption by living biofilms: Isothermal, chemical, physical and biological evaluation." *J. Environ. Manage.* 282 (Mar): 111950. https://doi.org/10.1016/j.jenvman.2021.111950.

Franco-Franklin, V., S. Moreno-Riascos, and T. Ghneim-Herrera. 2021. "Are endophytic bacteria an option for increasing heavy metal tolerance of plants? A meta-analysis of the effect size." *Front. Environ. Sci.* 8 (Jan): 603668. https://doi.org/10.3389/fenvs.2020.603668.

Goutam, J., J. Sharma, R. Singh, and D. Sharma. 2021. "Fungal-mediated bioremediation of heavy metal–polluted environment." In *Microbial rejuvenation of polluted environment*. Edited by D. G. Panpatte, and Y. K. Jhala, 51–76. Singapore: Springer.

Govarthanan, M., R. Mythili, T. Selvankumar, S. Kamala-Kannan, A. Rajasekar, and Y. C. Chang. 2016. "Bioremediation of heavy metals using an endophytic bacterium *Paenibacillus sp.* RM isolated from the roots of *Tridax procumbens*." *3 Biotech* 6 (2): 242. https://doi.org/10.1007/s13205-016-0560-1.

Guo, H., et al. 2010. "Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium *Bacillus sp.* L14." *Bioresour. Technol.* 101 (22): 8599–8605. https://doi.org/10.1016/j.biortech.2010.06.085.

Gupta, T., D. Chakraborty, and A. Sarkar. 2023. "Microbial enzymes and role in bioremediation of environmental pollutants: Prospects and challenges." In Vol. 2 of *Microbial degradation and detoxification of pollutants*. Berlin: Walter de Gruyter.

Ibrahim, A. A., A. G. Yusuf, G. Ismail, M. A. Ibrahim, A. R. Musa, and M. S. Sulaiman. 2021. "Conceptual background of bioaccumulation in environmental science." *World J. Adv. Pharm. Life Sci.* 1 (1): 035–041. https://doi.org/10.53346/wjapls.2021.1.1.0015.

Jeyakumar, P., C. Debnath, R. Vijayaraghavan, and M. Muthuraj. 2022. "Trends in bioremediation of heavy metal contaminations." *Environ. Eng. Res.* 28 (4): 220631. https://doi.org/10.4491/eer.2021.631.

Kashyap, S., R. Chandra, B. Kumar, and P. Verma. 2022. "Biosorption efficiency of nickel by various endophytic bacterial strains for removal of nickel from electroplating industry effluents: An operational study." *Ecotoxicology* 31 (4): 565–580. https://doi.org/10.1007/s10646-021-02445-y.

Li, K., and W. Ramakrishna. 2011. "Effect of multiple metal resistant bacteria from contaminated lake sediments on metal accumulation and plant growth." *J. Hazard. Mater.* 189 (1–2): 531–539. https://doi.org/10.1016/j.jhazmat.2011.02.075.

Liu, Q., Y. Wang, S. Sun, F. Tang, H. Chen, S. Chen, C. Zhao, and L. Li. 2023. "A novel chitosan-biochar immobilized microorganism strategy to enhance bioremediation of crude oil in soil." *Chemosphere* 313 (Feb): 137367. https://doi.org/10.1016/j.chemosphere.2022.137367.

Mahar, A., P. Wang, A. Ali, M. K. Awasthi, A. H. Lahori, Q. Wang, R. Li, and Z. Zhang. 2016. "Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review." *Ecotoxicol. Environ. Saf.* 126 (Apr): 111–121. https://doi.org/10.1016/j.ecoenv.2015.12.023.

Majhi, K., M. Let, U. Halder, and R. Bandopadhyay. 2023. "Copper adsorption potentiality of *Bacillus stercoris* GKSM6 and *Pseudomonas alcaliphila* GKSM11 isolated from Singhbhum copper mines." *Geomicrobiol. J.* 40 (2): 193–202. https://doi.org/10.1080/01490451.2022.2137603.

Maltsev, Y., S. Maltseva, and M. Kulikovskiy. 2023. "Toxic effect of copper on soil microalgae: Experimental data and critical review." *Int. J. Environ. Sci. Technol.* (Jan): 1–8. https://doi.org/10.1007/s13762-023-04766-3.

Manohari, R., and K. N. Yogalakshmi. 2016. "Optimization of copper (II) removal by response surface methodology using root nodule endophytic bacteria isolated from *Vigna unguiculata*" *Water Air Soil Pollut.* 227 (8): 285. https://doi.org/10.1007/s11270-016-2964-2.

Mello, I. S., S. Targanski, W. Pietro-Souza, F. F. F. Stachack, A. J. Terezo, and M. A. Soares. 2020. "Endophytic bacteria stimulate mercury phytoremediation by modulating its bioaccumulation and volatilization." *Ecotoxicol. Environ. Saf.* 202 (Oct): 110818. https://doi.org/10.1016/j.ecoenv.2020.110818.

Muyssen, B. T. A., and C. R. Janssen. 2002. "Accumulation and regulation of zinc in *Daphnia magna*: Links with homeostasis and toxicity." *Arch. Environ. Contam. Toxicol.* 43 (4): 492–496. https://doi.org/10.1007/s00244-002-1245-9.

Nguyen, K. B. T., and T. H. T. Phan. 2023. "Application of plant endophytic microorganisms (endophytes) in the treatment of heavy metal pollution in soils." In *Proc., Advances in Research on Water Resources and Environmental Systems, GTER 2022 Environmental Science and Engineering*, edited by P. L. Vo, D. A. Tran, T. L. Pham, T. Le, H. Thu, and N. Nguyen Viet. Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-031-17808-5_13.

Orozco, C. I., M. S. Freire, D. Gómez-Díaz, and J. González-Álvarez. 2023. "Removal of copper from aqueous solutions by biosorption onto pine sawdust." *Sustainable Chem. Pharm.* 32 (May): 101016. https://doi.org/10.1016/j.scp.2023.101016.

Özkoc, H. B., and Z. S. Taylan. 2010. "Assessment of various parameters of metal biology in marine microalgae *Phaeodactylum tricornutum* and *Dunaliella tertıolecta*." *Fresenius Environ. Bull.* 19 (12a): 2981–2986.

Parven, S., A. De, and A. Gupta. 2022. "Cu and Pb accumulation and removal from aqueous medium by *Enydra fluctuans* Lour. (Asteraceae)—A medicinal plant with potential for phytoremediation." *Environ. Sci. Pollut. Res.* 29 (44): 66902–66912. https://doi.org/10.1007/s11356-022-20483-9.

Peng, H., D. Li, J. Ye, H. Xu, W. Xie, Y. Zhang, M. Wu, L. Xu, Y. Liang, and W. Liu. 2019. "Biosorption behavior of the *Ochrobactrum* MT180101 on ionic copper and chelate copper." *J. Environ. Manage.* 235 (Apr): 224–230. https://doi.org/10.1016/j.jenvman.2019.01.060.

Priya, A. K., L. Gnanasekaran, K. Dutta, S. Rajendran, D. Balakrishnan, and M. Soto-Moscoso. 2022. "Biosorption of heavy metals by microorganisms: Evaluation of different underlying mechanisms." *Chemosphere* 307 (Nov): 135957. https://doi.org/10.1016/j.chemosphere.2022.135957.

Priya, E. M., and K. S. Tamilselvi. 2023. "Biotransformation of heavy metals by plant growth promoting endophytic bacteria: An assessment." *Indones. J. Social Environ. Issues* 4 (1): 36–44. https://doi.org/10.47540/ijsei.v4i1.741.

Rajeshkumar, R., S. Sahu, and J. R. Agharwal. 2012. "Biosorption of Cadmium (II) ions by the cadmium tolerant bacteria isolated from the chemical exposed soil of fireworks industry." *J. Pure Appl. Microbiol.* 6 (Jun): 781–787.

Rajkumar, M., N. Ae, and H. Freitas. 2009. "Endophytic bacteria and their potential to enhance heavy metal phytoextraction." *Chemosphere* 77 (2): 153–160. https://doi.org/10.1016/j.chemosphere.2009.06.047.

Rand, G. M., and S. R. Petrocelli. 1985. *Fundamentals of aquatic toxicology: Methods and applications*, 374–415. New York: Hemispheres.

Ribeiro, P. G., G. C. Martins, C. G. Moreira, C. de Oliveira, M. L. D. C. Andrade, T. S. Sales, W. F. T. Chagas, C. R. G. Labory, T. S. de Carvalho, and L. R. G. Guilherme. 2020. "Interactions of cadmium and zinc in high zinc tolerant native species *Andropogon gayanus* cultivated in hydroponics: Growth endpoints, metal bioaccumulation, and ultrastructural analysis." *Environ. Sci. Pollut. Res.* 27 (36): 45513–45526. https://doi.org/10.1007/s11356-020-10183-7.

Rubio-Santiago, J., et al. 2023. "Characterization of endophytic bacteria isolated from *Typha latifolia* and their effect in plants exposed to either Pb or Cd." *Plants* 12 (3): 498. https://doi.org/10.3390/plants12030498.

Sagadevan, S., I. Fatimah, T. C. Egbosiuba, S. F. Alshahateet, J. A. Lett, G. K. Weldegebrieal, M. V. Le, and M. R. Johan. 2022. "Photocatalytic efficiency of titanium dioxide for dyes and heavy metals removal from wastewater." *Bull. Chem. React. Eng. Catal.* 17 (2): 430–450. https://doi.org/10.9767/bcrec.17.2.13948.430-450.

Şentürk, İ., N. S. Eyceyurt Divarcı, and M. Öztürk. 2023. "Phytoremediation of nickel and chromium-containing industrial wastewaters by water lettuce (*Pistia stratiotes*)." *Int. J. Phytorem.* 25 (5): 550–561. https://doi.org/10.1080/15226514.2022.2092063.

Sharma, P., and S. Kumar. 2021. "Bioremediation of heavy metals from industrial effluents by endophytes and their metabolic activity: Recent advances." *Bioresour. Technol.* 339 (Nov): 125589. https://doi.org/10.1016/j.biortech.2021.125589.

Singh, V., et al. 2023. "Heavy metal contamination in the aquatic ecosystem: Toxicity and its remediation using eco-friendly approaches." *Toxics* 11 (2): 147. https://doi.org/10.3390/toxics11020147.

Sui, X., X. Wang, Y. Li, and H. Ji. 2021. "Remediation of petroleum-contaminated soils with microbial and microbial combined methods: Advances, mechanisms, and challenges." *Sustainability* 13 (16): 9267. https://doi.org/10.3390/su13169267.

Timková, I., J. Sedláková-Kaduková, and P. Pristaš. 2018. "Biosorption and bioaccumulation abilities of actinomycetes/streptomycetes isolated from metal contaminated sites." *Separations* 5 (4): 54. https://doi.org/10.3390/separations5040054.

USEPA. 1994. "Using toxicity tests in ecological risk assessment." *Intermittent Bull.* 2 (1): 1–12.

USEPA. 2002. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. EPA-821-R-02-013. Washington, DC: USEPA.

Varghese, R. 2012. "Biological removal of lead by *Bacillus sp.* obtained from metal contaminated industrial area." *J. Microbiol. Biotechnol. Food Sci.* 2 (2): 756–770.

Volaric, A., Z. Svircev, D. Tamindzija, and D. Radnovic. 2021. "Microbial bioremediation of heavy metals." *Hem. Ind.* 75 (2): 103–115. https://doi.org/10.2298/HEMIND200915010V.

Vulpe, C. B., M. A. Matica, R. Kovačević, D. Dascalu, Z. Stevanovic, A. Isvoran, V. Ostafe, and G. Menghiu. 2023. "Copper accumulation efficiency in different recombinant microorganism strains available for bioremediation of heavy metal-polluted waters." *Int. J. Mol. Sci.* 24 (8): 7575. https://doi.org/10.3390/ijms24087575.

Wang, Q., et al. 2020. "The endophytic bacterium *Sphingomonas* SaMR12 alleviates Cd stress in oilseed rape through regulation of the GSH-AsA cycle and antioxidative enzymes." *BMC Plant Biol.* 20 (1): 63. https://doi.org/10.1186/s12870-020-2273-1.

Wang, S., T. Liu, X. Xiao, and S. Luo. 2021. "Advances in microbial remediation for heavy metal treatment: A mini review." *J. Leather Sci. Eng.* 3 (1): 1–10. https://doi.org/10.1186/s42825-020-00042-z.

Wu, P., Z. Wang, A. Bhatnagar, P. Jeyakumar, H. Wang, Y. Wang, and X. Li. 2021. "Microorganisms-carbonaceous materials immobilized complexes: Synthesis, adaptability and environmental applications." *J. Hazard. Mater.* 416 (Aug): 125915. https://doi.org/10.1016/j.jhazmat.2021.125915.

Yadav, Y., R. Gothalwal, and R. K. Tenguriya. 2018. "Management of heavy metal pollution by using bacterial biomass." *Int. J. Biotech Trends Technol.* 8 (1): 15–27. https://doi.org/10.14445/22490183/IJBTT-V8I1P603.

Yao, Y., et al. 2022. "A field study on the composition, structure, and function of endophytic bacterial community of *Robinia pseudoacacia* at a composite heavy metals tailing." *Sci. Total Environ.* 850 (Dec): 157874. https://doi.org/10.1016/j.scitotenv.2022.157874.