

NATURE'S SYMPHONY

ISSN-E: 3007-2034

ISSN-P: 3007-2026

Website link: <https://www.tsfn.com/>

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To cite this article: Mughal, I.M. Irshad, S. and Mohsin, H. 2023. Isolation and Growth Optimization of Mercury-Resistant Bacteria Isolated from River Ravi. *Nature's Symphony*, 1(2):08-20.

DOI: <https://doi.org/10.69547/NS.12.02>

Article QR



Published online: 08 September 2023

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Isolation and Growth Optimization of Mercury-Resistant Bacteria Isolated from River Ravi

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Keywords: Mercury, Wastewater, Bioremediation, Mercury reduction, MIC

ABSTRACT

Mercury (Hg) is one of the most toxic heavy metals found on earth and has been reported as a contaminant from industrial and agricultural activities. Its release in the environment has led to extreme toxicity to all life forms. Various strategies like physico-chemical techniques have been proposed for Hg removal; bioremediation has proved to be most promising among all. This study focuses on the isolation of Hg resistant bacteria from Ravi River samples. Several biochemical tests were performed like gram staining, catalase, oxidase, sugar fermentation, antibiotic resistance, and motility test. Minimum inhibitory concentration (MIC) displayed that among all the isolates, Hg4 was able to resist up to 2500 ppm Hg followed by Hg6 and Hg7 which showed resistance till 2000 ppm Hg. These isolates were subjected to mercury reduction assay. Results revealed that Hg4, Hg6, and Hg7 bioremediated 59.82%, 40.82%, and 65.00% of mercury (Hg) respectively after 24 hours. In future, these isolates can be used for the bioremediation of Hg from contaminated sites which will help in creating a healthy environment.

1. INTRODUCTION

Our environment is globally very polluted by air, water, land, noise, and radioactive pollution due to anthropogenic activities (Appannagari, 2017). The emissions from automobiles, industrial waste, paints and dyes industries are the principal sources of pollution, which has led to the high prevalence of deadly diseases. Our underground water and surface water are contaminated by different toxic organic (fungicides, pesticides, insecticides, hydrocarbons, phenol, biphenyls, polychlorinated biphenyls, detergents, and oils) and inorganic (heavy metals which major heavy metals include Mercury (Hg), Nickel (Ni), Arsenic (As), Lead (Pb), Chromium (Cr),

and Cadmium (Cd)) pollutants (Qamar *et al.*, 2022). Heavy metals with more than 5 g/cm³ are used in battery manufacturing factories, medicine industries, and other chemical industries. Its various forms are present in underground and industrial wastewater, which are usually non-biodegradable but can be converted into non-toxic forms.

Mercury (Hg) is a well-known toxic heavy metal with a density of 13.5336g/cm³. Hg exists in several forms i.e. Hg⁰, Hg⁺, Hg²⁺, organic methyl, and ethyl Hg (Baldi *et al.*, 2017). The order of growing poisonousness is Hg⁰ < Hg²⁺, Hg⁺ < CH³-Hg (Chen *et al.*, 2020). The major sources of Hg contamination in the environment include natural and anthropogenic activities (Sundseth *et al.*, 2017). Natural

sources of Hg include forest fires, volcanoes, cinnabar, and fossil fuels such as petroleum and coal while major man-made sources are agricultural activities, urban emissions, discharges of combustion products, mining, and industrial waste, industrial into soil and water bodies (Amiri *et al.*, 2015). Contamination sites and the geographic extent of contamination are very important for the process of assessments (Eckley *et al.*, 2020). Hg contamination reaches industrial and mining sites from where it gains entry into water bodies resulting in Hg accumulation in the aquatic ecosystem. For example,

According to the guidelines of WHO, FDA, and EPA the legal limit of Hg is about 0.002 mg/L (Zhu *et al.*, 2017). The toxic effects of Hg include irritation in eyes and lungs, diarrhea, Down's syndrome, malfunctioning of reproductive systems in both males and females, dialogue shortcomings, memory loss, tremors, deafness, loss of visualization, character changes, paralysis, unconsciousness, neurological illnesses, congenital diseases, and even death (Genchi *et al.*, 2017). Literature has reported the direct release of Hg into Minamata Bay, Japan (Tohyama, 2020). that led to a disease called "Minamata disease" which is the poisoning of methyl mercury causing nervous system disorders by the consumption of large quantities of fish and shellfish that were heavily polluted with the poisonous Hg (Sakamoto *et al.*, 2018; Budnik and Casteleyn, 2019).

There are many methods physical and chemical which have been used in the past few decades for cleaning of contaminated water but were not eco-friendly. Biological methods serve as a promising method for the elimination of heavy metals from wastewater by biological tools (Goswami *et al.*, 2022). The major mechanisms involved are extracellular precipitation, biotransformation, biosorption, bioaccumulation, and biomineralization (Luka *et al.*, 2018).

Bacteria play an important role as a bioremediation tool for the removal of Hg from wastewater of industries. Two main mechanisms were involved in the bioaccumulation and bioremediation of Hg, i.e., enzymatic detoxification and degradation of Hg resulting in the formation of methylated Hg which is released in the environment (Matsui and Endo, 2018). *merA* gene encodes the Hg^{2+} reductase. Abbas *et al.* (2019) reported different types of enzyme-mediated degradation and remediation mechanisms in bacteria such as the formation of HgS , volatilization, biomineralization of Hg^{2+} and enzymatic reduction of Hg^{2+} to Hg^0 by mercuric reductase in the presence of NADPH. As a result of increased vapor pressure, Hg^0 will disperse into the system and environment. Different bacteria like *Staphylococcus*, *Bacillus*, and *Pseudomonas* were employed to convert Hg^{2+} to Hg^0 . *A. junii* and *P. stutzeri* are capable to bio-accumulating Hg about 38% and 45%, respectively.

Bio-sorption deals with the adsorption of Hg and accumulates on the surface and within the cell. The bio-sorption of Hg involves different types of chemical reactions such as chelation, ion exchange, and adsorption. The bacteria which are normally use these types of reactions and strategies of bio-sorption for the removal of Hg is *Pseudomonas* strain (Mahbub *et al.*, 2017). Microorganisms can reduce and remediate Hg^{2+} from the environment, especially from the water through the process of reduction and adsorption via the *mer* operon.

Hence, microorganisms and their metabolic pathways prove to be a potential strategy for the removal of toxic metals from polluted environments. The study aims to screen out Hg-resistant microorganisms from wastewater samples which can further be subjected to field experiments for Hg bioremediation.



2. METHODS

2.1 Sampling and Isolation of Mercury (Hg) Resistant Bacteria

The water samples were collected from the Ravi River and associated streams located in Lahore, Pakistan, which were used for the isolation of the Hg-resistant bacteria in a sterile wide container and transported to the laboratory for microbiological analysis. The pH and temperature of the sampling site were also recorded. 5ml water sample was added in 100ml autoclaved nutrient broth aseptically with 10ppm of Hg respectively. Then, the flask was incubated for enrichment at 37°C for 24 hours in a shaking incubator at 120rpm for microbial growth.

2.2 Selection and Biochemical Characterization of Hg-resistant Bacteria

To select Hg-resistant microorganisms, serial dilutions of the enrichment were performed and spread plates were incubated at 37°C for 24 hours. After incubation at 37°C, the Hg-resistant bacteria in the sample were observed as separate visible colonies on the surface of the media. The visible colonies were counted and expressed in terms of CFU/ml and selected and isolated depending on their size, shape, morphology, and color by quadrant streaking method.

2.3 Characterization of the Isolates

Various tests were performed for the biochemical characterization of isolates' bacterial colonies, including gram staining, catalase, oxidase, triple sugar iron (TSI), urease, citrate utilization, and MR-VP tests. All the tests were performed in duplicates with negative controls where applicable.

2.4 Minimum Inhibitory Concentration (MIC)

MIC for the isolated bacteria was determined by adding Hg of different concentrations of

250ppm, 500ppm, 750ppm, 1000ppm, 1250ppm, 1500ppm, 1750ppm, 2000ppm, and 2500ppm in 9 Erlenmeyer flasks. In this method, 10 ml of the nutrient broth was accompanied by different concentrations of Hg from 5,000 ppm Hg stock. Then, bacterial culture was taken from freshly prepared bacterial culture standardized by 0.5M McFarland standard were inoculated in each flask having different concentrations of Hg and incubated for 24 h at 37°C on shaking at 120rpm with negative controls. The optical density at 600nm was recorded using a spectrophotometer.

2.5 Salt Tolerance test

L-broth was prepared to have 3%, 5%, and 7% salt concentration with negative controls separately. Test tubes were autoclaved and allowed to cool at room temperature. Then, bacterial culture was taken from freshly prepared bacterial culture standardized by 0.5M McFarland standard were inoculated in each tube having different concentrations of salt and incubated at 37°C for 24 hours at 120rpm shaking with negative controls. The optical density at 600 nm was determined via a spectrophotometer.

2.6 The Effect of pH on Microbial Growth

L-broth was prepared in test tubes, autoclaved, and allowed to cool at room temperature. Then, 5M HCl and 5M NaOH were used to set the pH of the media at 3, 5, 7, 9, and 11. Then, bacterial culture was taken from freshly prepared bacterial culture standardized by 0.5M McFarland standard was inoculated in each test tube having different pH and subjected to incubation for 24 h at 37°C at 120rpm shaking with negative controls. The optical density at 600nm was recorded by a spectrophotometer.



2.7 Cross Metal Resistance

Cross metal resistance test is used to test the ability of bacterial isolates to grow on media supplemented with different heavy metals.

L-broth 0.5mM working solutions of copper chloride, sodium arsenate, cobalt chloride, and nickel chloride, while 0.25mM working solution of cadmium acetate and potassium dichromate was prepared followed by the inoculation of 100µL of bacterial culture that was taken from freshly prepared bacterial culture standardized by 0.5M McFarland standard was inoculated to each test tube aseptically. During all these procedures, negative controls having respective metals without microbial culture were maintained. Incubation was provided at 37°C for 24 hours.

2.8 Carbon Source Utilization

This test demonstrates the ability of isolates to utilize carbohydrates as sole carbon sources other than glucose. The tested carbohydrates used were fructose, mannitol, maltose, oxalate, and acetate. Bacteria could grow in a medium with these precursors to some extent showing turbidity.

L-broth was prepared in each of the 6 Erlenmeyer flasks having the above-mentioned carbon sources. After autoclaving, 100µL of bacterial culture was taken from freshly prepared bacterial culture standardized by 0.5M McFarland standard was inoculated to each test tube of every carbon source aseptically. Negative controls with respective substrate (acetate, fructose, maltose, ammonium oxalate, citrate, and mannitol) were set during all these procedures without microbial culture. Incubation was provided at 37°C for 24 hours.

2.9 Growth Curve

The growth curve experiment was set up for the three best isolates selected based on MIC and

performed in three 250ml Erlenmeyer flasks having 50ml of autoclaved nutrient broth with appropriate control. Bacterial culture was taken from freshly prepared bacterial culture standardized by 0.5M McFarland standard was inoculated in the flasks containing media. Optical density was measured at 600nm at different intervals of time i.e., 0h, 4h, 8h, 16h and 24h. 1ml of aliquot was drawn from the flask at each interval of time and took the OD at 600nm by spectrophotometer.

2.10 Standard Curve

A standard curve for selected bacterial isolates was drawn by colorimetric detection of mercury in media based on the reaction of mercury with dithizone. Different concentrations of Hg were used i.e. 0ppm, 25ppm, 50ppm, 200ppm, 400ppm, 1000ppm and 1200ppm in the medium. For this, 20% sodium sulfite was added to prevent dithizone from being oxidized. pH of media was maintained 1 by nitric acid (1ml HNO₃ in 9ml distilled water). 2.28ml of freshly prepared dithizone solution (0.001g dithizone in 100ml chloroform) was added and vortex for 2 minutes (Hg²⁺ gives orange color while Hg⁰ remains greenish). The supernatant was discarded, and the complex-colored layer is further analyzed at 540nm using UV-spectrophotometer.

2.11 Determination of Mercury Removal Potential of Isolates

The Hg removal experiment was set up and performed in a 250 ml flask having 50 ml of autoclaved N-broth. Bacterial culture was taken from freshly prepared bacterial culture standardized by 0.5M McFarland standard was inoculated in the medium supplemented with 1100ppm Hg. The flask without Hg was considered the control. All the flasks were incubated at 37°C on a shaking incubator at 120rpm. The flask containing nutrient broth with Hg was labeled as a negative control. The



growth of bacterial isolates and Hg removal from the medium was recorded at different time intervals of 0h, 8h, 16h, and 24h. Bacterial growth was determined by observing the optical density at 540nm. Aliquots of samples from the flasks (6ml culture with Hg) were withdrawn into the falcon tubes at time intervals of 0h, 8h, 16h, and 24h for calorimetric detection. All the falcon tubes were centrifuged at 8000rpm for 10 minutes followed by transfer of supernatant to fresh tubes for Hg quantification. Hg present in the supernatant was determined by dithizone assay as mentioned earlier. For the calculation of the removal of Hg, the following formula was used.

$$\% \text{Age of Hg removal} = (\text{Initial conc. of Hg} - \text{Final conc. of Hg}) \div \text{Initial conc. of Hg} \times 100$$

3.RESULTS

3.1 Morphological and Biochemical Characterization

The isolates were selected based on their morphology, shape, size and size as shown in table 1 and purified by quadrant streaking on agar plates. The purified isolates were subjected to several biochemical tests as described in table 2.

Table 1. Table shows the colony morphology of the isolates

Colony Morphology of Isolates							
Strains	Gram Stain	Pigment	Colonies	Margins	Elevation	Texture	Light passing
Hg2	+ve rods	Off White	Round, pinpoint	Entire	Flat	Rough	Opaque
Hg3	+ve cocci	White	Round, pinpoint	Entire	Elevated	Non-mucoid	Opaque
Hg4	+ve cocci	Orange	Round, pinpoint	Entire	Elevated	Non-mucoid	Opaque
Hg5	+ve cocci	Dark Yellow	Round, pinpoint	Entire	Flat	Rough	Opaque
Hg6	+ve rods	White	Round, pinpoint	Entire	Elevated	Non-mucoid	Opaque
Hg7	+ve cocci	Yellow	Round, pinpoint	Entire	Flat	Rough	Opaque
Hg8	+ve rods	Off White	Round, pinpoint	Entire	Flat	Rough	Opaque

Table 2. Biochemical Characterization of Isolates

Colony Morphology of Isolates					
Strains	TSI	Urease	Citrate utilization	MR test	VP test
Hg2	K/A/-/-	Negative	Positive	Negative	Positive
Hg3	K/A/-/-	Weak Positive	Positive	Positive	Negative
Hg4	A/A/-/-	Negative	Positive	Positive	Negative
Hg5	A/A/-/-	Positive	Negative	Positive	Positive
Hg6	K/A/-/-	Negative	Negative	Positive	Positive
Hg7	K/A/-/-	Weak Positive	Negative	Positive	Positive
Hg8	K/A/-/-	Negative	Positive	Positive	Negative



3.2 Minimum Inhibitory Concentration (MIC)

The experiment was carried out in flasks having different concentrations of Hg (250ppm-2500ppm) followed by inoculation of cultures of isolates for 24h in orbital shaker at 120rpm. MIC was checked for the isolates at nine concentrations of Hg i.e. 250ppm, 500ppm, 750ppm, 1000ppm, 1250ppm, 1500ppm, 1750ppm, 2000ppm and 2500ppm. It was observed that all the concentrations of Hg up to 1500ppm were resisted by all isolates. Hg2 was not able to grow at 1750ppm. Hg3, Hg5 and Hg8 were not showing growth at 2000ppm, while Hg6 and Hg7 were able to grow at 2000ppm and Hg4 was able to resist 2500ppm. So, the MIC for Hg2 is 1500ppm, while Hg3, Hg5 and Hg8 had MIC of 1750ppm and MIC for Hg6 and Hg7 is 2000ppm.

3.3 Salt Tolerance test

All the isolates exhibited maximum growth at 3% of NaCl concentration as shown in figure 1. The isolates Hg2 and Hg4 showed better growth at 3% and 5% of NaCl concentration, while showed no growth at 7% of NaCl concentration. The isolates Hg3 and Hg6 showed better growth at 3% of NaCl concentration, while showed no growth at 5% and 7% of NaCl concentration. The isolates Hg5 and Hg7 showed better growth at 3%, while little growth at 5% of NaCl concentration, and showed no growth at 7% of NaCl concentration. The isolate Hg8 showed better growth at 3% and 5% of NaCl concentration, while showed little growth at 7% of NaCl concentration.

3.4 pH Tolerance test

The isolates Hg2, Hg6, Hg7 and Hg8 showed maximum growth at pH7 and showed better growth at pH5, while showed no growth at pH3, pH9 and pH11. The isolate Hg3 showed maximum growth at pH7 and better growth at

pH5. It also showed little growth at pH3 while showed no growth at pH9 and pH11. The isolates Hg4 and Hg5 showed maximum growth at pH7 and better growth at pH5. It also showed little growth at pH3 and pH9, while showed no growth at pH11.

3.5 Cross Metal Resistance

The isolates Hg2 and Hg3 showed best resistance in the presence of Cu, Ni and Cr, while in the presence of As^{3+} showed better resistance and very little and no resistance in the presence of Cd and Co. Isolate Hg4 showed better resistance in the presence of Cu and Ni, while showed very little or no resistance in the presence of Cr, Cd, As^{3+} and Co. The isolate Hg5 showed better resistance in the presence of Chromium, while showed very little and no resistance in the presence of Cu, Cd, Ni, As^{3+} and Co. The isolate Hg6 showed best resistance in the presence of Cr, while in the presence of Ni and As^{3+} showed better resistance and very little and no resistance in the presence of Cu, Cd and Co. The isolate Hg7 showed best resistance in the presence of Cu, while in the presence of Cr showed better resistance and very little resistance in the presence of Cd, Ni and As^{3+} and no resistance in the presence of Co. The isolate Hg8 showed better resistance in the presence of Cu, As^{3+} , Ni and Cr, while in no resistance in the presence of Cd and Co aerobically.

3.6 Carbon Utilization test

The isolate Hg2 showed dense growth in the presence of fructose, while showed better growth in the presence of maltose and oxalate and very little growth in the presence of acetate, mannitol, and citrate. The isolate Hg3 showed dense growth in the presence of acetate and citrate, while showed better growth in the presence of mannitol, maltose and fructose, and very little growth in the presence of oxalate. The isolate Hg4 showed better growth in the presence of acetate, while very little



growth in the presence of mannitol, fructose, oxalate, maltose and citrate. The isolate Hg5 showed dense growth in the presence of citrate and maltose, while showed better growth in the presence of mannitol and very little growth in the presence of acetate, fructose and oxalate. The isolate Hg6 showed dense growth in the presence of fructose, while showed better growth in the presence of citrate, oxalate and maltose and very little growth in the presence of acetate, while no growth in the presence of mannitol. The isolate Hg7 showed dense growth in the presence of mannitol, while showed better growth in the presence of acetate, fructose, oxalate, maltose and citrate. The isolate Hg8 showed dense growth in the presence of fructose and citrate, while showed better growth in the presence of acetate and mannitol and very little growth in the presence of oxalate and maltose under aerobic conditions.

3.7 Determination of Mercury Removal Potential of Isolates

Metal reduction was obtained by a standard curve. It was shown that Hg4 bio-remediated 59.82%, while Hg6 showed bioremediation of 40.82% and Hg7 removed 65.00% of mercury (Hg) after 24 hours. It was also observed that Hg6 (18.82%) and Hg7 (24.82%) showed maximum bioremediation from 8-16 hours, while Hg4 (10%) showed maximum removal of mercury (Hg) from 0-4 hours.

4. DISCUSSION

Heavy metal pollution has led to harmful effects on all the biotic and abiotic components of our environment. Thus, it is best time to adopt effective strategies for the bioremediation of heavy metals. Microorganisms, especially bacteria, employ various mechanisms like oxidation, reduction, adsorption, absorption and accumulation for the removal of toxic metal species from the environment. The bacterial isolates under study in this project exhibited various characteristics on the basis of which they were screened.

Carbon sources provide energy for microbial growth and serve as electron donors in the electron transport chain (Meerbergen *et al.*, 2018). The results showed that fructose, citrate, and maltose were the best carbon sources because isolates showed high growth by using these sources which acted as electron donors through TCA cycle. The enzymes citrate synthase, isocitrate dehydrogenase, and malate dehydrogenase are the enzymes which regulate citric acid cycle and help in the assimilation of carbon sources. In the presence of mannitol, oxalate, and acetate, the isolates showed low growth because isolates might be having low ability to utilize these carbons as source of electrons.

High salt concentrations in the surroundings affect the physiological activities of bacteria. As salt causes loss of water from bacteria, it can affect cell membrane of bacteria, because cell membrane plays an important role for maintaining osmotic pressure and preventing the cell damage of bacteria. In the current study, Hg-resistant bacterial isolates were analyzed to check their ability to tolerate salts in varying concentrations of sodium chloride (NaCl) in different ranges as shown in figure 3.

Hg resistance is governed by the presence of *mer* operon. In the case of minimum inhibitory concentration (MIC) it was shown that all the isolates showed luxurious growth till 1500 ppm Hg as shown in figure 2. Hg2 showed high growth at 1500 ppm. Until 2000 ppm, Hg6 and Hg7 showed noticeable growth but Hg2, Hg3, Hg5, and Hg8 did not. At 2500 ppm of Hg concentration growth of all isolates was inhibited by Hg concentration except Hg4. The behavior of isolates strongly suggests the functioning of *mer* operon owing to Hg resistance.

All the isolates Hg2, Hg3, Hg4, Hg5, Hg6, Hg7 and Hg8 showed optimum and better growth at pH7. Figure 3 shows the growth trend of isolates. The results showed that all Hg resistant isolates were neutrophils, but some isolates were able to

survive at acidic Hg3, Hg4, and Hg5) and alkaline (Hg4 and Hg5) pH. i.e. Hg3, Hg4 and Hg5 were also acidophiles, and some isolates i.e. Hg4 and Hg5 were also alkaliphiles. These results displayed the versatility of isolates to grow at diverse conditions.

Cross Metal Resistance Test was performed to check whether the Hg resistant isolates had ability to resist the other metals or not. So, all isolates were tested for other multiple metals resistance such as Cadmium, Chromium, Cobalt, Nickel, Arsenate and Copper. All isolates were grown in the presence of these metals under aerobic conditions. The results in table 3 show the growth pattern of the isolates which is suggestive of the presence of various metal-resisting enzymes that the isolates can produce and confer resistance. Rehman *et al.* (2007) conducted a study in which they reported that bacterial strain *B. casei* was resistant to Hg at the concentration of 500µg/mL, while *P. aeruginosa* showed highest resistance at a concentration of 400µg Hg²⁺/mL. Their bacterial isolates were also checked for their resistance to various other heavy metals such as Cr, Cd, Cu, Pb, Zn and Ni.

Growth Curve Experiment was performed to optimize the growth of the bacteria at hourly bases as shown in figure 4. In the lag phase of growth curve all isolates Hg4, Hg6 and Hg7 showed very little growth from 0 hours to 4 hours, maybe due to the initial high concentration of Hg. The initial higher concentration normally inhibits the essential metabolic reactions and retards the growth (Abbas *et al.*, 2019). All three isolates Hg4, Hg6 and Hg7 showed exponential increase in growth from 4 hours to 8 hours while showed stationary growth or very little growth from 8 hours to 16 hours because at the stationary phase growth of bacteria decrease due to the lack of nutrients. The decline phase appeared from 16 hours to 24 hours due to the cell death in this phase. showed a study in which the growth of Hg resistant bacteria *B. vesicularis*,

N. mobilis, *F. necrogenes* and *F. aquatilis* for 58 hours which showed a even pattern of growth. The stationary phase that was the best time for the production of bacterial cell numbers of *B. vesicularis* and *N. mobilis*, was at 36 h, while that of *F. necrogenes* and *F. aquatilis* was at 42 h. They reported that the stationary phase difference presented occurred because of resistance against Hg.

Mercury Reduction Experiment was performed to determine Hg reduction i.e. 0-4 h, 4- 8 h, 8-16 h, and 16 to 24 h as shown in Figure 4. Hg4, Hg6, and Hg7 were selected for the Hg reduction experiment based on MIC; because these three isolates showed growth at higher concentrations of Hg. It was shown that Hg4 bio-remediated 59.82%, while Hg6 showed bioremediation of 40.82% and Hg7 removed 65.00% of mercury (Hg) after 24 hours. It was also observed that Hg6 (18.82%) and Hg7 (24.82%) showed maximum bioremediation from 8-16 hours, while Hg4 (10%) showed maximum removal of mercury (Hg) from 0-4 hours. This reduction is due to the presence of *mer* operon which is comprised of *merA*, *merR*, *merP*, *merD*, *merC*, *merT*, *merG*, *merE*, and *merF* genes. Among these, *merA*, *merR*, *merC*, *merP*, *merE*, and *merT*, play major roles in the Hg²⁺ reduction (Pugazhendhi *et al.*, 2018). *merR* gene acts as a promoter for *mer* operon acting downstream and is tempted by Hg²⁺. *merC*, *merP*, *merE*, and *merT* genes code for transporter proteins, whereas *merA* gene translates Hg²⁺ reductase for conversion of Hg²⁺ into Hg⁰. Therefore, the *merA* gene is significant for optimizing Hg bioremediation in bacteria (Von Canstein *et al.*, 1999). Thus, these isolates hold great potential to be used as bioremediating tools to create a healthier ecosystem.

5. CONCLUSION

This study serves as a potential bedrock over which Hg4, Hg6, and Hg7 can be used for Hg bioremediation at large scales. Being able to consume various carbon sources and resist



multiple heavy metals, these strains can further be taken towards the assessment of their bio-removal ability of Cr (VI) under different abiotic conditions concentrations, etc.

Conflict of interest:

The authors declare no conflict of interest.

Table 3. Results for cross-metal resistance based on turbidity

Isolates	Cross Metal Resistance					
	Turbidity					
	Cu	Cr	Cd	As ⁺⁵	Co	Ni
Hg2	+++	+++	+	++	-	+++
Hg3	+++	+++	-	++	-	+++
Hg4	++	+	-	-	-	++
Hg5	+	++	-	-	-	-
Hg6	-	+++	-	++	-	++
Hg7	+++	++	+	+	-	+
Hg8	++	++	-	++	-	++

Dense growth (+++), Better growth (++), Little growth (+) and No growth (-).

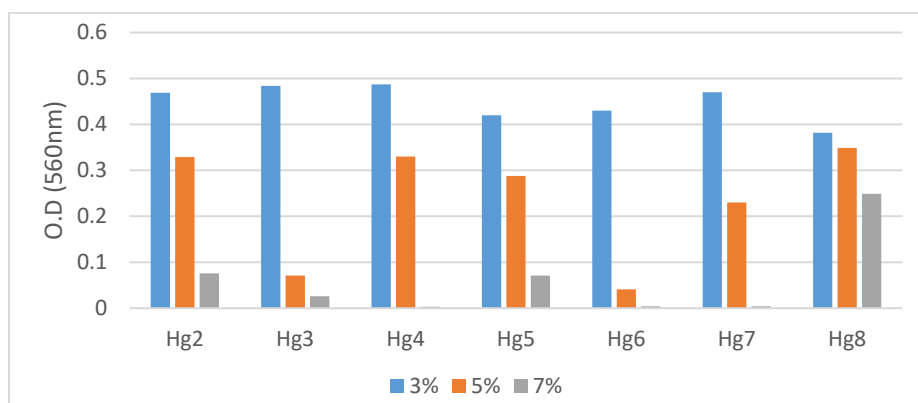


Figure 1. Growth of Hg-resistant isolates on varying salt concentration

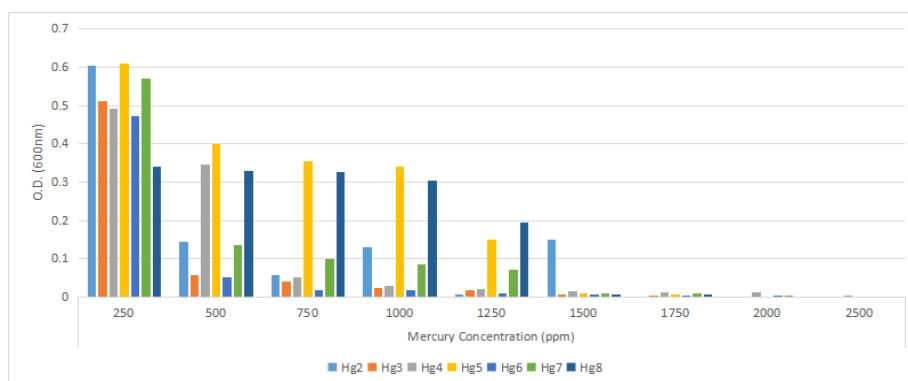


Figure 2. Minimum inhibitory concentration of mercury for Hg-resistant isolates



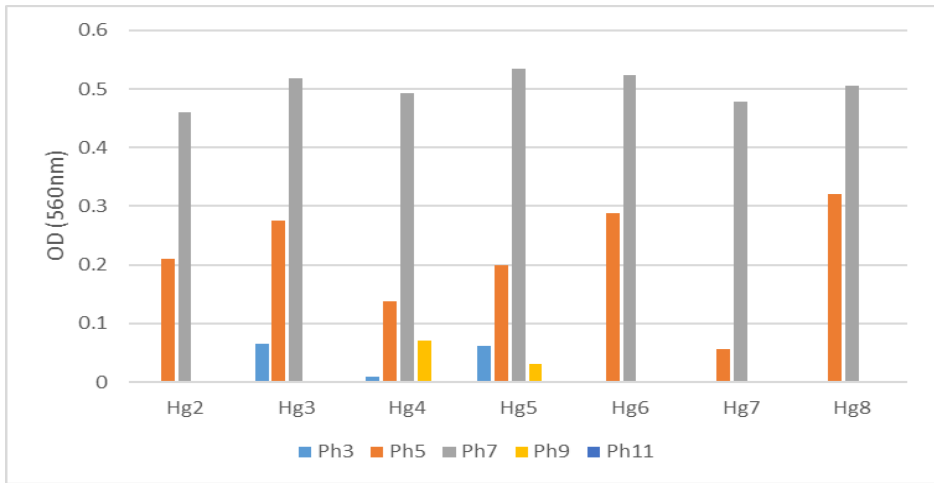


Figure 3. Growth of mercury resistant isolates on varying pH range

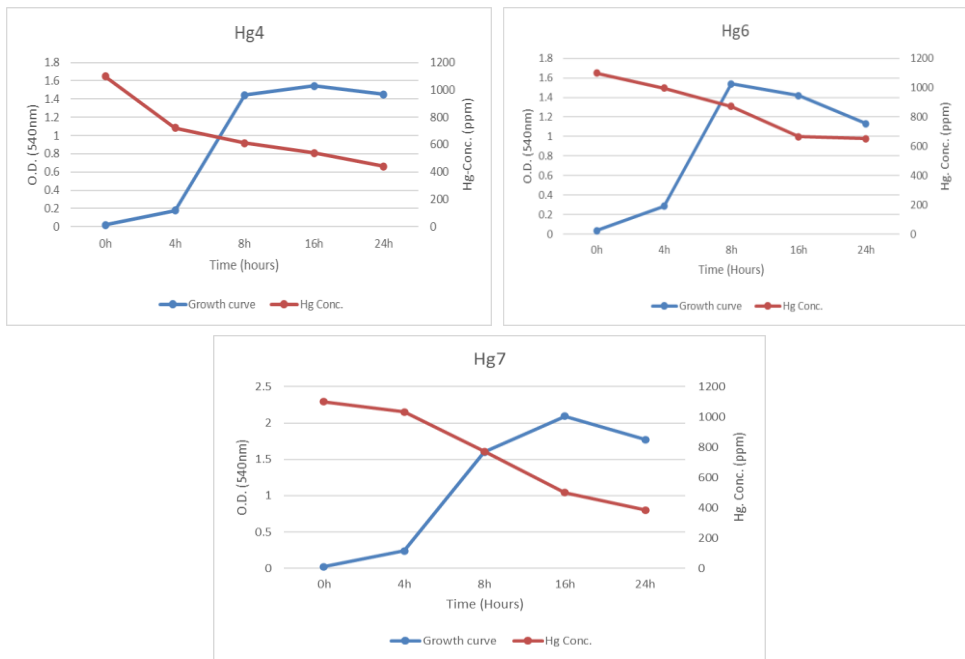


Figure 4. Figure shows the growth curves and the Hg reduction potential of the selected isolates

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