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Bioremediation of heavy metals from synthetic water by endophytic bacteria isolated from floating hydrophytes

Nariman N. El-Sebaaye [a], Ashraf A. Elsayed [a], Yasser A. El-Amier [a,*]

[a]Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

Abstract

One of the most pressing environmental issues worldwide today is heavy metal pollution. This study aimed at isolating endophytic bacteria from the internal cells of the roots, stems, and leaves of *Lemna gibba* and *Eichhornia crassipes* collected from a permanent pond in the Nile Delta, to remove various heavy metals (Cu, Cr, and Pb). Of the 44 total isolates, 34 isolates were considered positive for exopolysaccharides. The halotolerance test revealed that all the isolates tolerated NaCl stress up to 15%, and when the bacteria were stressed by growth in 20% and 25% NaCl, 21 and 17, respectively, were able to grow. The growth kinetics of the isolates were examined with Pb and Cr metal (150 and 300 mg L^{-1}) independently. To estimate the Pb ion minimum inhibitory concentration, four Pb-tolerant bacterial isolates were chosen. The isolates were grown in LB medium with 100-500 mg L^{-1} Pb metal ions and measured at 600 nm at predetermined time intervals (8, 12, 24, 48, and 72 h). All bacterial stains survived 100–500 ppm lead concentrations. In this work, the effectiveness of a single isolate, 2Pe, in removing Pb was evaluated. At an ion concentration of 20 mgL^{-1}, isolate (2Pe) removal efficiency was 21.65%. The endophytic isolate (2Pe) was described morphologically and biochemically, and its name was identified by 16S rRNA sequencing. In the end, humans, animals, plants, and everything else in the ecosystem are all put under stress by the accumulation of heavy metals in the environment. To ensure efficient and cost-effective procedures, it is necessary to have a thorough awareness of the process and the available options for remediation at each stage.

Keywords*:* Hydrophytes, *Lemna*, *Eichhornia*, Endophytic Bacteria, Heavy Metals, Bioremediation.

1. Introduction

Heavy metal contamination of aquatic ecosystems is becoming a serious issue for both the environment and public health. Many sectors, including mining, metal plating facilities, and tanneries, frequently release heavy metals into the environment, which can contaminate freshwater and the marine environment [1,2]. Because of their toxicity and pervasiveness in the environment, these metals may accumulate to dangerous levels in water, which can threaten the health of cattle, aquatic life, and humans [3,4]. Prolonged exposure to heavy metals by dermal contact, inhalation, or consumption of products containing heavy metals causes a wide range of disorders in humans and animals. Several public health organizations have reported that millions of people throughout the world are afflicted with ailments linked to heavy metal exposure [5].

Heavy metals may be broken down in a number of ways, some of which are environmentally beneficial and economically feasible, while others are neither. No method can guarantee that heavy metals have declined to zero [6,5]. Heavy metal salts are often water-soluble and cannot be physically separated. Secondary pollution is brought through physiochemical mechanisms to the treatment area [7,8]. Microbes' ability to degrade heavy metals into less hazardous forms has been studied for decades. It is better, cheaper, and environmentally friendly [9]. Microbe-produced organic acids with low molecular weights degrade heavy metals and soil particles containing heavy metals. Heavy metals are transformed into more stable, less mobile, or inert forms by precipitation, biosorption, and microbial enzymatic transformation [5,10,11].

*Corresponding author e-mail: yasran@mans.edu.eg (Y.E-A.).
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Plant-associated bacteria help hosts adapt to changing environments [12]. Epiphytes are plant-associated bacteria recovered from rhizoplane and phylloplane surfaces, while endophytes live inside tissues without harming the host [13,14]. These bacteria are present in most plant species, span several bacterial phyla, and promote plant development and disease control [15,16]. Recent research reveals that plants living in polluted places may degrade xenobiotic chemicals [17-19] and phytoremediation toxic metals [20].

Studies on both animals and humans have shown that exposure to heavy metals may have detrimental effects on reproductive health and growth. The adverse effects of prolonged exposure to heavy metal pollutants include mutagenicity, carcinogenicity, teratogenicity, immunosuppression, poor physical condition, and decreased fertility. Lead is everywhere and dangerous in high doses. Constant exposure to high-lead water may eventually lead to problems with the neurological system, kidneys, brain development, cancer, and anaemia. Lead in water does not decompose [21]. Toxic levels of chromium may induce inflammation and ulcers in the stomach and small intestine, anaemia, and harm to the reproductive system [9,22]. Copper is a necessary trace element for all forms of life, although it may be harmful in large doses. Mycocadium, the brain, liver, and pancreas all accumulate the increased copper (II) ions [23]. Headache, nausea, vomiting, diarrhoea, trouble breathing, liver and renal failure, and death will result [24].

Few studies have looked at the heavy metal removal capability of endophytic communities of heavy metal accumulator plants during phytoremediation [25,26], despite the presumed prevalence of endogenous bacterial endophytes that breakdown hydrocarbons. So, the purpose of this study was to examine the ability of endophytic bacteria isolated from floating hydrophytes, such as *Eichhornia crassipes* and *Lemna gibba*, to remove various heavy metals (Cu, Cr, and Pb).

2. Materials and Methods

2.1. Plant material collection

Adult duckweed and water hyacinth plants (*Lemna gibba* and *Eichhornia crassipes*) were obtained from a permanent pond in a village 30 km from Mansoura City, Dakahlia Province, in March-May 2022. The plant was identified according to Boulos [27]. Healthy plants were collected and placed in zip-lock plastic bags to store the material until isolation then transferred to Microbiology Laboratory, Faculty of Science, Mansoura University.

2.2. Isolation and Purification of Endophytes

Bacon and Hinton [28] method for isolating and purifying endophytes from LB agar media was followed (2.5 g of peptone, 1.25 g of yeast extract, 2.5 g of sodium chloride, 3.75 g of agar and 250 ml of distilled water). Dos Santos et al. [29] developed a technique in which plant samples were rinsed with running water, then cut into four sections: root, stem, leaf, and capitula, before being surface-sterilized. After soaking the plant parts in 70% ethanol (C_2H_5OH) for 30 seconds, then in 0.5% sodium hypochlorite (NaOCl) for 2-3 minutes, and lastly in sterile distilled water (Dil.H_2O) for 10 minutes, we were able to kill any bacteria or fungi on the surface (2-3 times). Then, the plant matter was dried between the folds of sterile filter sheets. The cut ends of surface-sterilized segments were removed and put in a suitable LB agar medium, with the cut surface contacting the agar, using a flame-sterilized scalpel. After 48 hours of incubation at 35 degrees Celsius, the highest number of bacterial endophyte colonies was recorded.

2.3. Exopolysaccharide Test

In a qualitative analysis of exopolysaccharide (EPS) synthesis, endophytic bacterial isolates were evaluated on nutritional agar medium with a high sucrose concentration (5%). The plates with the isolates on them were incubated at 28 ℃ for two days. One could see the thick, sticky substance that had grown over the stripe. This thick emulsion was confirmed by the isolates to be a result of the presence of polysaccharides [30].

2.4. Halotolerance test

The forty-four isolates were cultivated at 28 °C for 2 days on LB agar medium containing 0, 5, 10, 15, 20, and 25% NaCl to select the halotolerant isolates and rank the isolates by their degree of tolerance to NaCl concentrations.

2.5. Heavy metals tolerance test

Isolates' resistance to heavy metals was measured using the agar-dilution technique. Isolates were plated on Luria-Bertani (LB) agar with varying doses of heavy metals (Cu; 50-250 mg/l, Cr; 50-300 mg/l, and Pb; 50-400 mg/l). Overnight, the plates were kept at 28 degrees Celsius and checked for bacterial growth. The least inhibitory concentration was defined as the lowest concentration of metal that fully stopped bacterial growth (MIC). After autoclaving and chilling to 50 °C, filter-sterilized stock solutions of each metal salt were applied to LB agar [31].

2.6. Effect of heavy metals on growth kinetics of the isolate

High-tolerance bacterial isolates were cultured in LB broth at 28 ℃, with vigorous shaking (150 revolutions per minute) for 24 hours. In brief, the initial inoculation cells were grown to an optical density (OD) at 600 nm of 1.0 before being incubated for 72 hours at 28 °C and 150 rpm in 100 µL of LB liquid medium with various Pb and Cr concentrations (150 and 300 mg/l). At regular intervals (0, 8, 12, 24, 48, and 72 hrs.), the cells' absorbance was measured at 600 nm [32]. There were three separate runs for each experiment. The growth of the isolate in the absence of metals served as a control in this study.

2.7. Determination of minimum inhibitory concentration of lead

To assess the Pb tolerance of four different bacterial isolates, 100 ml of LB broth was added to a 250 ml flask, and the flask was placed in a shaking incubator at 150 rpm for 24 hours at 28 °C. Once inoculated cells reached an OD 600 of 1.0, 100 µL of the cells were incubated at 28 °C with 150 rpm in 100 mL of LB liquid medium with various doses of Pb (100, 200, 300, 400, and 500 mg/l) for 72 hours. At predetermined time intervals (8, 12, 24, 48, and 72 h), the optical density at 600 nm (UV-1800, Shimadzu, Japan) was measured to assess bacterial growth. The trials were repeated three times for accuracy. The control for this experiment was the isolate's growth in the absence of metals.

2.8. Screening for the most potential biosorbent bacteria

The potential of a single bacterial strain as a biosorbent for Pb^{2+} removal from synthetic wastewater samples was investigated. During early MIC testing, it was discovered to be resistant to massive quantities of Pb^{2+} ions. The bioremediation capacity of the isolated 2Pe was determined by batch experiments. In summary, 100 ml of LB broth supplemented with 20 mg/l of Pb was inoculated with individual and mixed 2Pe isolates in 250 ml flasks. The flasks were kept in a 28 °C incubator with a 150-rpm agitator for 72 hours. After 72 hours, samples were taken, centrifuged (10,000 rpm for 5 minutes), filtered (0.22 ml), and analyzed with AAS to determine the concentration of heavy metals that had been left behind. In order to determine the standard error in the design and serve as a control to verify that the biosorption capacity was connected to the presence of bacteria, LB medium supplemented with Pb^{2+} ions were utilized. Heavy metal solution produced in ppm concentration was used in the screening studies. The heavy metal solution was infected with the immobilized most promising bacterial isolates (batch mode), and the residual heavy metal concentrations were measured (using the atomic absorption technique), allowing the percentage of heavy metal removal to be estimated.

2.9. Characterization of Endophytic Bacteria

2.9.1. Morphological characterization

The isolate was described to establish the morphology of the bacterial cells based on distinguishing features such as cell shape, colony colour, and texture. The classic Gram staining approach described by Aneja et al. [33], Cappuccino and Sherman [34], and Collee and Enright [35] was used to determine this.

2.9.2. Biochemical characterization

The selected endophytic bacterial strains were biochemically characterized using standard methods such as the catalase, amylase, lipase, protease, cellulase, nitrate reductase, and indole tests; and functionally characterized using standard methods such as the phosphate solubilization test, hydrogen sulphide production test, indole production test, and gibberellin acid production test [34].

2.9.3. 16S rRNA gene sequencing

MicroSeq® 500 16SrRNA Bacterial Identification Kits were used for the molecular characterization of isolated bacteria. With a total volume of 20l (7l purified PCR product and 13l sequencing module), the sequencing processes were performed in a 9700 thermal cycler for 10 seconds at 96 °C, 5 seconds at 50 °C, and 4 seconds at 60 °C (25 cycles). The Dye ExTM 2.0 Spin Kit was then used to spin off the cycle sequencing reaction's surplus of dye terminators and primers (Qiagen PN 63204).

The sequences were analyzed using Finch TV (version 1.4.0) and MEGA-X (version 10.2.5) software, and phylogenetic trees were constructed with the closest reported type of strain sequences in Seaview. The sequences of the isolates identified in this study were deposited in the GenBank database run by the National Center for Biotechnology Information.

3. Results and Discussion

To survive and even flourish, plants may form relationships with other organisms in their habitat. Beneficial symbiotic partnerships between microorganisms and plants are among the most significant relationships that living things may have [36]. There is a type of bacteria known as plant-beneficial bacteria, and they support their host plants in many ways, including making them more resistant to biotic and abiotic challenges that would otherwise stunt their development [37,38].

3.1. Isolation and purification of endophyte isolates

In this study, forty-four bacterial isolates were obtained from two floating hydrophytes plants (*Eichhornia crassipes* and *Lemna gibba*) collected from ponds in Nile Delta. The endophytic bacterial isolates grown on LB agar medium under aseptic conditions were selected, purified according to differences in their morphology as shown in Figure 1 a-c. The study of population diversity of bacterial endophytes isolated from *E. crassipes* showed more diversity of species than *L. gibba*. The tissues (roots, stems, and leaves) of two different floating hydrophytes were used to isolate the strains. Our research showed that the endophytic population, which originated in the plant's own tissues, was most dense in the leaves (Figure 1). Apart from the capacity of bacteria to colonies plants as endophytes, host plants and environmental conditions may have a noteworthy influence on endophytic diversity. Host plant age, ecological location, genotype, and even tissue type determines endophytic bacteria [15,39]. Host plant development phases also affect endophytic diversity, with nutrient-rich stages having more bacteria [40]. Climate also affects plant endophytic colonizers. Penuelas et al. [41] found that climate change changed the number and composition of endophytic bacteria in leaf tissue. The strategy utilized to examine these bacteria also impacts plant endophytic diversity. The sterilizing agent, concentration, and treatment period can affect the bacteria retrieved from a plant [15,39].

3.2. Exopolysaccharides

Exopolysaccharide (EPs) are hydrated molecules with 97% water in the matrix, which protects microorganisms and plants from drying out. The capacity of endophytic bacterial isolates to generate polysaccharides was examined in a plate experiment. The visibly thick and viscous mass that developed over the stripe was regarded as a favorable outcome for polysaccharide synthesis. In this study, 34 of the 44 isolates were considered positive (Table 1). Certain endophytes are known to create EPs outside of cell surfaces under hostile circumstances, ensuring water holding capacity and nutrient absorption around the plant roots and protecting them from salt and desiccation [42,43].

3.3. Halotolerance test

Plants share physiological responses to abiotic stressors such as drought, salt, floods, heat, cold, and heavy metals [44,45]. Host plants may be shielded by endophytic bacteria, which achieve this by biosynthesizing chemicals that promote host stress tolerance [46] and by rapidly activating host stress response systems after stress exposure (induced systemic tolerance). In this work, the halotolerance test of 44 endophytic bacterial isolates revealed that all the isolates tolerated NaCl stress up to 15% and were therefore classified as highly halotolerance bacterial isolates (Table 2). However, when the bacteria were stressed by growth in 20% and 25% NaCl, 21 and 17, respectively, were able to grow. The inoculation of plants with certain endophytes has

been shown to increase the plants' resistance to salt stress and promote their development in saline environments [47,48].

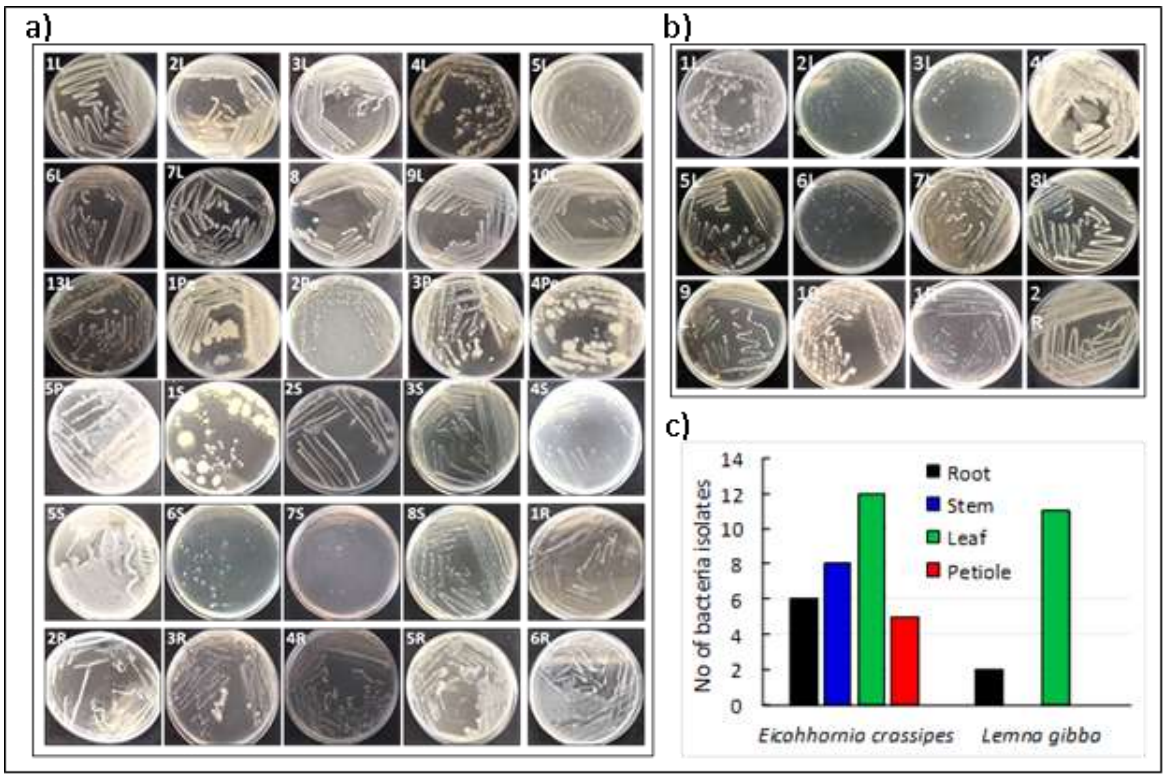

Figure 1. Bacterial endophytes isolated from **a)** *Eichhornia crassipes*, **b)** *Lemna gibba* L on L.B agar medium, and **c)** Number of the endophytic bacteria isolated from different tissues of the selected hydrophytes (L: Leaf, Pe: Petiole, S: Stem, and R: Root).

Table 1. Quantitative screening of the endophytic bacterial isolates from *E. crassipes* and *L. gibba* for production of exopolysaccharide.

Plant organ	Code of isolate	Exopolysaccharide	Plant organ	Code of isolate	Exopolysaccharide
Eichhornia crassipes					
Leaf	1L	-	Stem	1S	-
	2L	-		2S	-
	3L	+		3S	-
	4L	+		4S	+
	5L	-		5S	+
	6L	+		6S	+
	7L	+		7S	+
	8L	+		8S	+
	9L	-	Root	1R	-
	10L	+		2R	+
	11L	+		3R	+
	12L	-		4R	+
Petiole	1Pe	+		5R	+
	2Pe	+		6R	-
	3Pe	+			
	4Pe	+			
	5Pe	+			
Lemna gibba					
Leaf	1L	+	Root	1R	+

2L	+	2R	+
3L	+		
4L	+		
5L	+		
6L	+		
7L	+		
8L	+		
9L	+		
10L	+		
11L	+		

Table 2. Halotolerance test of endophytic bacterial isolates isolated from *E. crassipes* and *L. gibba* using different concentrations of sodium chloride.

Plant	Plant organ	Code of isolate	Halotolerance test				
			5%	10%	15%	20%	25%
Eichhornia crassipes	Leaf	1L	+	+	+	+	+
		2L	+	+	+	+	-
		3L	+	+	+	+	+
		4L	+	+	+	+	+
		5L	+	+	+	-	-
		6L	+	+	+	-	-
		7L	+	+	+	+	+
		8L	+	+	+	+	+
		9L	+	+	+	-	-
		10L	+	+	+	-	-
		11L	+	+	+	-	-
		12L	+	+	+	+	+
	Petiole	1Pe	+	+	+	+	+
		2Pe	+	+	+	+	+
		3Pe	+	+	+	+	+
		4Pe	+	+	+	+	-
		5Pe	+	+	+	-	-
	Stem	1S	+	+	+	+	+
		2S	+	+	+	+	-
		3S	+	+	+	-	-
		4S	+	+	+	+	+
		5S	+	+	+	+	-
		6S	+	+	+	-	-
		7S	+	+	+	+	+
		8S	+	+	+	+	+
	Root	1R	+	+	+	-	-
		2R	+	+	+	-	-
		3R	+	+	+	-	-
		4R	+	+	+	-	-
		5R	+	+	+	-	-
		6R	+	+	+	-	-
Lemna gibba	Leaf	1L	+	+	+	-	-
		2L	+	+	+	-	-
		3L	+	+	+	+	+
		4L	+	+	+	-	-
		5L	+	+	+	+	+
		6L	+	+	+	-	-
		7L	+	+	+	-	-
		8L	+	+	+	-	-
		9L	+	+	+	-	-
		10L	+	+	+	-	-
		11L	+	+	+	+	+

	Root	1R	+	+	+	-	-
		2R	+	+	+	+	+

3.4. Heavy metal tolerance test

Heavy metal bioremediation and agriculture are two important biotechnological applications of this effect, although there have been few efforts to do a thorough overview of studies in this area. Plants' resistance to heavy metals may be boosted by endophytic bacteria [49]. In this work, 44 endophytic bacterial isolates were streaked on various concentrations of heavy metals individually (Cu, Cr, and Pb) on LB agar plates. Results indicate that the 44 bacterial isolates can grow at 50, 100, and 150 ppm (Tables 3-5) for Cu, Cr and Pb, respectively. Whereas Cu metal 18 and 16 bacterial isolates from 44 bacterial isolate able to grow at 200 and 250 ppm, respectively (Table 5). For Cr metal, 19 and 15 bacterial isolate were able to grow at 200 and 300 ppm, respectively (Table 4). On the other hand, Pb metal 44, 23 and 12 bacterial isolates from 44 bacterial isolates can grow on LB agar plates at 200, 250 and 300 ppm concentration, respectively (Table 3).

Table 3. Lead tolerance test of endophytic bacterial isolates isolated from *E. crassipes* and *L. gibba* using different concentrations of Pb $(C_2H_3O_2).3H_2O$.

Plant organ	Code	Pb tolerance test (ppm)						Plant organ	Code	Pb tolerance test (ppm)					
		50	100	150	200	250	300			50	100	150	200	250	300
Eichhornia crassipes															
Leaf	1L	+	+	+	+	+	+	Stem	1S	+	+	+	+	-	-
	2L	+	+	+	+	+	-		2S	+	+	+	+	+	+
	3L	+	+	+	+	+	-		3S	+	+	+	+	-	-
	4L	+	+	+	+	+	-		4S	+	+	+	+	-	-
	5L	+	+	+	+	+	-		5S	+	+	+	+	-	-
	6L	+	+	+	+	-	+		6S	+	+	+	+	-	-
	7L	+	+	+	+	-	-		7S	+	+	+	+	-	-
	8L	+	+	+	+	+	-		8S	+	+	+	+	-	-
	9L	+	+	+	+	+	-	Root	1R	+	+	+	+	+	-
	10L	+	+	+	+	-	-		2R	+	+	+	+	-	-
	11L	+	+	+	+	+	+		3R	+	+	+	+	-	-
	12L	+	+	+	+	-	-		4R	+	+	+	+	-	-
Petiole	1Pe	+	+	+	+	+	+		5R	+	+	+	+	-	-
	2Pe	+	+	+	+	+	+		6R	+	+	+	+	+	-
	3Pe	+	+	+	+	-	-								
	4Pe	+	+	+	+	+	+								
	5Pe	+	+	+	+	+	+								
Lemna gibba															
Leaf	1L	+	+	+	+	-	-	Root	1R	+	+	+	+	-	-
	2L	+	+	+	+	-	-		2R	+	+	+	+	-	-
	3L	+	+	+	+	+	-								
	4L	+	+	+	+	+	-								
	5L	+	+	+	+	+	-								
	6L	+	+	+	+	+	+								
	7L	+	+	+	+	-	-								
	8L	+	+	+	+	+	+								
	9L	+	+	+	+	+	+								
	10L	+	+	+	+	+	+								
	11L	+	+	+	+	+	-								

Table 4. Chromium tolerance test of endophytic bacterial isolates isolated from *E. crassipes* and *L. gibba* using different concentrations of $K_2Cr_2O_7$.

Plant organ	Code	Cr tolerance test (ppm)					Plant organ	Code	Cr tolerance test (ppm)				
		50	100	150	200	300			50	100	150	200	300
Eichhornia crassipes													

Leaf	1L	+	+	+	+	+	Stem	1S	+	+	+	+	+
	2L	+	+	+	+	+		2S	+	+	+	-	-
	3L	+	+	+	-	-		3S	+	+	+	-	-
	4L	+	+	+	+	+		4S	+	+	+	-	-
	5L	+	+	+	-	-		5S	+	+	+	-	-
	6L	+	+	+	+	+		6S	+	+	+	-	-
	7L	+	+	+	+	+		7S	+	+	+	+	-
	8L	+	+	+	+	+		8S	+	+	+	+	+
	9L	+	+	+	+	-	Root	1R	+	+	+	-	-
	10L	+	+	+	+	+		2R	+	+	+	-	-
	11L	+	+	+	-	-		3R	+	+	+	-	-
	12L	+	+	+	+	-		4R	+	+	+	-	-
Petiole	1Pe	+	+	+	+	-		5R	+	+	+	-	-
	2Pe	+	+	+	-	-		6R	+	+	+	-	-
	3Pe	+	+	+	-	-							
	4Pe	+	+	+	-	-							
	5Pe	+	+	+	-	-							
Lemna gibba													
Leaf	1L	+	+	+	-	-	Root	1R	+	+	+	-	-
	2L	+	+	+	-	-		2R	+	+	+	+	+
	3L	+	+	+	-	-							
	4L	+	+	+	-	-							
	5L	+	+	+	-	-							
	6L	+	+	+	+	+							
	7L	+	+	+	+	+							
	8L	+	+	+	-	-							
	9L	+	+	+	+	+							
	10L	+	+	+	+	+							
	11L	+	+	+	+	+							

Table 5. Copper tolerance test of endophytic bacterial isolates isolated from *E. crassipes* and *L. gibba* using different concentrations of $CuSO_4.5H_2O$.

Plant organ	Code	Cu tolerance test (ppm)					Plant organ	Code	Cu tolerance test (ppm)				
		50	100	150	200	250			50	100	150	200	250
Eichhornia crassipes													
Leaf	1L	+	+	+	-	-	Stem	1S	+	+	+	-	-
	2L	+	+	+	-	-		2S	+	+	+	+	+
	3L	+	+	+	-	-		3S	+	+	+	-	-
	4L	+	+	+	+	+		4S	+	+	+	-	-
	5L	+	+	+	+	+		5S	+	+	+	-	-
	6L	+	+	+	+	+		6S	+	+	+	+	+
	7L	+	+	+	+	-		7S	+	+	+	+	-
	8L	+	+	+	+	+		8S	+	+	+	+	+
	9L	+	+	+	-	-	Root	1R	+	+	+	+	+
	10L	+	+	+	-	-		2R	+	+	-	-	-
	11L	+	+	+	+	+		3R	+	+	-	-	-
	12L	+	+	-	-	-		4R	+	+	+	-	-
Petiole	1Pe	+	+	+	-	-		5R	+	+	+	-	-
	2Pe	+	+	+	-	-		6R	+	+	+	-	-
	3Pe	+	+	+	-	+							
	4Pe	+	+	+	-	-							
	5Pe	+	+	+	-	-							
Lemna gibba													
Leaf	1L	+	+	+	-	-	Root	1R	+	+	+	-	-
	2L	+	+	+	+	+		2R	+	+	+	-	-
	3L	+	+	+	+	+							
	4L	+	+	+	+	+							

5L	+	+	+	+	+
6L	+	+	+	-	-
7L	+	+	+	+	-
8L	+	+	+	-	-
9L	+	+	+	-	-
10L	+	+	+	+	+
11L	+	+	+	+	+

3.5. Effect of heavy metals on growth kinetics of the isolate

Heavy metals have the potential to have a considerable influence on the kinetics of both substrate biodegradation and microorganism growth, including lag times and specific growth rates [52,53]. To examine the influence of heavy metals on the growth kinetics of Pb and Cr ion isolates, the isolates' growth ability was evaluated in LB medium supplemented with (150 and 300 mg L^{-1}) Pb and Cr metal separately. Heavy metal tolerance test bacterial isolates that can withstand the stress of Cr and Pb ions were chosen to study the influence of Cr and Pb ions on growth kinetics. Figure 2 depicts the growth profiles of the isolates in the presence of metals. There was a slight differential in growth response depending on the metal. Differences in growth profiles may be related to metal toxicity and bioavailability [54]. The control for this experiment was the growth of the isolate without metals.

Heavy metal impacts on bacteria differ depending on the heavy metal type [55], metal speciation [56], metal concentration [57], and microbe type [58]. Other environmental factors, like pH, buffers, and the presence of other metallic [60], nonmetallic [61], or organic molecules [62], have also been shown to affect the interaction.

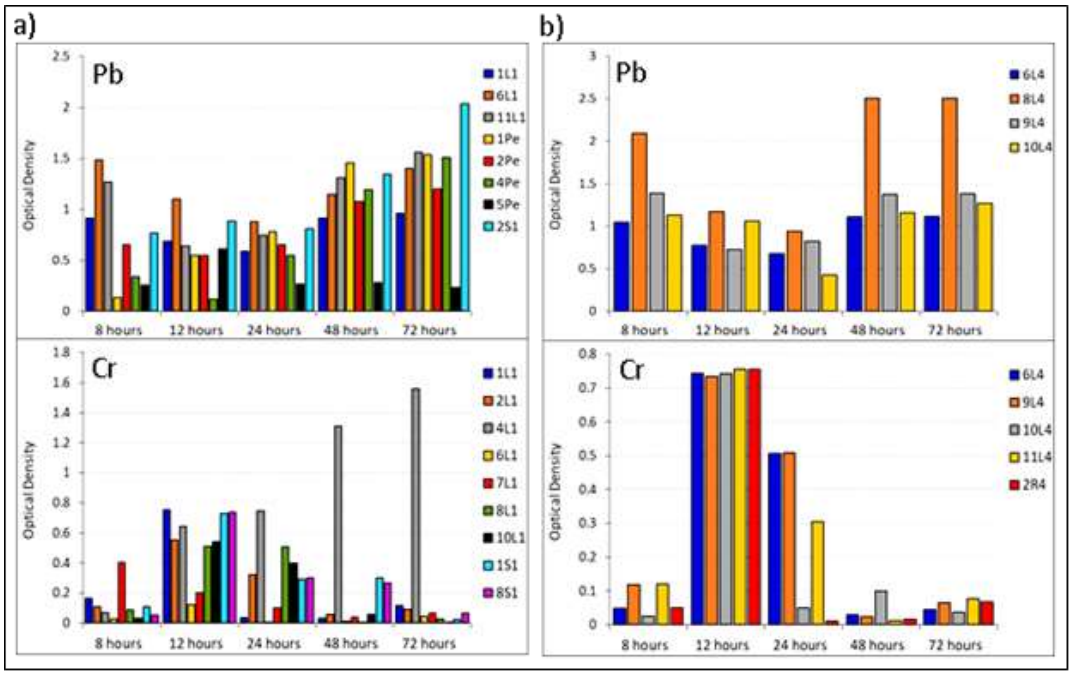

Figure 2. Effect of heavy metals on growth kinetics of the bacterial isolate. a) bacterial isolate from *E. crassipes*, and b) bacterial isolate from *L. gibba*.

3.6. Determination of MIC (Minimum Inhibitory Concentration) of lead

The concentration of heavy metal-resistant bacteria isolates was evaluated by gradually increasing the quantity of heavy metal in the growing medium. Increase in turbidity was found at all Pb concentrations $(C_2H_3O_2).3H_2O$ [63]. Based on the influence of heavy metals on the isolates' growth

kinetics, four strains of Pb-tolerant bacterial isolates were chosen to estimate the lowest inhibitory concentration of Pb ions. The isolates' growth capacity was examined in LB medium modified with (100-500 mg L^{-1}) Pb metal ions, and OD at 600 nm was measured at predefined time intervals (8, 12, 24, 48, and 72 hrs.). The control for this experiment was the growth of the isolate without metals.

All the bacterial stains survived lead concentrations ranging from 100 to 500 ppm. Bacterial strains thrive at such concentrations. Since lead (Pb) is particularly persistent in water and soil, accumulates in the top eight inches of ground, and is very immobile, it has a wide range of negative effects on living animals (morphological, physiological, and biochemical) [64,65]. Even though it is a naturally occurring element, soil concentrations have increased due to human activities such as mining, fossil fuel burning, and manufacturing [66,67]. It was also discovered that the test in nutrient media was sensitive at concentrations 10 to 1000 times lower than those obtained in solid media when determining the sensitivity of environmental bacteria to the different divalent metal ions.

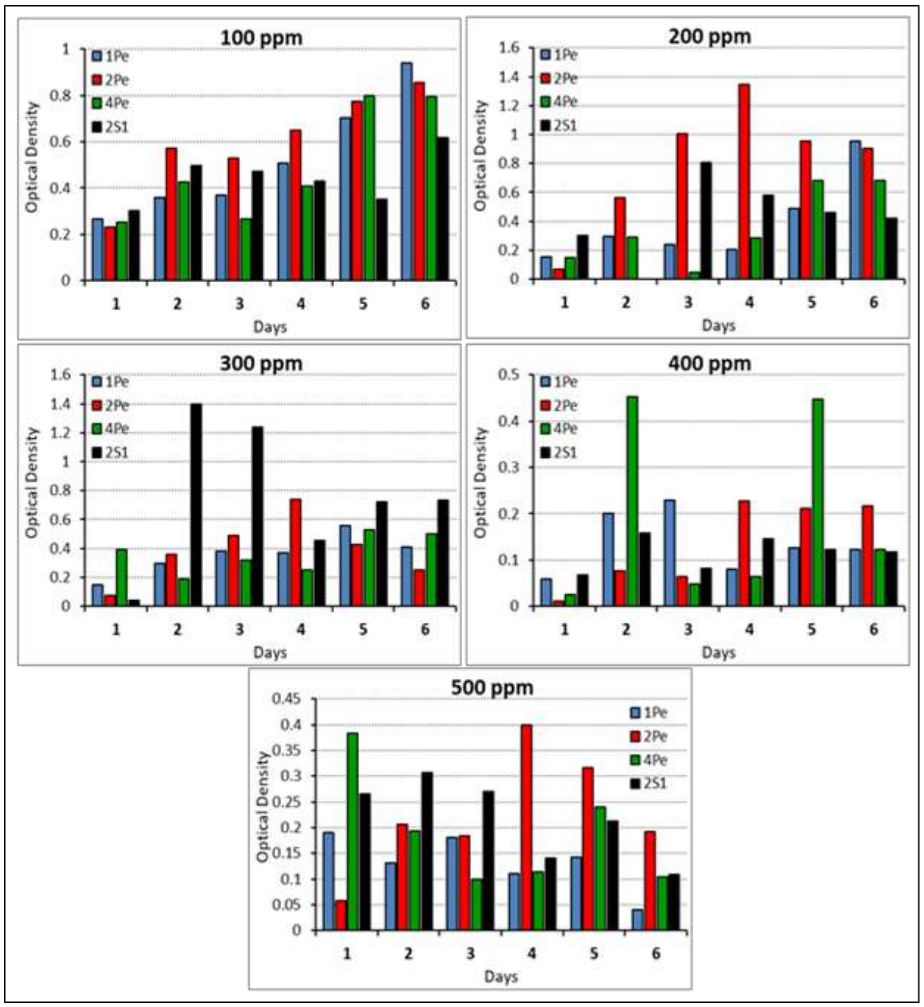

Figure 3. Effect of different concentrations of Pb on growth kinetics of the bacterial isolate (MIC).

3.7. Screening for the most potential biosorbent bacteria

Metals contained in aquatic effluents in trace concentrations afford a huge opportunity for biological treatment (using bacteria) of pollutants. Bacteria, fungus, yeast, and algae are just a few of the many microbes known to accumulate and/or absorb heavy metals on their surfaces. Bioremediation of heavy metals may be achieved by harnessing the detoxifying potential of these resistant microbes.

These bacteria may be used in environmental processes including biosorption, bioaccumulation, and bioprecipitation to clean up heavy metal-containing industrial effluents [68,69]. In this study, the Pb-removal efficiency of one isolate, 2Pe, was assessed. The removal efficiency of the isolate at an ion concentration of 20 mgL^{-1} was 21.65%. Comparatively, isolated 2Pe has a higher Pb-removal efficiency of 21.65%. [70] observed that *Enterobacter* sp. and *Klebsiella* sp. isolated from industrial effluents decreased Pb when evaluated in vitro, and our results corroborate these findings.

3.8. Characterization of Endophytic Bacteria

Colony morphology was one method for determining whether endophytes varied. The most dominant and otherwise interesting (in terms of colony morphology) isolates were chosen for the investigation. There is a wide variety of Gram-negative and Gram-positive bacteria among the endophytic bacteria [71]. The pure isolate (2Pe) from the petiole of *E. crassipes* was characterized morphologically, showing that colony shape is irregular, filamentous margin, raised in elevation, smooth texture, and creamy colored, and was scanned microscopically according to cell shape, whereas isolates were circular in shape and Gram-positive bacteria. It has been noted by previous researchers that Gram-negative bacteria tend to dominate in plant tissues [72,73]. These bacterial species may have developed with the plant to thrive in its nutrient-poor environment. Isolated Gram-positive bacteria strains generate spores in response to changes in pH, temperature, and salinity, which may provide them with a competitive edge.

Biochemistry was used to describe the pure isolate (2Pe) from the petiole of E. crassipes based on enzyme activity and function. The isolate demonstrated a wide range of enzyme production abilities. Whereas amylase, cellulase, protease, and nitrogen fixation produce positive results, lipase and H_2S provide negative effects. The results we obtained are consistent with those obtained by Elbeltagy et al. [72] and Sultan et al. [38]. The processes by which endophytic bacteria gain entry to and remain inside the host plant are not fully understood but may include hydrolytic enzymes and cellulases [74,75]. There has been no comprehensive study of the enzymes secreted by endophytes. As described by *Azoarcus* sp. [76] and *Enterobacter asburiae* JM22, these enzymes function as virulence factors for plant pathogenic bacteria and may be implicated in the invasion of host plants by endophytes [77].

The conventional way of identifying bacteria, which is based on the phenotypic traits of the bacteria, is often not as accurate as the identification methods that are based on the genotype of the bacterium. Based on 16S rRNA gene sequence analysis, the isolated strain was identified as *Bacillus velezensis* strain CBMB205 (NR_116240.1). There are both variable and conserved segments in the 16S rRNA gene sequence, which totals about 1,550 base pairs in length. The 16S rRNA gene is big enough and has enough interspecific polymorphisms to offer distinctive and reliable data [78].

4. Conclusion

Heavy metal pollution comes in numerous forms, both naturally occurring and anthropogenic. It is important to rid the world of heavy metals since they are poisonous and cannot be broken down by natural processes. When wastewater from factories leaks into the ground or water supply, the government must intervene immediately, continue to monitor the situation, and ensure that the effluent is properly treated. Biological agent bioremediation is superior to conventional treatment procedures since it is more effective, less expensive, and better for the environment. When metals and microbes interact, it affects how the microbes behave, which may have consequences for tasks including growth, colonization, and biofilm formation in remediation. One of the most versatile microorganisms is bacteria, which has huge bioremediation potential and should be studied and used extensively. Several different strategies are used by bacteria, each tailored to the unique heavy metals, environmental factors, and bacterial species at play. Lastly, further study is required to determine the best way to implement these laboratory possibilities in commercial settings.

5. Conflicts of interest

"There are no conflicts to declare."

6. Acknowledgments

"None"

7. References

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