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Lead-Resistant Bacterial Isolates from Industrial Discharges: Characterization and Bioremediation Insights

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Abstract

Heavy metal pollution, particularly lead, poses severe environmental and health risks. Microbial bioremediation offers an ecofriendly and effective strategy to mitigate such contamination. The present work focused on exploring indigenous bacterial isolates capable of withstanding and reducing lead concentrations. Five isolates, designated SLW4, SLW5, LTS7, SLS1, and AN4, were obtained from industrial effluents. Their morphological, physiological, and biochemical characteristics were examined. Optimum growth conditions were identified at 37°C and pH 7. Based on biochemical profiling, SLW4 and LTS7 were identified as *Klebsiella* sp., SLW5 as *Yersinia* sp., SLS1 as *Vibrio* sp., and AN4 as *Pseudomonas* sp. Minimum inhibitory concentration (MIC) values were determined, and growth kinetics were assessed. Lead bioremediation was quantified using atomic absorption spectrophotometry at 24 and 48 hours. SLS1 exhibited the highest MIC of 650 µg/ml, while AN4 showed the lowest (600 µg/ml). Growth analysis revealed SLW4 with the longest exponential phase and AN4 the shortest. At 24 hours, AN4 achieved the highest lead reduction (74.04%), while SLW4 recorded the lowest (71.93%). After 48 hours, SLW4 demonstrated maximum removal (80.97%) and LTS7 the least (78.85%).The isolates exhibited substantial lead resistance and bioremediation potential, with efficiency increasing over time. These findings highlight their applicability in environmentally sustainable strategies for lead detoxification.

Introduction

Environmental pollutants are persistent danger that impacts human health [1]. The environment has been seriously polluted with several pollutants which include inorganic ions, organic pollution, organometallic compounds, radioactive isotopes, gaseous pollutants, nanoparticles and heavy metals [2].

Lead (Pb) is one of the most crucial environmental pollutants among heavy metals, and several researches have demonstrated that lead is a trigger for health issues. Lead even in small quantities, is the maximum risky heavy metal [3] which induces acute and chronic effects on human health [4]. Most prominent and stable forms of lead are lead hydroxyl compounds and Pb^{2+} and this Pb^{2+} is most reactive form and produce polynuclear as well as mononuclear hydroxides and oxides. Lead found with other metals in compound forms and may be obtained from natural sources *i.e.,* autochthonous and allochthonous. In soil lead is found in small quantities as pyromorphite, $[Pb_5 (PO_4)_3Cl]$,

Massicot (PbO), Litharge, crocoite ($PbCrO_4$), Anglesite ($PbSO_4$) and cerusite ($PbCO_3$) [5]. Lead is one of the non-essential heavy metals. It is dangerous to all organisms even at low levels, due to interactions with huge biomolecules and by way of inactivation of a chain of enzymes. Lead has a tendency to accumulate in soils due to its low solubility and relative freedom from microbial remediation and it may be available to the food webs in the future [6].

Lead is contaminating the environment through extraction of metals, melting processes, mineral rocks weathering and industrial activities, combustion of coal, waste disposal, agriculture as well as atmospheric deposition, arsenate-based insecticides, pigments, fertilizers and lead batteries [7].

Lead also has sever effects on microorganisms by nucleic acid degradation, cell membrane destruction, effecting enzymatic activities, cell division, protein denaturation, metabolic respiration that results in microorganism's death. Bacteria have various strategies against lead contamination such as siderophores on cell surface, accumulation of lead in cell, attaching to other living organisms, absorption, metal deposition and extracellular polymers on cell surface. Bacteria also have physiochemical complex mechanisms for lead removal *i.e.,* precipitation, ion exchange, electrostatic interaction and redox mechanisms. Microorganisms also have mechanical mechanisms against heavy metals include methylation, metal-ligand destruction, oxidation, enzymatic destruction, organometallic degradation, demethylation, chelation, permeability inhibition and metal removal. Microorganisms have anionic system that allows them to bind metal cations [8, 9].

Currently, the only way to eliminate lead contamination in soil is through physical removal and destruction of contaminated soil. In recent years, extensive study of bacteria has revealed remarkable capabilities for detoxifying and transforming environmental pollutants [10, 11]. Despite the fact that numerous strategies have been applied to document and address this difficulty but it stays a distressing problem. Surroundings and people both are affected by these risks globally. A unique technique should be designed in order to guard surroundings and human beings from the disadvantageous reactions of environmental pollutants, and bioremediation is one of these strategies [12].

Bioremediation, which has emerged as a relatively and comparatively cheap technique, can show to be a powerful device to counter the sick-outcomes of pollution and render the tainted soil much less polluted and freed from poisonous or recalcitrant compounds [13, 14]. Bioremediation is an environmentally useful and long-time period approach which could be a ways extra successful and efficient at eliminating heavy metals from diverse parts of the surroundings. Different types of bioremediation are; degradation, mineralization, detoxification, transformation of toxic metal form to less toxic metal form etc. The use of bioremediation technique depends upon many factors like site characteristics, cost, and concentration of pollutants [15]. Bioremediation can be applied in *ex-situ* as well as in-situ. *In situ* bioremediation, applied directly at polluted sites. *In situ* bioremediation has further types *i.e*., biosparging, bioventing, intrinsic and bio augmentation or engineered bioremediations.

The primary objectives of this study were to isolate and characterize lead-resistant bacteria from industrial effluents, assess their minimum inhibitory concentration (MIC) for lead, and evaluate the metal-removal potential of their planktonic cells using atomic absorption spectrophotometry.

Materials and Methods

Sample Collection

Two types of samples including industrial effluents and soil were collected from four different industrial estates of Punjab, Pakistan. The sites included tannery industries Sialkot, Quaid-e-Azam industrial estate Kot Lakh pat Lahore, Sunder industrial estate, Lahore and Shalimar link road lead batteries site Lahore, Punjab, Pakistan. Physicochemical parameters such as temperatures and pH of effluents were also noted at the time of sampling.

Isolation of lead resistant bacteria

Isolation of lead resistant bacteria was done on lead nitrate [Pb $(NO_3)_2$] supplemented nutrient agar plates Serial dilutions from 10^{-1} to10^{-6} were prepared for the wastewater and soil samples for bacterial isolations. These dilutions were plated on agar plates for isolation of lead resistant bacteria. The plates were incubated at 37°C for 24 hours. Morphologically different colonies were selected from different plates and purified by quadrant streaking on Pb $(NO_3)_2$ supplemented nutrient agar plates.

Determination of minimum inhibitory concentration (MIC) for lead (Pb^{2+})

A broth dilution method was used to examine the minimum inhibitory concentration of isolated organisms for lead by following the protocol of Haroun et al., (2017) [16]. For this purpose, nutrient broth was prepared and autoclaved (10 ml per test tube). Tubes were supplemented with Pb^{2+} from 100 µg/ml to 1200 µg/ml with an interval of 25 µg/ml in between each concentration. Standardized inoculum ($O.D_{600}$ = 0.1) of overnight bacterial cultures were prepared. An inoculum of 100 µl of standardized culture (OD_{600}= 0.1) was added in Pb^{2+} supplemented media test tubes. The experiment was performed in triplicates with one negative control for each concentration of Pb^{2+}. The test tubes were incubated for 24 hours at 37°C. MIC was noted as the highest concentration of Pb^{2+} at which visible growth of microorganism could observed.

Morphological characterization of bacterial isolates

Single colonies of purified bacterial isolates were obtained by quadrant streaking for the study of colony morphology such as size, form, texture, color, elevation and margin. Cell morphology was studied by using Gram staining technique [17].

Biochemical characterization

The bacterial isolates were characterized biochemically by following the protocols described in Cappuccino and Sherman [17].

Physiological Characterization of Pb^{2+} resistant bacteria [18]

Determination of optimum temperature

The bacterial growth is greatly influenced by external environmental temperature. A little fluctuation may inhibit the growth or enzyme activity as well as change the behavior of bacteria. To check the optimum temperature for bacterial growth, four different temperatures were selected *i.e.,* 4°C, 25°C, 37°C, 45°C. For this purpose, nutrient broth was prepared and autoclaved. Overnight bacterial cultures were prepared and standardized with optical density of 0.1 (OD_{600}= 0.1). An inoculum of 100 µl of standardized cultures (OD_{600}= 0.1) was added in each test tube. The experiment was performed in triplicates with one negative control for each temperature. Then tubes were incubated at their designated temperatures 4°C, 25°C, 37°C, 45°C for 24 hours. After incubation period optical density of isolated bacterial cultures were noted at 600 nm using spectrophotometer (*Spec ORD 200 plus*) taking nutrient broth as blank.

Determination of optimum pH

To determine the optimum pH for bacterial growth three different pH values were selected *i.e.,* 5, 7 and 9. For this purpose nutrient broth was prepared and autoclaved. Overnight bacterial cultures were prepared of each isolated bacteria and standardized with optical density of 0.1 (OD_{600}= 0.1). An inoculum of 100 µl of standardized culture (OD_{600n}= 0.1) was added in each test tube. The experiment was performed in triplicates with one negative control for each bacterium. Then all tubes were incubated at 37°C temperature for 24 hours. After incubation period optical density of isolated bacterial cultures were noted at 600nm using spectrophotometer (*Spec ORD 200 plus*) taking nutrient broth as blank.

Study of bacterial growth curve [19]

To determine the bacterial growth curve, nutrient broth was prepared in flasks (100 ml media per flask). Overnight bacterial cultures were standardized with optical density of 0.1 (OD_{600}= 0.1). An inoculum of 100 µl of standardized culture (OD_{600}= 0.1) was added in each experimental flask and incubated at optimum temperature of the bacterial isolates (37°C) for 24 hours' time interval. Bacterial cultures were taken after every 1 hour time interval and optical density was determined at 600 nm using spectrophotometer (*Spec ORD 200 plus*) taking nutrient broth as blank. After that graphs were plotted by taking time on X-axis and optical density on Y-axis for each isolate.

Estimation of Pb^{2+} biosorption by bacterial isolates using atomic absorption spectrophotometry

For estimation of Pb^{2+} biosorption ability of bacterial isolates, 24 hours old incubated bacterial cultures were standardized at 600 nm (OD_{600}= 0.1) for the inoculum. Nutrient broth media was prepared (100 ml media in each flask) and autoclaved. After that media was supplemented with Pb^{2+} (100 µg/ml). These prepared flasks were then inoculated with 1% inoculum of standardized cultures (OD_{600}=0.1) under sterilized conditions. Test is performed in triplicates with one negative control using same concentration of Pb^{2+} but without inoculum of microbe. The flasks were incubated at 37°C and 100 rpm for 24 hours as well as for 48 hours. After incubation period of 24 hours each culture and control was

centrifuged at 5000 rpm for 20 minutes and careful transformation of supernatant was done in sterile tubes and refrigerated until the evaluation of Pb^{2+} by atomic absorption spectrophotometry. Similarly after 48 hours

the same process was repeated with the culture and control for the estimation of Pb^{2+} by atomic absorption spectrophotometry [20].

Results

Physiochemical characteristics of samples

The physicochemical characteristics of samples have been given in table 1. It was noted that the temperature for wastewater samples was from 39°C to 43°C and pH ranged from 4 to 7. The humidity of the sampling areas ranged from 16% to 23% on the day of sampling (table 1).

Table 1: Physiochemical characteristics of wastewater samples

Physiochemical characteristics of wastewater samples					
Samples code	**Sampling sites**	**pH of the sample**	**Temperature of the sample**	**Color of the sample**	**Humidity in environment**
LTW	Wastewater sample from leather tanneries Sialkot	6	39°C	Pale Yellow	23%
SIW	Wastewater sample from Sundar industrial estate Lahore	7	41°C	Blackish	18%
SLW	Wastewater sample from Shalimar link road Lahore lead batteries	4	43°C	Blackish	16%
AN	Wastewater sample from leather tanneries Kasur	7	40°C	Yellowish	21%

Isolation, purification and characterization of isolated bacteria

From soil and wastewater samples, cultured on Pb^{2+} supplemented nutrient agar plates, total 12 bacterial isolates that showed resistance against Pb^{2+} were selected based on different morphological characteristics. Out of these 12 bacterial isolates 04 bacterial isolates were selected from soil samples and 08 were selected from wastewater samples (table 2). These selected bacterial isolates were then sub cultured on Lead supplemented (100 µg/ml) nutrient agar plates for purification through several rounds of streaking. These purified bacterial isolates were then used to study their morphological characteristics.

Table 2: Bacterial isolates selected from different samples

Samples	**Selected isolates**	**Codes for the bacterial isolates**
Soil sample from leather tanneries Sialkot (LTS)	1	LTS7
Wastewater sample from Sundar industrial estate Lahore(SIW)	1	SIW2
Wastewater sample from Shalimar link road Lahore lead batteries (SLW)	3	SLW4, SLW5,SLW7
Soil sample from Shalimar link road Lahore lead batteries (SLS)	3	SLS1,SLS2.SLS4
Wastewater sample from leather tanneries Kasur	4	AN1.AN2, AN4,AN5

Maximum Tolerance Concentration (MTC) of isolated bacteria for Pb^{2+}

MTC of bacterial isolates for Pb^{2+} did not show much difference in values among the bacterial isolates. SLS1showed the highest MTC value that was 650 µg/ml. SLW4, SLW5 and LTS7 showed MIC equal to 625 µg/ml while AN4's MTC was 600 µg/ml. The bacterial isolates SLW7, SLS2, SLS4 and SIW2 showed MTC equal to 575 µg/ml and AN2, AN5 showed 550 µg/ml MIC values while the lowest MTC value 525 µg/ml was observed for the bacterial isolate AN1. Based on these MIC values, SLW4, SLW5, SLS1, LTS7 and AN4 were selected for further studies.

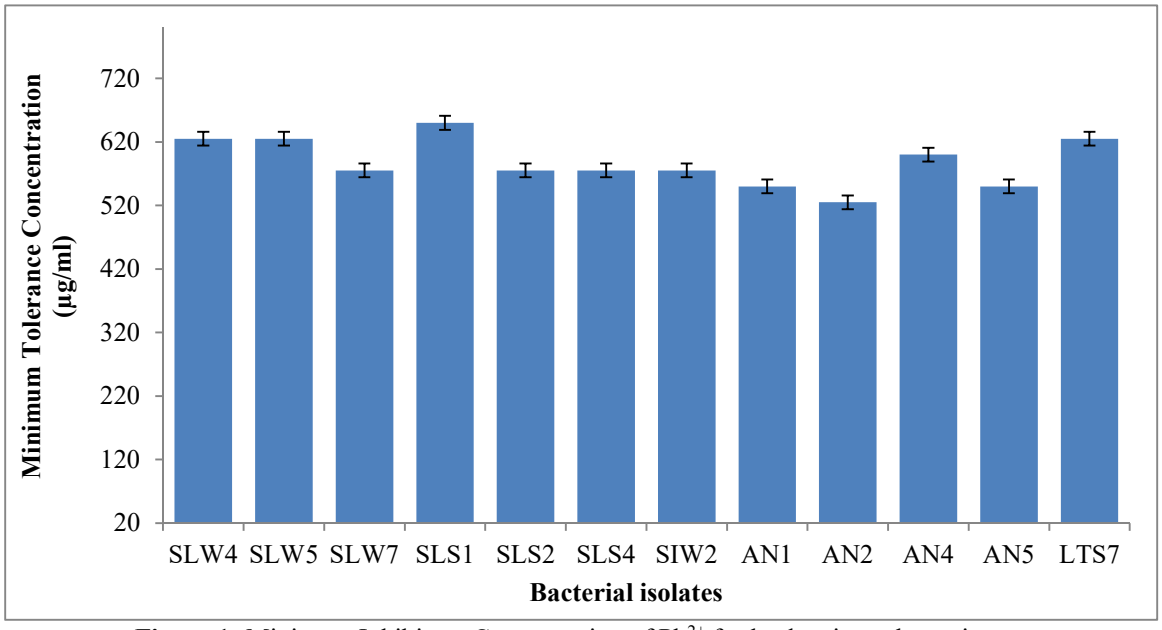

Figure 1: Minimum Inhibitory Concentration of Pb^{2+} for lead resistant bacteria

Morphological characteristics of isolated bacteria

Morphological characteristics of the selected bacterial isolates have been given in table 3 below.

Table 3: Morphological characteristics of Lead (Pb^{2+}) resistant isolates

Morphological characteristics of isolated bacteria									
Bacterial isolate	**Size**	**Form/ Shape**	**Appearance**	**Texture**	**Pigmentation**	**Elevation**	**Margin**	**Gram's Staining**	**Cell's shape**
SLW4	Large	Irregular	Smooth	Mucoid	White	Raised	Undulate	Negative	Bacilli
SLW5	Small	Irregular	Smooth	Butyrous	White	Raised	Undulate	Negative	Bacilli
SLS1	Large	Circular	Smooth	Moist	White	Raised	Entire	Negative	Bacilli
LTS7	Large	Irregular	Smooth	Mucoid	White	Raised	Undulate	Negative	Bacilli
AN4	Large	Circular	Rough	Mucoid	Greenish	Flat	Undulate	Negative	Bacilli

Biochemical characterization of Pb^{2+} resistant bacterial isolates

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Biochemical results of bacterial isolates have been given in table 4

Table 4: Biochemical characterization of Pb^{2+} resistant bacterial isolates

Bacterial isolates						
Biochemical Tests	**SLW4**		**SLW5**	**SLS1**	**LTS7**	**AN4**
Catalase	Positive		Positive	Positive	Positive	Positive
Oxidase	Negative		Negative	Positive	Negative	Positive
Indole	Negative		Negative	Positive	Negative	Negative
Methyl Red	Negative		Positive	Negative	Negative	Negative
Voges Proskaeur	Positive		Negative	Positive	Positive	Positive
Citrate	Positive		Negative	Positive	Positive	Positive
Glucose	TSI	Positive	Positive	Positive	Positive	Positive
Lactose/ Sucrose		Positive	Positive	Positive	Positive	Positive
H_2S Gas		Negative	Negative	Negative	Negative	Negative
Starch	Negative		Positive	Positive	Negative	Positive
Urease	Positive		Negative	Negative	Positive	Negative
Gelatin	Negative		Negative	Positive	Negative	Positive
Motility	Negative		Negative	Positive	Negative	Positive

Physiological characterization of Pb^{+2} resistant bacteria

Determination of optimum temperature and pH range for Pb^{+2} resistant bacteria

Temperature at which a microorganism shows the maximum growth is called its optimum temperature. All the bacterial isolates showed maximum growth at 37°C (figure 2 A). The pH at which a microorganism shows the maximum growth is called its optimum pH. All of the bacterial isolates showed the maximum growth at pH 7 (figure 2 B).

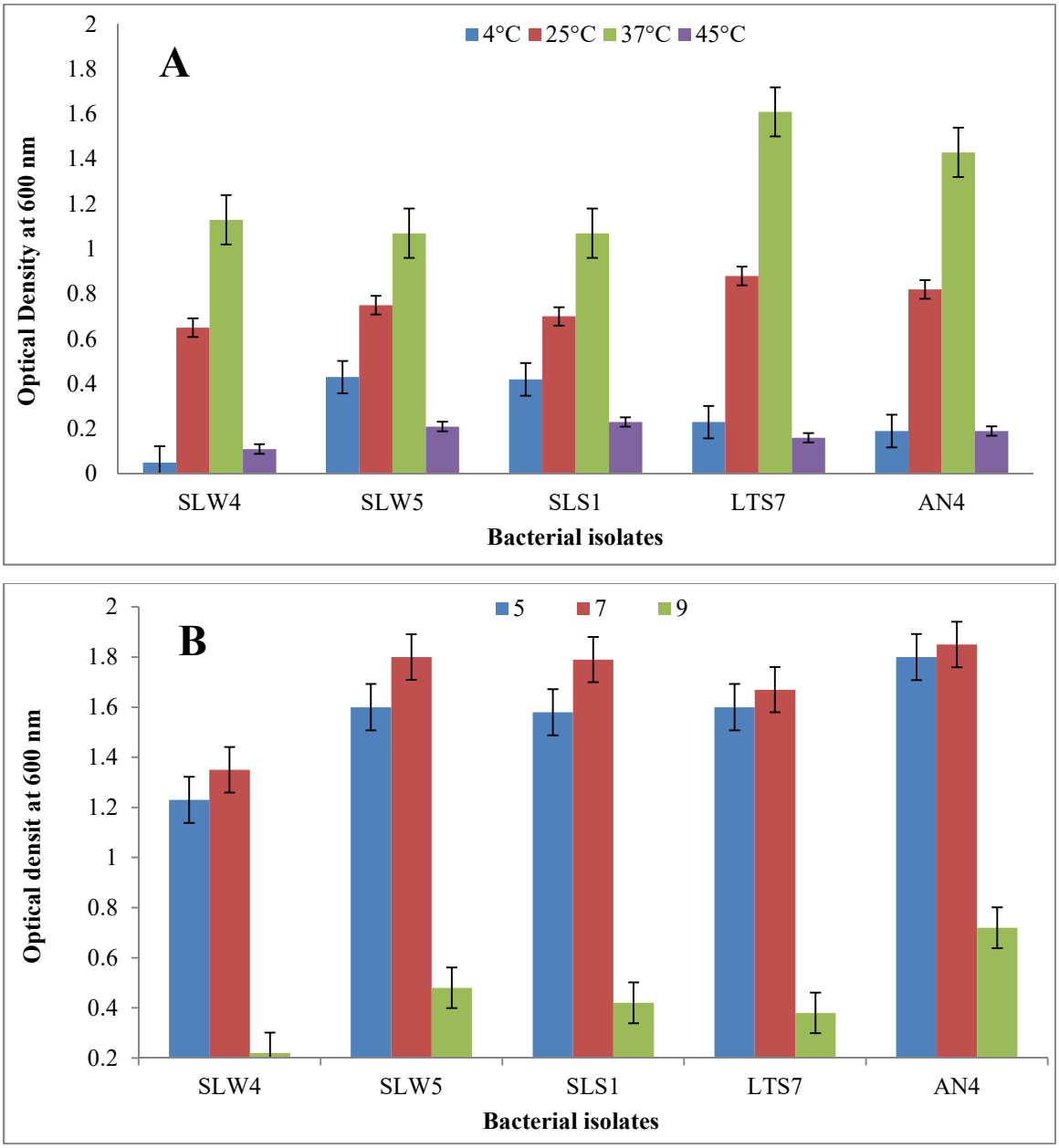
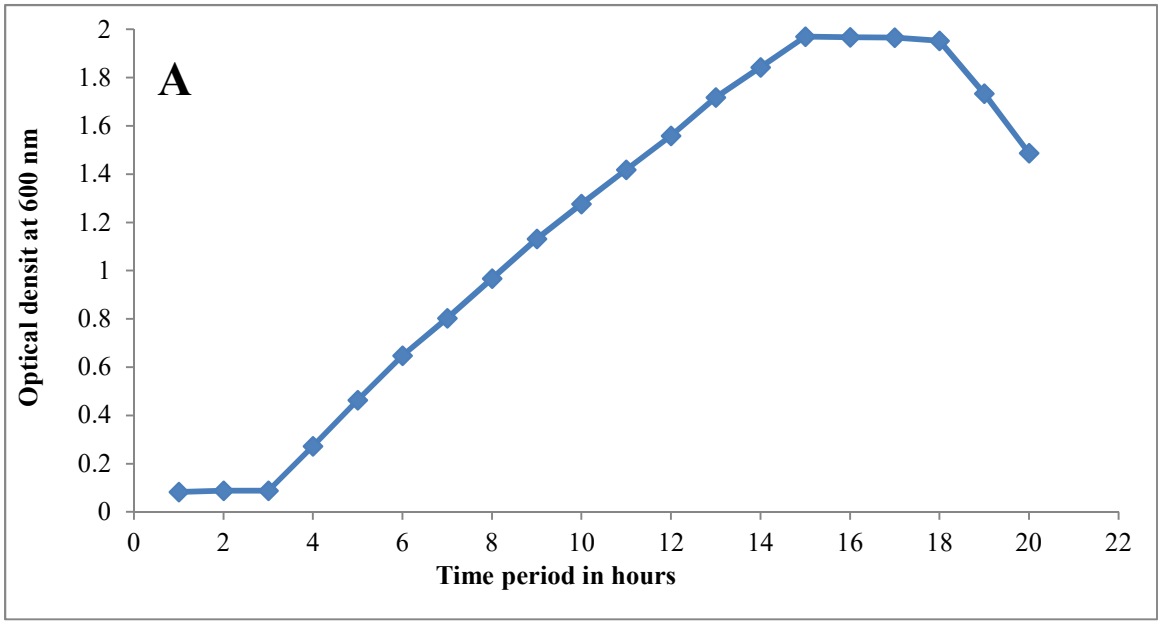
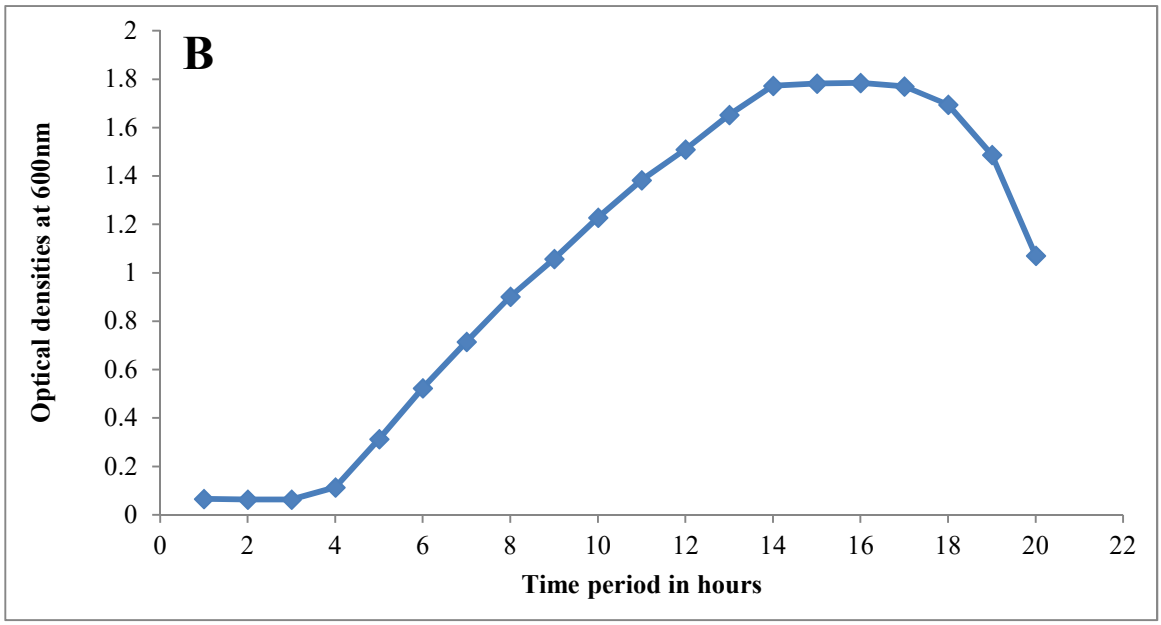


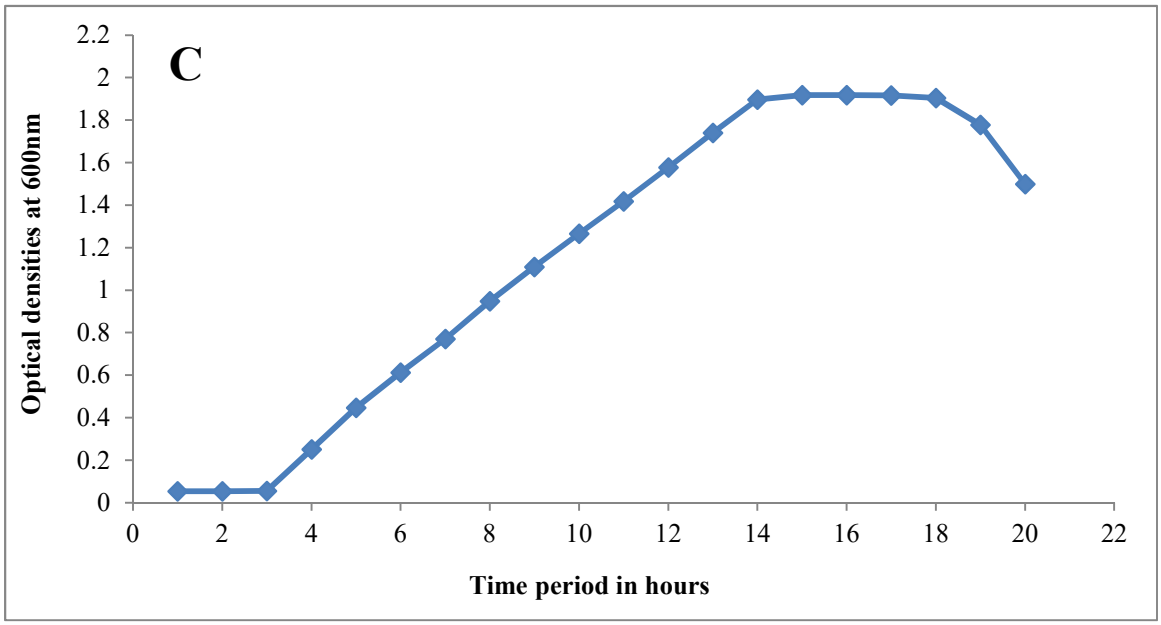
Figure 2: Effect of temperature (A) and pH (B) on the growth of Pb^{2+} resistant bacterial isolates

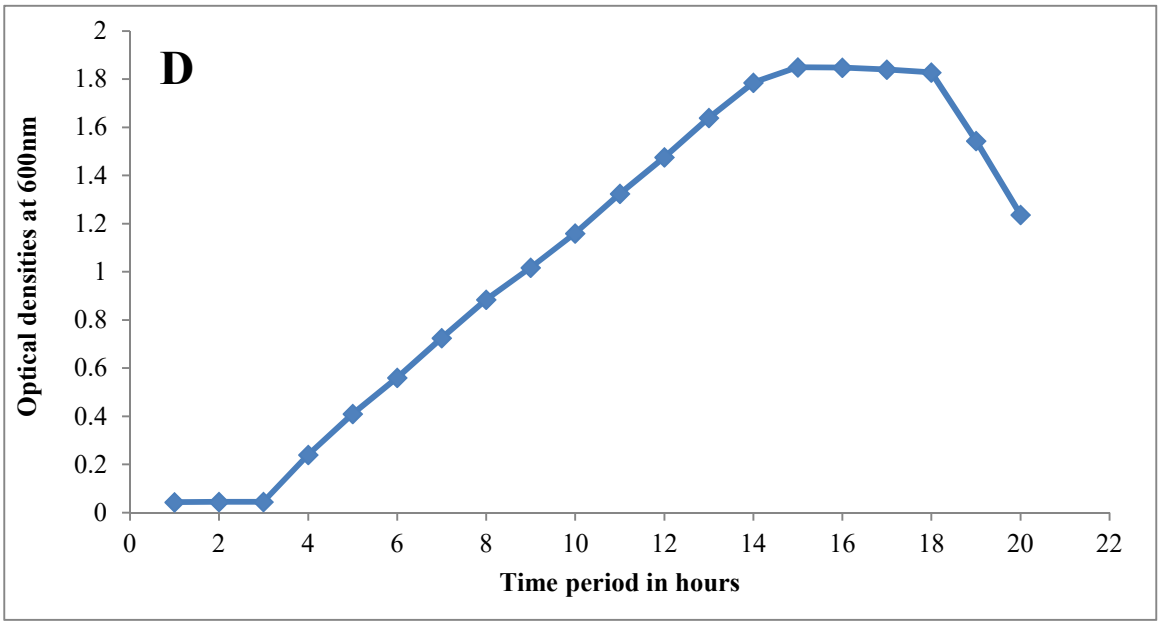
Determination of growth curve for Pb^{2+} resistant bacterial isolates

Growth curve was observed for all bacterial isolates. It was observed that SLW4 remained in lag phase for about 2 and half hours and then continuous increase in optical density was observed up to 14 hours. After that no major change in readings was observed up to 17 hours, indicating stationary phase and then rapid decline in optical density was observed, indicating death or decline phase. Bacterial isolate SLW5 shows 3 hours lag phase and then log phase was observed up to 13 hours. After that stationary phase was observed for 3 hours and after 16 hours incubation time period decline period had started. Bacterial isolates SLS1 and LTS7's lag phase duration was 2 hours and 11 hours log phase duration was observed. After 13 hours' time period stationary phase was observed that was last for 4 hours. After 17 hours' time period optical density values decreased that was the indication of decline phase. AN4's lag phase was 2 hours and log phase time duration was 10 hours. After 12 hours incubation period time stationary phase was observed that was last for 3 hours and after 16 hours' time period decline phase had been started (Figure 3 A-E).




B
Optical densities at 600nm
Time period in hours
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
2
4
6
8
10
12
14
16
18
20
22


C
Optical densities at 600nm
Time period in hours
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
0
2
4
6
8
10
12
14
16
18
20
22



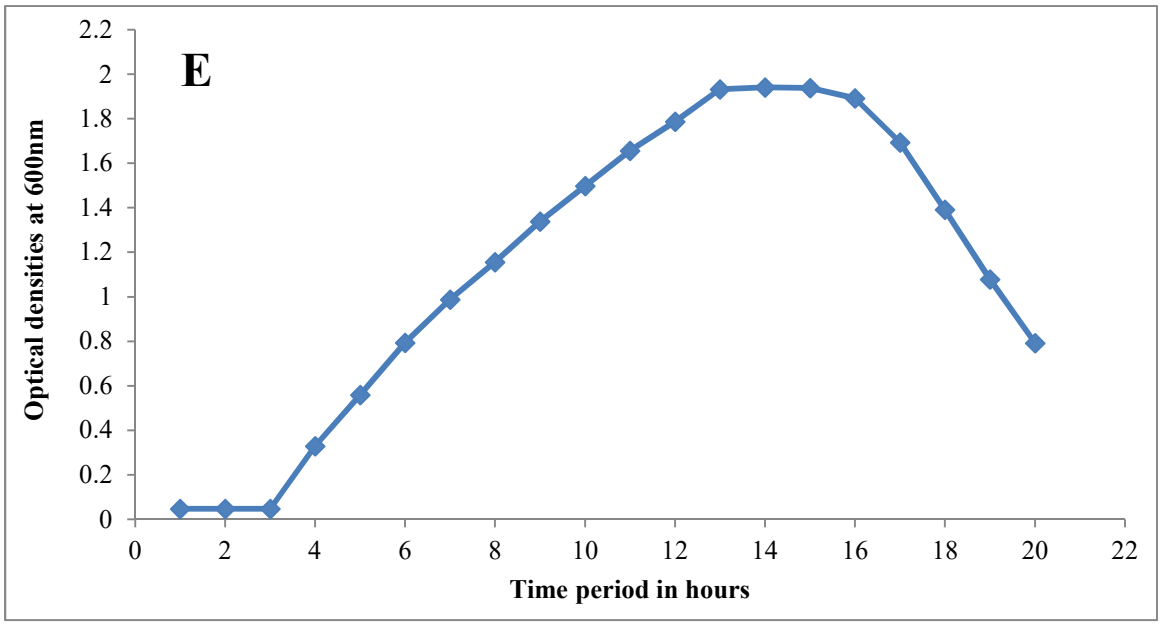


Figure 3: Growth curves for SLW4 (A), for SLW5 (B), for SLS1 (C), for LTS7 (D), and for AN4 (E)

Pb^{2+} biosorption by bacterial isolates

Pb^{2+} biosorption potential of bacterial isolates was determined in planktonic growth after 24 and 48 hours of incubation time through atomic absorption spectroscopy. The figure 9 given below shows that bacterial isolates AN4, SLS1, SLW5, LTS7 and SLW4 biosorbed 74.04 µg/ml, 73.38 µg/ml, 72.14 µg/ml, 73.07 µg/ml and 71.93 µg/ml Pb^{2+} from culture media respectively, after 24 hours in their planktonic mode of growth. The bacterial isolates AN4, SLS1, SLW5, LTS7 and SLW4 biosorbed 78.97 µg/ml, 79.91 µg/ml, 80.87 µg/ml, 78.85 µg/ml and 80.97 µg/ml Pb^{2+} from culture media respectively after 48 hours (Figure 4).

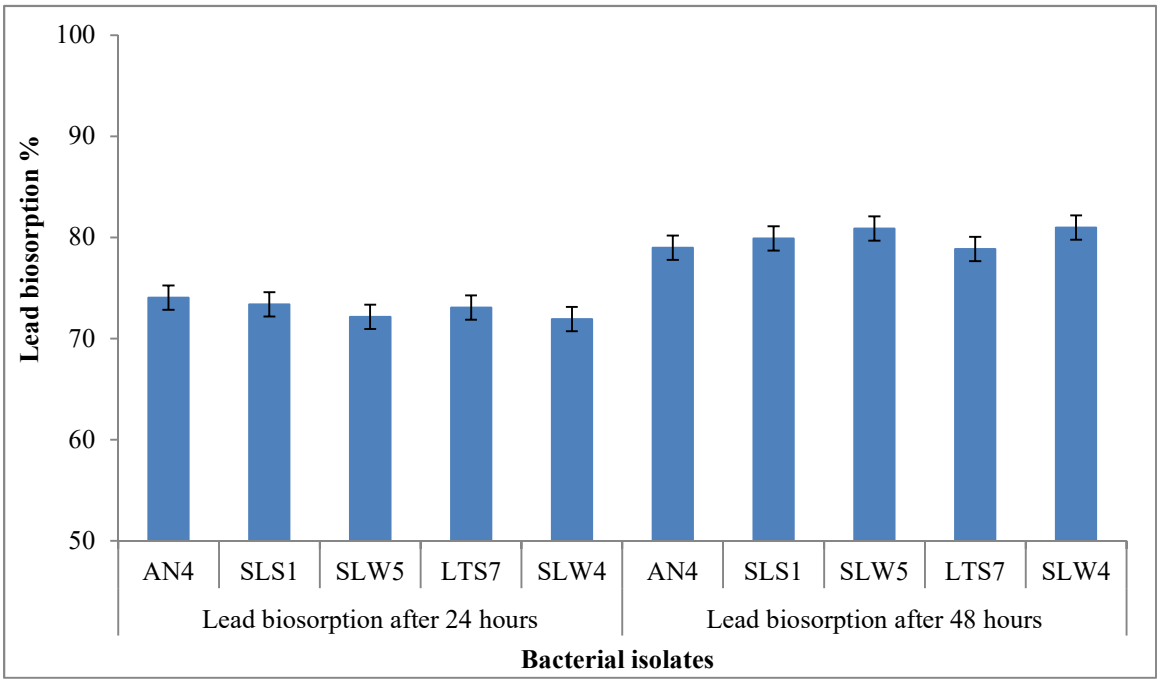


Figure 4: Pb^{2+} biosorption by bacterial isolates in planktonic mode after 24 hours and 48 hours

Identification of bacterial isolates on the basis of biochemical characterization

On the basis of biochemical characterization, it can be predicted that the bacterial isolate AN4 belonged to genus *Pseudomonas*, SLW4 and LTS7 belonged to genus *Klebsiella*, SLW5 belonged to genus *Yersenia* while SLS1 belonged to genus *Vibrio*.

Discussion

Heavy metals do not degrade by physical and chemical agents. These heavy metals not only affect the growth but also physiological activities of living organisms [15]. Among all these heavy metals, lead is one of the most dangerous heavy metals after arsenic because it cause serious health hazards even in very small quantity. Lead is a mutagenic metal that affects various organs as well as organ systems and cause cancer as lead is a possible human carcinogen [21]. There are several ways to eliminate lead but the best way to eliminate lead contamination is through bioremediation that is carried out by bacteria and fungi [12]. Bioremediation has been developed as a relatively conservative method and can demonstrate as a successful instrument to counter the deleterious effects of contamination and render the sullied sites less contaminated [22] and is also an environmentally useful and long-time period approach which could be a successful and efficient way of eliminating heavy metals from diverse parts of the surroundings.

This study has been carried out by isolating lead resistant bacteria from soil and industrial effluents collected from industrial areas of two different cities (Lahore and Sialkot) of Punjab, Pakistan. On the basis of physical appearance of colonies, 12 bacterial isolates were selected; 04 isolates (LTS7, SLS1, SLS2 and SLS4) from soil samples and 08 isolates (SIW2, SLW4, SLW5, SLW7, AN1, AN2, AN4 and AN5) from wastewater samples. Two bacterial isolates were gram positive (SLW7 and AN1) while 10 bacterial isolates were gram negative.

The bacterial isolates were subjected to determine the maximum tolerance limit of lead for further studies. Minimum inhibitory concentrations of Pb^{+2} for bacterial isolates have much similar values. The bacterial isolates, SLW4, SLW5, SLS1, LTS7 and AN4 showed the highest MIC. SLS1 showed 650 µg/ml MIC. MIC for SLW7 was 575 µg/ml and for AN1, the MIC was 525 µg/ml. Pandey *et al.,* (2018) noted 900 µg/ml MIC for lead by gram positive *Bacilli* [23] whereas according to Gupta *et al.,* (2024) 1000 µg/ml maximum tolerance of lead was experimented for *Bacillus subtilis* [24]. SLW5, SLW4 and LTS7

showed MIC equal to 625 µg/ml. According to Saleem *et al.,* (2015) maximum tolerance limit was 1600 µg/ml for Pb by predicted lead resistant bacteria *Enterococcus, Salmonella, Shigella, Bacillus, Enterobacter* and *Klebsiella* [6] and according to Amoozegar *et al.,* (2025) genus *Halomonas* showed MIC of 560 µg/ml [25]. Maximum tolerance limit of AN4 was 600 µg/ml whereas Jaafar (2020) experimented 1800 µg/ml MIC for lead by *Pseudomonas pentosaceus* [26]. Similarly Gummersheimer and Giblin (2003) studied four lead resistant bacteria; two belonged to genus *Corynebacteria* and two belonged to genus *pseudomonas*, had maximum tolerance limit 226 µg/ml [10]. Kafilzadeh *et al.,* (2012) investigated 1400 µg/ml maximum tolerance limit for *pseudomonas* sp., *corynebacterium* sp. and *Bacillus* sp. whereas 700 µg/ml for *staphylococcus* sp. and *E. coli* for lead. [27] noted 1000 mg/L MIC of *Staphylococcus* sp. for lead [27].

On the basis of maximum tolerance limit/MIC values, five bacterial isolates (SLW4, SLW5, SLS1, LTS7 and AN4) were selected for further studies to check the potential for lead biosorption by planktonic bacterial isolates. Then physiological and biochemical characteristics were determined. Physiologically all the isolates showed maximum growth at 37℃ and 7 pH. Biochemically the isolates were predicted to be the member of four genera of *Enterobacteriaceae* family. AN4 belong to genus *Pseudomonas,* SLW5 belong to genus *Yersenia* and SLS1 belong to genus *Vibrio* whereas two bacterial isolates, SLW4 and LTS7 belong to genus *Klebsiella*. Growth curves of these selected bacterial isolates were determined. SLW4 showed longest log phase period of 12 hours whereas SLW5 and AN4 showed minimum log phase period of about 10-11 hours. Similarly SLW5 took the longest period of 3 hours at lag phase to enter into log phase while all other isolates took just 2 hours in lag phase and then started rapid cell division.

Atomic absorption spectrophotometer technique was used to evaluate the biosorption of lead heavy metal. Lead biosorption potential of isolated bacteria was examined in indigenous growth at 24 hours and 48 hours of incubation time period using atomic absorption spectrophotometer. The bacterial isolates SLW4, LTS7, SLW5, SLS1 and AN4 biosorbed lead metal 71.93%, 73.07%, 72.14%, 73.38% and 74.04% respectively from cultural media in their indigenous mode of growth after 24 hours. The highest biosorption was done by AN4 isolate after that SLS1, which biosorbed 73.38% lead less than AN4 (74.04%) but more than other bacterial isolates. Similarly, LTS7 biosorbed more lead 73.07% as compare to SLW5 which biosorbed 72.14% lead. The least results of biosorption at 24 hours incubation time period was shown by SLW4 71.93% only. The isolated bacteria SLW4, LTS7, SLW5, SLS1 and AN4 removed 80.97%, 78.85%, 80.87%, 79.91% and 78.97% lead respectively after 48 hours. The highest value of biosorption 80.97% was noted by SLW4 at 48 hours incubation time period whereas least biosorption value 78.85% was noted by LTS7 isolate. SLW5 also biosorbed lead heavy metal at 48 hours incubation time period very well 80.87% close to the highest value 80.97% shown by SLW4. SLW5 biosorption potential (72.14%; second least value) was good at 48 hours' time period as compare to 24 hours' time period (80.87%; second highest value) among these isolates. Similarly, SLS1 biosorbed more lead 79.91% as compare to AN4 78.97%. AN4 although biosorbed more lead as compare to LTS7 but the difference in biosorption potential was much close 78.97% and 78.85% respectively between these two bacterial isolates after 48 hours incubation time period. AN4, which showed the maximum value at 24 hours' time period, after 48 hours incubation time period was near to the least biosorption value. SLW4 showed much better results at 48 hours incubation time period as compare after 24 hours' time period among all these bacterial isolates. However, the results were showing that all these isolated bacteria had biosorbed more lead at 48 hours than at 24 hours. It was also noted that biosorption of lead increased with the passage of time with these bacterial isolates.

Some other researchers also have done work on bioremediation of lead *i.e.,* Pandey *et al.,* (2018) investigated the accumulation of lead using atomic absorption spectrophotometry at 12 hours, 18 hours, 24 hours and 48 hours and resulted that the maximum efficiency was 72.3% after 24 hours [23]. According to Saleem *et al.,* (2015) absorption of lead was 87.2% with Pb Fa-458 (*Bacillus*) after 24 hours [6] whereas, result for lead removal by *Staphylococcus* sp. was 83% at 48 hours [28]. Karn *et al.,* (2018) studied *Aspergillus* sp., a lead resistant fungi, noted that the specie could transform the lead 68.12% and 76.52% at 24 hours and 48 hours incubation time period

respectively [29]. According to an experiment the lead precipitation by microbes at various ranges from 80 ppm to 1000 ppm [4]. Their study showed that after 11 days incubation in 500 ppm lead concentration media, 99% lead had been removed and similarly 87% lead removal had been occurred in media supplemented with 1000 ppm lead after 22 days. A group of researchers isolated four lead resistant, gram negative bacterial strains (A, B, C and D); two strains were found *Bacillus* sp. and two were *cocci* [30]. They could remediate the lead up to 64.4% in seven days. Moreover, these planktonic bacteria might biosorbed Pb^{2+} concentration ranging from 7475 ppm to 3488 ppm after seven days. Strain A reduced lead 55.8% (bioremediated lead concentration from 6908 to 3854 mg/L), Strain B remediated 64.46% lead (lead concentration reduced from 7333 to 4727 mg/L), strain C remediated 56.9% lead (lead concentration reduced from 6690 to 3809 mg/L) and strain D remedited 54.6% lead (lead concentration reduced from 6380 to 3488 mg/L). Strain B represented the highest reduction 64.46% of lead heavy metal. According to researchers result for lead removal by *Bacillus subtilis* strain MTCC2423 at 1000 ppm was 99.99% in 6 days [24] and according to another group of researchers *Bacillus cereus* could bio-accumulated the lead 54.54% [3]. The results of [26] for lead heavy metal using *E. coli, Staphylococcus* sp., *Corynebacterium* sp., *pseudomonas* sp. and *Bacillus* sp. were 60.35%, 64.82%, 86.64%, 87.97% and 89.66% respectively. Similarly a research was done under anaerobic conditions in which more than 90% lead was being reduced in 7 days, using local consortium of bacteria [31]. In the study, lead biosorption was 91.3% at 1 hour by genus *Halomonas* [25]. A group reported 100% lead removal with *Pararhodobacter* sp., *Rhodobacter spharoides* removed 90.31% and *Terrabacter tumescens* removed 100% lead [32]. Similarly *Enterobacter cloacae* removed 68.1% lead and lead bioremediation *by Sporosarcina pasteurii* was 100% [33]. A group also studied biosorption of lead using *Bacillus licheniformis* and estimated lead biosorption through atomic absorption spectrophotometer [34]. They noted that reduction of lead increased from 74.94% to 89.39% with the elevation of lead concentration. Lead bioremediation result by *Pseudomonas pentosaceus* was 62.10% to 68.39% with lead concentration in media from 25 to 50 ppm respectively [26].

Conclusion

Lead is a potent environmental toxin causing deleterious effect on health of living beings, producing acute and chronic diseases. Bioremediation is an ecofriendly and cost effective tool to counter lead contamination. The results of atomic absorption spectrophotometer for lead removal from the culture media by the selected bacterial isolates showed an efficiency of 71.93% to 80.97% in planktonic mode of growth. This is a clear indication of microbes' usefulness in bioremediation process. So these isolated bacteria, AN4 (*Pseudomonas*), SLS1 (*Vibrio*), SLW5 (*Yersinia*), LTS7 and SLW4 (Both belong to genus *Klebsiella*) can be used as a useful tool for the removal of lead from contaminated sites.

Future Perspectives / Recommendations

In future this research can be expanded to the pilot scale and in situ bioremediation technology. Moreover, the molecular level studies can add more significance to this research.

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Author Contribution

Adnan Shoukat designed and performed the experimental work and also prepared the manuscript draft, Muhammad Arslan Azhar helped in the preparation of this manuscript and supervised the overall project.

Conflict of Interest

Authors declare no conflict of interest.

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