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Removal of Some Heavy Metals from Industrial Wastewater by Actinomycetes Isolated From Contaminated Soil

Fatima Mohammed Bakran, Magda Mohammed Aly,
Nidal Mohammed Omar Zabermawi
Biology Department, Faculty of Science, King Abdulaziz University Jeddah, Saudi Arabia,

Abstract: *This study was aimed to examine the ability of bacteria to removeheavy metals. Out of 23 isolates, recovered from different contaminated soil area from industrial wastewater Treatment plant (IWWTP), located in Jeddah, strain FM1 and FM2 was the most potent in removing of heavy metals. The 16S rRNA sequence of metal tolerant bacterial isolate FM1 showed the highest (98%) similarity with Streptomyces toxytricini and the second isolate FM2 was showed the highest (98%) similarity with Streptomyces sp. The growth of the previous isolate was determined after 5 days at 30 ºCin the presence of different heavy metals concentrations, 3000- 5500 mg/l of lead acetate. The most resistant isolate for lead was isolate FM1 (MIC 5200 mg/l). Moreover, the efficiency of biosorption in liquid medium was tested by the metal ion concentration analyzed using Plasma Atomic Emission Spectrometer ICPE-9000. The adsorption of heavy metal ions on the adsorbent could be influenced by several operating conditions, such as, initial metal ion concentration, solution pH, contact time, adsorbent dose, temperature and on the ionic strength of the aqueous solution. For the tested isolate, the optimum pH for lead removal was at pH 6-8. On the other hand, the optimum temperature for lead removal was 28ºC. Biosorbent mass of 0-1.5 g/l was tested for removal of lead. The result showed that the adsorption capacities against heavy metals, Pb^{++} was increased with increasing the weight of the used dry biomass. The result showed that FM1 and FM2 biomass has a good potential to be used in removal of metal ions. Their use in real life situation can alleviate pollution and increase the quality of water for human consumption and sanitary purposes.*

Keywords*: Biosorption, Maximum Tolerance Concentration, Biomass, Streptomyces, Plasma Atomic Emission Spectrometer, Biosorption, Dead, Industrial Wastewater Treatment Plant*

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I. Introduction

Pollution is one of the world's greatest challenge, because the global increase in human development is rapidly changing our world (DeFries *et al*., 2004) . Mixed pollution resulting from organic and inorganic compounds were founded to be in high concentration in many areas particularly industrial zones, oil storage areas, waste recycling sites and sediments near roads, for instances, several heavy metals were founded in the water of rivers (Polti et al., 2007, Mansour, 2012). The most common widespread pollutants are heavy metals which are of great concern as they are non – biodegradable and thus persist in the nature (Liu et al., 2005).

Heavy metals toxicity depended on the absorb dose, the rout of exposure and duration of exposure, for example, from drinking water polluted with lead, acute exposure can result in loss of appetite, hypertension, renal dysfunction, fatigue, arthritis and hallucination. Chronic exposure to lead can result in mental retardation,birth defects, psychosis, autism, allergies, weight loss, hyperactivity,paralysis, muscular weakness, and may even cause death(Martin and Griswold, 2009).

Conventional solutions to the problem of heavy metals pollution are many different processes that have been used for theremoval of toxic heavy metals. They include oxidation and reduction,chemical precipitation, filtration, evaporation, ion-exchangeand reverse osmosis (Kikuchi and Tanaka, 2012). Many of them,however, may not be very effective or are too expensive (Krishnani et al., 2008). Resent techniques, like using microorganisms to study their natural mechanism which help in removal of heavy metals from harmful effluent has been explored, and the most famous known technique is bioremediation. Bioremediation is the use of living microorganisms for removal of pollutant from the biosphere including soil and water or wastewater. (Samal and Kotiyal, 2013, EPA, 2016). Bioremediation is an effective process to reduce environmental pollution due to heavy metals(del Carmen Vargas-Garcia et al., 2012).The bioremediation techniques represent the most effective technique and superior to conventional techniques because it is lesser economic cost,cause no environmental pollution and more effective to the removal of heavy metals and other pollutants.

This study aimed to demonstrate bacterial bioremediation ability in removing heavy metal such as lead.

II. Materials and Methods

Collection of Soil Samples and Isolation Heavy Metal Resistance Bacterial:

For the present investigation, soil samples (total 14) were collected from different contaminated soil area in Industrial Wastewater Treatment Plant (WWTP) located in Jeddah. Each sample was collected in sterile test tubes and stored at 4°C until used for isolation of actinomycetes.Collected samples were serially diluted and inoculated onto one culturing medium (Shirling and Gottlieb, 1966), Starch nitrate agar medium (20.0 g soluble starch, 1.0g K_2HPO_4, 0.5g $MgSO_4.7H_2O$, 0.5g NaCl, 3.00g $CaCO_3$, 0.01g $FeSO_4.7H_2O$, 2.0 g KNO_3, 1.0 ml trace salt solution, 20 g agar) prepared with 1 L distilled water. Then, about one gram of each soil samples was suspended in 9 ml of sterilized distilled water and serial dilution was done to obtain dilutions up to 10^{-7}. One ml suspension of each 10^{-5} to 10^{-7} dilutions, were separately added to the surface of a platecontaining starch nitrate agar medium and incubated at 30°C for 7 days. The obtained bacteria colonies were transferred to new plates until obtained of pure colonies which purified by streaking on soiled agar medium.The selected bacterial colonies were inoculated on Starch nitrate agar medium by streaking plate at 30℃ for 7 days. Until pure cultures were obtained, then transferred to slants Starch nitrate agar and preserved directly to a refrigerator at 4℃. For long preservation (more than six months) strains were kept in starch broth plus 50% sterile glycerol and stored at -20℃ until used (Ramesh and Mathivanan, 2009, Kumar and Kannabiran, 2010).

Preparation of metal solutions

The heavy metals salt solutions were prepared from analytical-grade chemicals such as lead acetate $Pb(CH_3COO)_23H_2O$,sterilized separately for 15 min at 110°C (Saurav and Kannabiran, 2009) and preserved at 4℃ until used. The concentrations of each metal ion were prepared from stock solutions of 100,000 mg/l. Fresh dilutions were used for each study. The pH of each test solution was adjusted to the required value by using 1 M NaOH and 1N HCl (Latha et al., 2015).

Screening test for heavy metals resistance bacteria (Primary qualitative assay)

The actinobacteria isolate FM1 and FM2 were screened for heavy metal resistant activity in starch nitrate agar medium, after preparing of the medium it was sterilized by autoclaving at 121°C for 15 min, for lead acetate, 4000 mg/l was added. The bacterial isolate was screened on medium containing the tested concentration of Pb^{++}. The growth (+++, ++, +, -) were determined after 5 days. Moreover, the most resistance isolated were screened on medium contain different concentration of lead acetate (Koushalshahi et al., 2012, Daboor et al., 2014). This method was used to determine the minimum inhibitory concentration (MIC) for each heavy metal. This method was used to give a rapid screening, but it is a qualitative estimation (Abbas and Edwards, 1989).

Identification of the selected bacterial isolates FM1 and FM2

Morphological, physiological and biochemical characterization of the bacterial isolate FM1and FM2

Strain was preliminarily identified according to traditional morphological criteria, including morphology and growth pattern onstarch nitrate agar for example.Characteristics of the bacterial colonies on the agar plate, morphology of substrate and aerial hyphae, the morphology of spores, color of the produced pigment were carried out(Goodfellow and Haynes, 1984). Also, the selected bacterial isolate FM1and FM2 were cultivated on different media plates for example; Yeast extract -malt extract agar (ISP-2), In-organic salts-starch iron agar (ISP-4), and Tyrosine agar (ISP-7) plates and identified according to morphology, physiology and biochemical characters. The cellular morphology of the bacterial isolates FM1 and FM2were examined under light microscope and scanning electron microscope (Lalucat *et al.,* 2006). Moreover, biochemical characterization of the isolates was carried out as described in International actinomycetes isolates Project including biochemical identification tests such as catalase, citrate oxidase, indole production(Pridham and Lyons Jr, 1961).

Molecular Identification

Genomic DNA from each isolates were obtained using the QIAamp DNA Mini Kit (Weisburg *et al.,* 1991).Then, the concentration and purity of DNA were determined.Princess Al-Jawhara Center of Excellence in Research of Hereditary Disorders, the 16S rDNA gene was amplified by PCR using the forward primer 5'-AGTTTGATCATGGTCAG-3' and the reverse primer 5'-GGTTACCTTGTTACGACT-3'. The DNA sequence was compared to the GenBank database at the National Center for Biotechnology Information (NCBI) using the BLAST program(Saitou and Nei, 1987).

Determination of antibiotic sensitivity and resistance pattern of the selected isolates:

Antibiotic sensitivity and resistance of the bacterial isolates FM1 and FM2 have been tested on Mueller Hinton agar plates using the ten following antibiotics: Amikacin(30 µg/ml), Ceftazidime(30 µg/ml), Aztreonam (30 µg/ml), Piperacillin (100 µg/ml), Imipenem (10 µg/ml), Ciprofloxacin (5 µg/ml). The inhibition zone has

been recorded after 5 days of incubation at 30°C. After incubation, the antibiotic inhibition zone diameters (IZD) were measured in mm. The strains were classified as being resistant, intermediate resistant or susceptible to an antibiotic.
Sensitive (S)≥ 21mm, Intermediate (I): 16-20 mm) and resistant (R) ≤ 15mm (Thokchom and Joshi, 2012).

Biomass preparation of actinobacteria for biosorbent process

The biomass of actinobacteria (biosorbent) was prepared by (Latha et al., 2015). The tested isolates FM1 and FM2 were cultivated in 500 ml flasks containing 100 ml of starch nitrite broth medium and was kept shaking in an orbital rotary shaker at 130 rpmfor 10 days at 30℃. Then, the culture was harvested by centrifugation at 4500 rpm for 15 min and was washed three times with distilled water. The pellet was kept in glass petri dishes and dried at 70℃ for 24 h. After the biomass was dried, it wascrushed in a blender to powder. Then, it was used for further studies.

Biosorption of Lead Acetate Pb^{++} at different metals concentrations

The lead removal ability of the actinobacterial isolates FM1 and FM2 were determined by measuring the level of lead uptake following the method of Saurav and Kannabiran (2011) with slight modifications. In the stopper conical flasks (100 ml), 25 mL of Pb^{++} metal salt solution of lead acetate with different concentrations (3000-5500 mg/l) was added and 0.25 g of the dried biomass of the tested actinobacteria was resuspended and the pH was adjusted to 7.0. Then the flasks were kept shaking in an orbital rotary shaker at 130 rpm for one week. The contents of the flasks were centrifugation, then filtered through Whatman no.1 filter paper and the filtrates were analyzed for lead concentration by Plasma Atomic Emission Spectrometer ICPE-9000. The metal removal efficiency (MRE) was calculated by using the following equation:

$$\% \text{ of MRE or \% Biosorption} = C_i - C_f / C_i \times 100$$

Where MRE is metal removal efficiency, C_i represents the initial lead metal ion and $C_f$represents the final lead metal ion concentration.

Biosorption of Lead Acetate Pb^{++} with different biomass of the tested bacteria

Biosorption experiments were carried out with different concentrations (0.5, 1.0, 2.0, 2.5, 3.0 g/l of biomass obtained from strain FM1 and FM2 for removal of Pb^{++}, at pH 7. The Stoppard conical flasks (100 ml), containing 25 ml of metal salt solution (800 ppm of Pb^{++}) at pH 7, with appropriate biomass concentration, were kept shaking at 130 rpm for one week. The contents of the flask were filtered through filter paper and the filtrate was analyzed for metal concentration by Plasma Atomic Emission Spectrometer ICPE-9000. Percentage metal removal and specific metal uptake (Q) were calculated (Volesky and May-Phillips, 1995).

$$\text{Percentage metal removal or \% Biosorption} = C_i - C_f / C_i \times 100$$

$$\text{Specific metal uptake (mg metal/biomass weight): } Q = [V \times (C_i - C_f)] \div [1000 \times M]$$

Where: Q = specific metal uptake (mg metal/g biosorbent), V = volume of metal solution (ml), C_i = initial concentration of metal in solution (mg/l), C_f = final concentration of metal in solution (mg/l), M = mass of biosorbent

Effect of pH on heavy metal removal

The dry biomass (0.25 g) of eachActinomycete isolates FM1 and FM2were inoculated into a series of 100 ml conical flasks containing either 25 ml of distilled water with 800 mg /l of lead acetate. The pH was varied from 4 to 10 (4, 6, 7, 8 and 10). The pH of the medium was adjusted using dilute HCl or NaOH. Then, all flaks were incubated at 30℃ and 130 rpm for 5 days.The percentage biosorption of metal ions was calculated at the all tested pH values.

Effect of temperature on heavy metal removal by dry biomass

To detect the optimum temperature, the represented dry biomass of the tested bacteriawas incubated at different temperatures (24, 28, 30, 35 and 45◦C). The cultures were shaken in a rotary shaker (130 rpm) at different temperatures. After 5 days of incubation, heavy metal removal and biomass were measured. Based upon the heavy metal removal by dry biomass the optimaltemperature was determined.

Concentration of heavy metals residuals in the bacterial biomass

To detect the residual of heavy metals found in the biomass of the isolates which they involved in bioremediation technique, the biomass fragments were collected after centrifugation at 4500 rpm in 15 minutes. then the sample pallet was collected and oven-dried at 70◦C about 24 h. After that, each sample (0.5 g) were then digested through addition of 5ml of hydrochloric (HCl) acid and 5 ml sulphuric acid (H_2SO_4). After

heating gently until the sample was digested (formation of a clear solution above the residue), the volume was adjusted to 10ml with distilled water, pH was adjusted to 7 with 5 M NaOH and the solutions were analyzed for metal concentrationPb^{++}using Plasma Atomic Emission Spectrometer (ICPE-9000).

Statistical Analysis

All values are the mean of three reading and were expressed as mean ± slandered division (SD).Statistical analysis was performed for each two samples using t-test as described in statistical programing and $P \leq 0.05$ was considered significant.

III. Results

Isolation and purification of the actinomycetes isolates

In this study, from contaminated soil samples, twenty-three bacterial isolates were purified and maintained on starch nitrate medium. All isolates showed good growth and different colors e.g. gray, yellow, black, green and brownon starch nitrate medium.

Screening for metal tolerance isolate

In this experiment, screening for metal tolerance isolates was carried out by adding different concentration of lead acetate in solid medium and selected thebest tolerance microbe to lead acetate on solid medium.So, two bacterial isolates were selected out of 23 bacterial isolates, named FM1and FM2, gave heavy growth at 4000 ppm of Pb^{++}.

Minimum inhibitory concentration (MIC) of Lead

In this study, the actinomycetes isolates from soil were explored for their bioremediation capabilities to provethat they have potential in bringing down the intensity of heavy metals in media. The inhibitory effects of Pb^{++} on bacterial growth were investigated on starch nitrate agar medium. The MIC of Pb^{+} for FM1 and FM2 were 5200, 5000mg/l, respectively. The growth of selected microorganism decreased with increasing the concentration of lead metal, while high growth was observed in the control (bacterial isolate inoculation without heavy metal). In fact, longer lag phases were observed as the concentrations of heavy metal was increasedin the medium.The results showed that the isolates FM1 was resistant to Pb^{++} (5200 mg/l) than isolate FM2 (5000 mg/l) as in Table1.

Table 1: Actinomycete isolate FM1 and FM2 tolerance to different lead concentrations.

Concentration of the tested heavy metal, pb++ (mg/l)												
Isolates	1000	1500	2000	2500	3000	3500	4000	4200	4500	5000	5200	5300
FM 1	+ + +	+++	+++	+ ++	+ ++	+ ++	+ ++	++	+	+	+/-	-
FM 2	+ + +	+++	+++	+++	+ ++	+ ++	+ ++	++	+	+	-	-

+++: high growth, ++: moderate growth, +: low growth, +/- very low growth -: no growth

Morphological and biochemical characterization of the resistant isolate

The actinobacteria with potential heavy metal biosorption ability were selected for identification. The morphological, culture and biochemical characteristics of the strains were investigated and recorded in the Table 2. The isolate was Gram positive, not acid fast, with non-motile cells and substrate and aerial mycelium were well developed. The color of the isolate, on starch nitrate was pink. The growth on solid mediumexamined under light microscope. The aerial mycelia were well developed and pear a long chain of spores. The spore had cylindrical shape and are arranged in long chains. The substrate mycelia were not divided, and it was well developed branched filament. Resistance to antibiotics was determined on Mueller Hinton agar plates. Results in table 3 showed the effect of six antibiotics on the examined bacteria.

Table 2. Morphological characteristics of the selected isolates

Characteristics	FM1	FM2
Gram stain	Gram-positive	Gram-positive
Colony size	Discrete	Discrete
Acid fast stain	Negative	Negative
Motility	Absent	Absent
Respiration	Aerobic or Facilitative Aerobic	Aerobic or Facilitative Aerobic
Substrate Mycelium	Branched	Branched
Spore chain	Long	Very long
Motile spores	Absent	Absent
Aerial mycelium	Present	Present

Sporangia	Absent	Absent
Optimum growth temperature	30 °C	35 °C
Optimum pH range	6.5 - 8.0	6.0 - 7.0
Melanin pigment	Positive	Positive
Catalase	Positive	Positive

Table 3. Inhibition zone of antibiotic susceptibility of the two bacterial strains:

Bacterial Strains	**Zone of Inhibition (mm)**					
	AK	**CAZ**	**ATM**	**PRL**	**IMI**	**CIP**
FM1	S (30.3)	R (0)	R (0)	I (15.3)	S (26)	I (20.3)
FM2	S (32.3)	R (0)	R (0)	R (0)	S (24.33)	I (20.41)

AK: Amikacin, CAZ:Ceftazidime, IMI: Imipenem, CIP: Ciprofloxacin, ATM: Aztreonam PRL: Piperacillin. Inhibition zone diameters were measured inclusive of the diameter of the discs. S: Sensitive (≥21mm); I: Intermediate (16-20 mm); R: Resistant (≤15).

Molecular Identification

The partial sequencing of 16S rRNA gene of the strain FM1 on both directions yielded nucleotide sequence length of 1248 base pairs. The BLAST search of 16S rDNA sequence of the strain showed the highest (98%) similarity with *Streptomyces toxytricini* strain ZZ446, *Streptomyces.* sp strain SD18, and *Streptomyces globosus*. The second strain shown the partial sequencing of 16S rRNA gene of the strain on both directions yielded 16S rDNA nucleotide sequence length with 1300 base pairs. The BLAST search of 16S rDNA sequence of the strain showed the highest (98%) similarity with *Streptomyces* sp. strain 64-LR13-2, *Streptomyces sp.* strain HBUM83834, and *Streptomyces sp. Strain ELO60 (*Figures 1, 2)

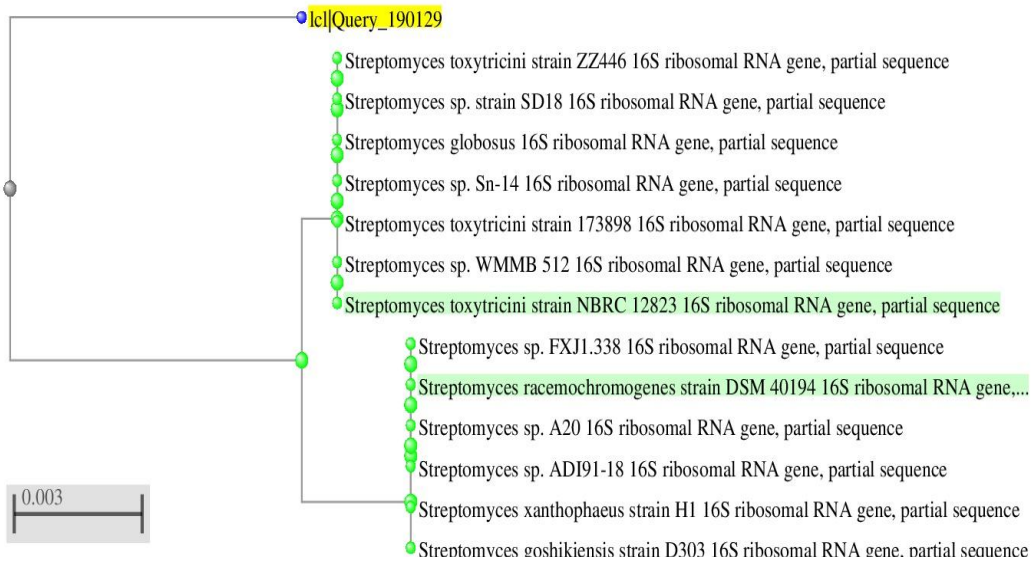

Figure1. Phylogenetic tree based on 16s rRNA for *Streptomyces toxytricini.* (FM1)

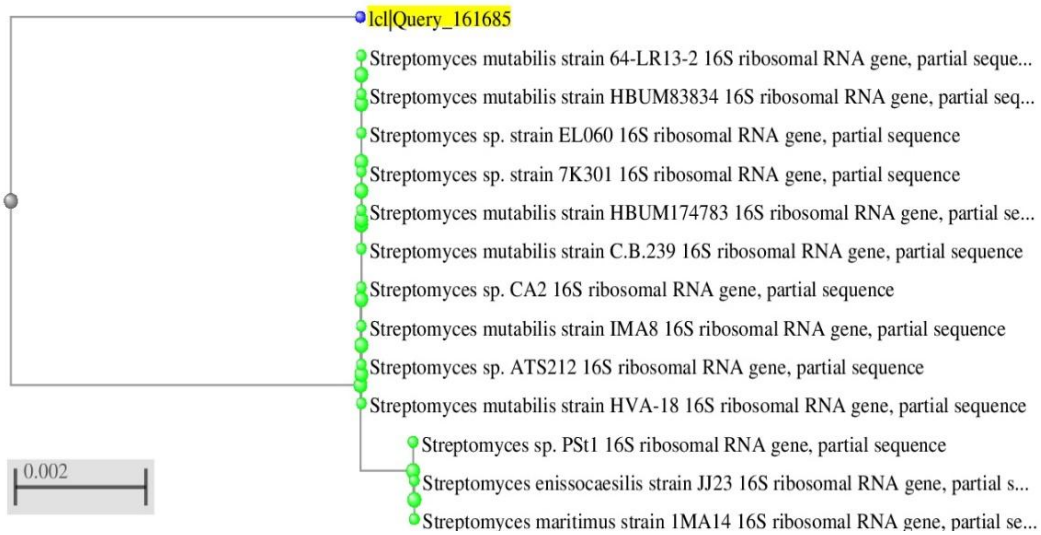

Figure 2. Phylogenetic tree based on 16s rRNA for *Streptomyces* sp. FM2

Adsorption of Heavy Metal $Pb^{++}$from Synthetic Metal Solutions by Dead Biomass of FM1 Isolate

The biomass of actinobacterial biosorbent material was prepared dried, crushed to powder and weighted (about 3 g of dead dry biomass was obtained from each 1000 ml of the starch nitrate broth medium). The dried biosorbent was kept at 4 ℃until used for further studies.

Biosorption of Lead Acetate Pb^{++} With Different Metal Concentrations

The percentage of adsorption was a function of the initial metal concentration. The amounts of metal uptake q (mg/g) by the dead biomass of FM1 at a different metal concentration $Pb^{+}$was shown in table 9. Increase lead metal concentration resulted in an increase in the capacity of metal adsorption from 99.06% to 99.81% with a maximum adsorption was 99.95% in 800 mg/lof lead.On the other hand, the amounts of metal uptake q (mg/g) by the dead biomass of FM2 isolate at a different lead increasecapacity of metal adsorption from 99.57% to 99.99% with a maximum adsorption was 99.99% in 600 and 1000 mg/l of lead (Figure 3).

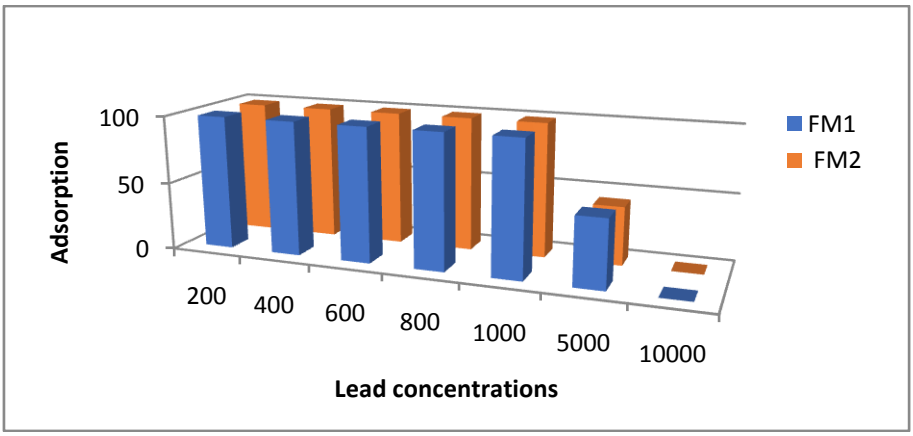

Figure 3. Capacity of lead adsorption by FM1 and FM2 isolates grown in different concentrations of lead.

Biosorption of Lead Acetate Pb^{++} with different biomass concentration.

The effect of biosorbent concentration on the sorption of $Pb^{++}$was tested and the results are shown in figure.4. The biosorption of $Pb^{++}$was strongly influenced by the biosorbent concentration. As the biosorbent weight of the isolate FM1 was increased from 0.12g to 1.50 g, the removal percentage increased from 98.09% to 99.70% for Pb^{++}. Moreover, increasing biosorbentweight of the isolate FM2 from 0.12g to 1.50 g, increased the removal percentage from 99.78%to 99.85% for Pb^{++}.

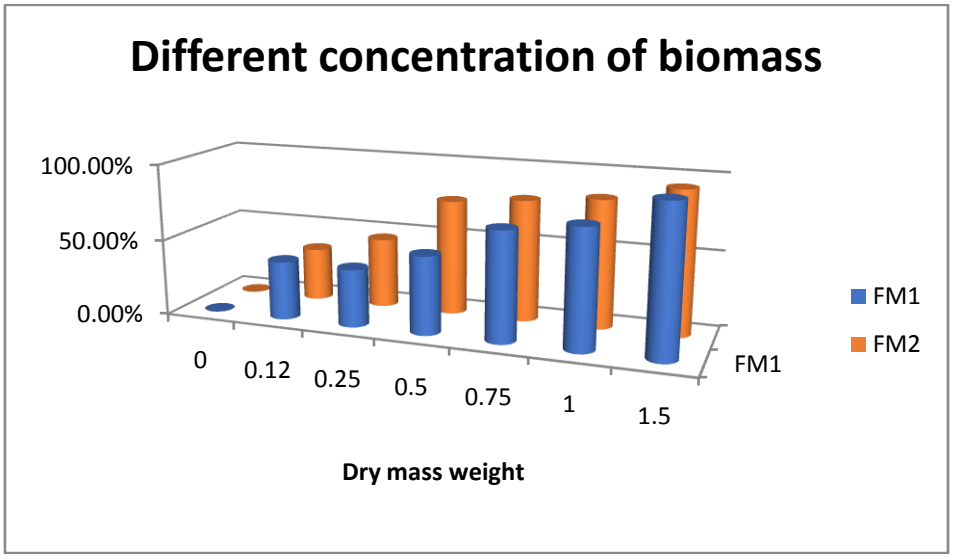

Figure 4: Biosorption of Lead Acetate with different biomass

Effect of different pH value on biosorption of Lead Acetate by bacterial dry mass

The result showed the effect of pH on the adsorption on different concentration of lead by dead biomass of the isolate FM1. Biosorption capacity was analyzed over a pH range from 4to 10. The highest biosorption rate of lead was 99.98% by isolate FM1 at pH 8. Also, the highest biosorption capacity of lead by isolate FM2 was 99.82% at pH8 (**Figure 5)**

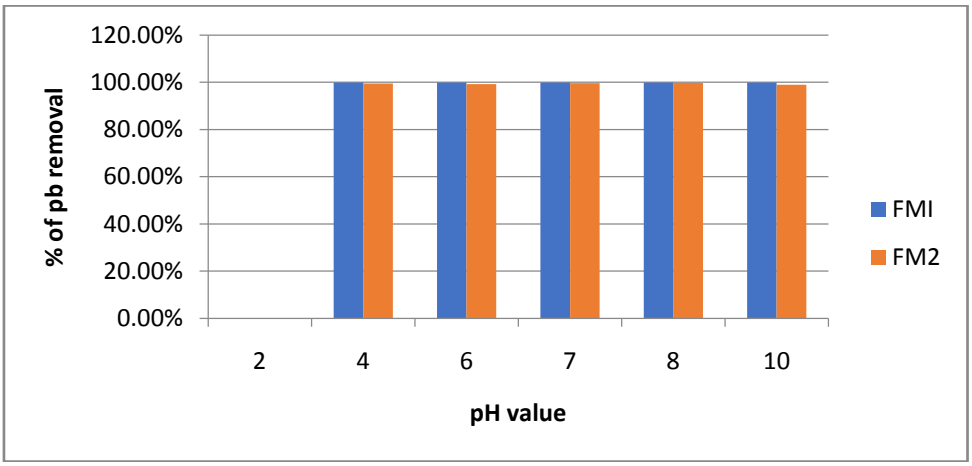

Figure 5: Biosorption of Lead Acetate at different pH values

Effect of Different Temperatures on biosorption of Lead Acetate by bacterial dry mass

The result showed the effect of different temperatureson the adsorption of lead by the dead biomass of the isolateFM1. Biosorption capacity was analyzed over a temperature range from 24℃ to 45℃. The highest biosorption rate of lead was 99.81% by isolate FM1at 37℃.One the other hand, the highest biosorption capacity of lead by isolate FM2 was 99.69 % at 45℃. Figure.6

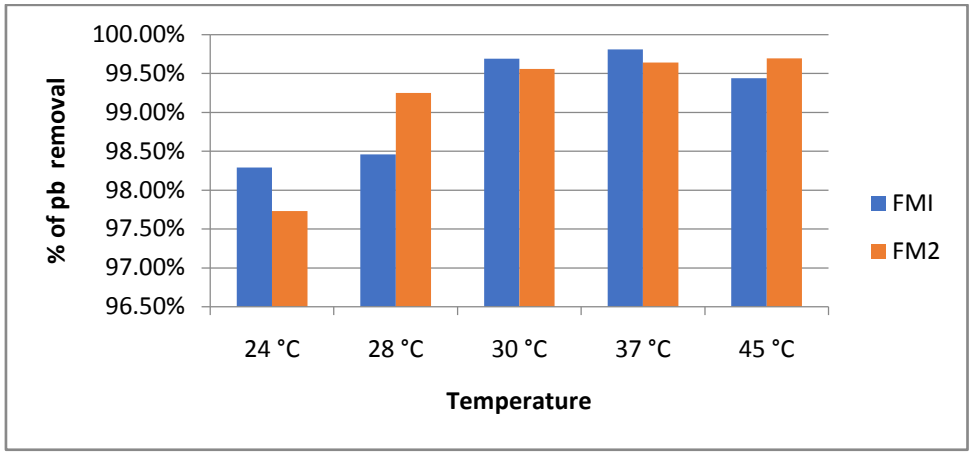

Figure 6: Biosorption of Lead Acetate at different temperature

Concentration of heavy metal residue in the bacterial biomass of isolate FM1

Total metal content is important because it determined the rate of the metals in the dead bacterial biomass, thus the potential of each biomass for metal uptake was determined. The bacterial biomass, used for removal of lead metal, was digested using different acid solution for example HCl, then analyzed for total metal (Pb^{++}). The result showed the highest lead uptake by isolate FM1 and FM2 about 228-299 mg/mg dry in solution with 3000-5000 mg/l of lead (Table 4).

Table 4. Concentration of Lead residuals within the bacterial isolate's biomass.

Lead (Pb) concentration, mg/l	Lead (Pb) concentration, mg/mg dry weight	
	FM1	FM2
3000	**297.70**	**228.9**
3500	**298.98**	**235.36**
4000	**299.76**	**239.49**
4500	**299.84**	**297.00**
5000	**299.88**	**295.64**

IV. Discussion

Environmental pollution particularly from heavy metals and minerals in the wastewater is the most severe problem in the world. Due to extensive anthropogenic activities such as industrial operations particularly mining, agricultural processes and disposal of industrial waste materials; their concentration has increased to dangerous levels. Heavy metals in industrial effluent include copper, nickel, chromium, lead, zinc, arsenic, cadmium, selenium and uranium . However, these methods have several disadvantages such as high reagent requirement, unpredictable metal ion removal, generation of toxic sludge etc. Adsorption process being very simple, economical, effective and versatile has become the most preferred methods for removal of toxic contaminants from wastewater (Pawar and Bhosale, 2018).

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least five times greater than that of water. Because of their high degree of toxicity, metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to the US Environmental Protection Agency and the International Agency for Research on Cancer. thus, Unconventional methods like using microorganisms to study their natural mechanisms which help in reducing the toxicity of harmful effluents has been explored. Bioremediation is the "use of living organisms (primarily microorganisms) for removal of a pollutant from the biosphere"(Samal and Kotiyal, 2013). Bioremediation is an effective process to reduce environmental pollution due to heavy metals(Handa and Jadhav, 2017)

This study aimed to demonstrate the effect of bioremediation technique in the removal of heavy metals pollutants in industrial sewage water such as lead, these pollutants could reach to the underground water which causes harmful effects on human health, animals, and plants.

In the current work, thirty-two bacteria of the family actinomycetes were isolated from different contaminated soil area, Industrial Wastewater Treatment Plant (WWTP) located in Jeddah, Kingdom of Saudi Arabia. The different bacterial isolates were obtained, purified and maintained on starch nitrate agar medium at

30℃ and pH7. All isolated showed good growth and different colors. Similar isolates with different colors and shapes were isolated before (Williams and Cross, 1971, Kumar *et al.*, 2014).

All the obtained isolates were screened on medium with different concentration of lead metal. Two isolated were selected.according due to the specific adaptation strategies of some *Streptomyces*strains, they can cope with high heavy metal concentrations. Growth of bacteria in the presence of heavy metals was recorded before(Schmidt *et al.*, 2005, Hopwood, 2006). Melanin-like pigment produced by some *Streptomyces* during growth in nickel containing media was reported to bind to the heavy metal and the capacity of this pigment to form nickel complexes was determined (Kothe et al., 2010). Other researches support this study, actinorhodin related blue pigments produced by *Streptomyces coelicolor* can be used to form new complexes with heavy metals (Kothe *et al.*, 2005, Bystrykh *et al.*, 1996)that might be very convenient in the removal of heavy metals. It was mentioned that several morphological, physiological and reproductive characteristics coding on major genome of *Streptomyces* would allow its species to occupy extreme environments or contaminated environment (Kothe and Varma, 2012). Since these properties are shared by this species of the genus, it can be interpreted as an evidence for the inheritance of resistance to heavy metals.

In the current study, the ability of isolate to resistant different concentration of lead was recorded by measuring the minimum inhibitory concentration (MIC) at which no colony growth was recorded (Koushalshahi et al., 2012, Daboor et al., 2014). The result showed that MIC of $Pb^{++}$was 5200 mg/l for the isolate FM1. Similarly, another study showed two strains of *Bacillus sphaericus*, resistant to two concentrations of chromium 10 mg/l and 30 mg/l (Velásquez and Dussan, 2009). According to Van Nostrand *et al.*, (2007), four actinobacterial strains were isolated, among them two of them of the genus *Streptomyces* was able to grow on 85.2 mmol/l of nickel (Idris *et al.*, 2004).

In the current study, the selected isolates were identified by physicochemical tests, biochemistry and optical microscopy. The strain was found to belong to the genus *Streptomyces*. A similar study reported three strains showed the highest resistances against nickel and zinc belong to one species, *Streptomyces mirabilis* (Schmidt *et al.*, 2009). Morphological characteristics are very important to identification different *Streptomyces* strains by using microscopic examination. The selected isolates produced aerial mycelia and chains of conidia (Laidi *et al.*, 2006). The color of the aerial and substrate mycelia varied depending on the type of the media used (Agwa *et al.*, 2000). The 16S rRNA sequence of metal tolerant bacterial isolateFM1 showed the highest (98%) similarity with *Streptomyces toxytricini* and the second isolate FM2 wasshowed the highest (98%) similarity with *Streptomyces sp. FM2*. Many authors used the previous methods to identify bacteria. The 16S rDNA sequence was compared to the GenBank database at the NCBI using the BLAST program. According to the obtained results, UR10 was identified as *Streptomyces exfoliatus* UR10, which is a new uricase producer. Similarly, in India, 4 strains belonging to *Streptomyces exfoliatus* were isolated from mangrove ecosystems (Gupta *et al.*, 2009).

Many authors also reported the utilization of resistant bacteria in the removal of heavy metals. It has been reported that the biosorption capacity of metal-resistant strains were found to be higher than that of non-resistant strains (Wnorowski, 1991, Sundar *et al.*, 2011). The percentage of adsorption was a function of the initial metal concentration. The amounts of metal uptake q (mg/g) by the dead biomass of FM1 at a different metal concentration Pb^{+} are determined. The increase to lead metal concentration resulted in increase in the capacity of metal adsorption from 99.06% to 99.81% with a maximum adsorption was 99.95% at 800 mg/l of lead.

Amoroso et al. (1998) have reported that metal resistance and biosorption capability may be widespread among actinomycetes growing in contaminated environments. Richards *et al.* (2002)have studied the heavy-metal resistance patterns of *Frankia* strains.Bakhti *et al.* (2008)investigated the effect of metal ion concentration on adsorption of silver using dried activated *S. rimosus* biomass. Uptake of the metal increased from 12 to 63mg/gwith increasing silver concentration from 40 to 200mg/l. Chergui *et al.* (2007) reported that uptake of each of the metal, Copper; Zinc; Chromium by *S. rimosus* increased with increasing metal ion concentration. The metal ion removal was highly concentration dependent.Mameri *et al.* (1999) also investigated the effect of initial zinc metal concentration on Zn(II)sorption using activated *S. rimosus* biomass. They found that the biosorptive capacity increased to a maximum of 80 mg/g at a residual zinc ion concentration of 200 mg/l. The increase in the biosorbent's loading capacity as a function of metal ion concentration was believed to be due to a high driving force for mass transfer. In agreement with this, a more concentrated solution should display better adsorption performance(Sahmoune, 2018). Also, other study reported the biomasses of *Streptomyces* species were reported to be capable of biosorption of heavy metals (Mosbah and Sahmoune, 2013).

The biosorption of Pb^{++} was strongly influenced the biosorbent concentration. As the biosorbent of FM1 isolate concentration was increased from 0.12g to 1.50 g the removal percentage increased from 98.09% to 99.70% for Pb^{++}. An increase in the biomass concentration generally increases the amount of solute biosorbed, due to the increased surface area of the biosorbent, which in turn increases the number of binding sites. Conversely, the quantity of biosorbed solute per unit weight of biosorbent decrease with increasing biosorbent

dosage, which may be due to the complex interaction of several factors. An important factor at high sorbent dosages is that the available solute is insufficient to completely cover the available exchangeable sites on the biosorbent, usually resulting in low solute uptake (Vijayaraghavan and Yun, 2008). Selatnia et al. (2004) reported that the removal efficiency of lead by *S. rimosus* decreased from 90 to 30% with increasing adsorbent dose from 1 to 10g/l. In contrast, (Tassist *et al.,* 2010)obtained the opposite behavior on the removal efficiency of Aluminum by *S. rimosus.* Authors revealed that the removal efficiency of Aluminum by *S. rimosus* biomass increased from 45 to 98% with increasing adsorbent dose from 5 to 30g/l.

There are many reports on the use of dead and living cells in heavy metals' biosorption, but both biomasses show biosorptive capacity (Brady *et al.,* 1994). Dead biomass offers several advantages over living organisms because dead biomass is not affected by a high concentration of pollution. The results of many studies have indicated that dead biomass has a higher capacity and biosorption efficiency of heavy metals than living biomass (Marqués *et al.,* 1991).The efficiency of biosorption (E%) increased with the increasing biomass concentrations to certain levels as a result of the equilibrium limitations (Madacha *et al.,* 2006) or due to the biomass granules which are agglomerated (Selatnia et al., 2004). Al-Garni (2005) found that the biosorption efficiency of Pb^{++}increased from 10.4 to 47 % and from 11.2 to 49.9 % as biomass concentration increased from 1 to 10 g/l of *Citrobacter freundii* and *Klebsiella pneumoniae*, respectively. However, the biosorption capacity (Qmax) decreased from 50 to 22.9 mg g^{-1} and from 54 to 24 mg g^{-1} of Pb^{++} as the concentrations of biomass increased from 1 to 10 g /l. Abdel-Monem *et al.* (2010) found that the increase in biomass concentrations of *Bacillus subtilis*117W and *Burkholderia cepacia* complex 120S showed an increase in the total amount of Ni^{2++} removed.

The result in current study showed the effect of pH on the adsorption on different concentration of lead by selected isolates dead biomass. The effect of pH depends on other factors such as the concentration of metal ions, the physiological state of biomass, the type of bacteria (Gram-positive or -negative) and the type of heavy metal (Kaewchai and Prasertsan, 2002, Abdel-Monem *et al.,* 2010).Mahamadia and Torto (2007)found that the biosorption of nickel ions is apparently negligible at low pH values and increases with therise in pH.

This study showed the effect of different temperature range of on the adsorption on different concentration of lead by dead biomass of the selected isolates. Biosorption capacity was analyzed over a temperature range from 24℃ to 45℃. The highest biosorption rate of lead was 99.81% & 99.69 % for FM1 and FM2 isolates at 37℃, 45℃, respectively. Temperature seems to affect biosorption only to a lesser extent within the range from 20 to 40°C(Krim *et al.,* 2006). Physical damage to the biosorbent can be expected at higher temperatures. An increase in temperature has been found to reduce the biosorption capacity of the biomass *S. rimosus* (Krim *et al.,* 2006). The structures of cell walls play an important role in the adsorption process of metal ions. This may be due to the presence of positively charged cations of metal ions, which connect to negatively charged functional groups in capsules or polymers on the cell wall by means electrostatic reactions (Gourdon *et al.,* 1990). Gram-positive bacteria exhibit more advantages in the biosorption process compared with Gram-negative bacteria(Malik *et al.,* 1998) becauseGram-positivebacteria have thick peptidoglycan cell walls that make them potentially more suitable for biosorption (Wernérus *et al.,* 2002).

V. Conclusions

Soil samples from different contaminated soil area are rich with Actinomycete.Out of Thirty-two isolates resistant to one heavy metals, lead, one isolate showed high resistant and ability to grow in different concentration of lead. The isolates FM1 and FM2 were the maximum tolerance concentration (MTC) for Pb^{++} were 5200, 5000 mg/l, respectively. The bacteria isolates were belonging to genus *Streptomyces*. The dry biomass was used for heavy metal removal at different conditions. It was found pH, temperature, weight of the used cell biomass and time are factors affecting removal activities. The obtained bacterial isolate was used for removal of the tested heavy metal (Pb^{++}) from the collected waste water. Generally, removal of lead metal ions increased with increased adsorbent dose and contact time. However, favorable adsorption conditions may be different for different adsorbents and metal ions. If it is possible to develop such biosorbents from natural product, then these adsorbents may offer significant advantages over currently available commercially expensive activated carbons, and in addition contribute to an overall waste minimization strategy.

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