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# Biosorption of lead by *Pseudomonas spp* isolated from Petroleum soils, Khouzestan

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## ABSTRACT

The aim of this study is providing a solution for biological treatment which was began with separation resistant strains against lead. For this study, Khouzestan of Petroleum soils samples were collected under sterile conditions and after transporting to the laboratory, consecutive dilutions prepared in sterile normal saline up to $10^{-10}$ and cultured on LB agar containing 5mgl$^{-1}$lead nitrate by the use of spreading method. After 24h of incubation, resistant colonies separated. In order to screen, gram negative bacteria cultured on Mcconkey agar and by the help of biochemical tests, 10 *Pseudomonas* strains were isolated in the medium which contains 100mgl$^{-1}$ lead. The minimum inhibitory concentration in the medium containing 100-2100mgl$^{-1}$ of lead was used for separating the desired strains. The dominant strains were resistant against 2000mgl$^{-1}$ lead. Antimicrobial susceptibility testing was performed to assess the strain drug resistance. Then, LB broth containing 100mgl$^{-1}$lead and$1.5 * 10^8$/ml bacteria was used and after 24h of incubation, the sample was centrifuged and supernatant was used for atomic absorption spectroscopy analysis. The strain, *Mso$_2$*, was the best strain which eliminated lead from culture medium up to 50%.This strain can be a good candidate for detoxification of the environment.
**KEYWORDS**: Biosorption, Lead, *Pseudomonas Spp*, Petroleum Soils, Detoxification

## INTRODUCTION

Heavy metals forms a part of soil pollutions in oil areas [1]. Elements such as lead, not only isn't necessary for biological life, but also they are highly toxic [2].One of the basic questions about heavy metals is that they aren't metabolized inside the body. In fact, these elements after entering the body not repelled and accumulated in tissues such as fat, muscles, bones which causes a number of diseases and complications in the body. Toxic heavy metals in plants and animals and their entry in to the food chain highlight its danger and create many ecological effects [3, 4].Concerns about lead role in children's mental retardation caused reducing its use in the world [5].Reverse osmosis, electro dialysis, ultra filtration, ion exchange, chemical precipitation, physical absorption, phytoremediation, forming complex and bioabsorption are methods of removing these metals from their environment. Bioabsorption is a high efficiency and low cost technology for removing toxic metals from environment which is a reversible process and can also be useful in the extraction of metals from waste waters [6, 7, 8].This method can be done by the use of alive and killed cells and factors such aspH, ionic strength, temperature, time, biomass density, existing of organic ligands and presence of the other metals and organic compounds in the environment are effective on it [6, 9]. Algae, fungi and bacteria are as bioabsorbents and their cell walls contain functional groups such as amine, hydroxyl, carboxyl and sulfates which serve as the mental binding site. Among bacteria, *Pseudomonas* is efficient in heavy metal biological absorption [10, 11].The aim of this study was to separate and identify superior strains of *Pseudomonas* strains for removing lead from lead containing liquid.

## MATERIALS AND METHODS

### Sampling

Oil contaminated soils from five cite in Khouzestan (Maroun, Masjed Suleiman, Ahwaz, Zavirchari and Salie) was transferred under sterile conditions within 24hto laboratory in ice boxes.

### Isolation and purification of strains

To isolate resistance strain of lead in oily soils LB agar and Mcconkeyagar were used. At first 10gr of each soil samples adds to the Erlenmeyer flasks containing 100ml sterile normal saline and after homogenization, was used for preparation of dilution to$10^{-10}$.Then, 0.1ml of the appropriate dilution in LB agar containing 5mgl$^{-1}$metal was cultured by spreading method. After 24h of incubation at 37°C, colonies morphology was studied and then Mcconkey agar was used

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for screening of gram negative bacteria. Isolated strains were used for studying the shape and gram reaction. In order to identify the strains, catalase, oxidase, oxidation of carbohydrates, nitrate reduction and MRVP tests were used.

**Minimum inhibitory concentration test**

For this purpose, the bacterial strains were cultured on the medium which containing 100-2100mgl$^{-1}$ of lead and their resistances to lead were investigated.

**Gel diffusion test**

In order to determine the antibacterial susceptibility of strains the standard amount of bacteria was cultured on Mueller Hinton agar. Kanamycin (10µg), tetracycline(30µg), ampicillin(10µg), erythromycin(15µg) and chloramphenicol (30µg) discs were used in this test and zone diameter was measured after 24h.

**Determination of optimal conditions for growth**

The best conditions for bacterial growth in presence of metal with factors such as temperature, rate of shaking and pH were measured. In this stage, LB broth containing 100mgl$^{-1}$ lead used and after adding standard of bacterial suspension, were kept for 24h in shaking incubator at temperatures of 25, 30, 37 and 40°C, pH of 5, 6, 7, 8 and 9 and shaking rate of 100, 150 and 200rpm.Then the absorption of samples was measured at 600nm [2, 12].It is noted that all of the tests were repeated three times.

**Metal removal test**

For this purpose, turbidity equivalent to 0.5 McFarland standards of bacteria, added to LB broth containing 100mgl$^{-1}$ lead and after 24h of incubation under optimal conditions, the samples were centrifuged in 20′, 4000rpm and supernatant was used for atomic absorption spectroscopy analysis.

## RESULTS

Totally, in the first, 24 strains of bacteria on the basis of colonies shapes were separated and isolated 10 strains by growing on Mcconkey agar. On the basis of gram staining and biochemical tests showed that, these strains are *Pseudomonas* (Table1).

**Table1.**Biochemical reactions of strains

| Strain | Catalase | Oxidase | TSI | VP | MR | Nitrate reduction | Gram staining |
|---|---|---|---|---|---|---|---|
| **All of them** | + | + | R/R | - | - | + | - |

Ten isolated *Pseudomonas* which are resistant against lead, were studied and $Mso_2$was the best resistant strain and grew up to 2000mgl$^{-1}$ of lead.*Mso*$_2$in terms of antibiotic sensitivity test showed that it is resistance against chloramphenicol and erythromycin and sensitive to tetracycline, ampicillin and kanamycin (Figure1).

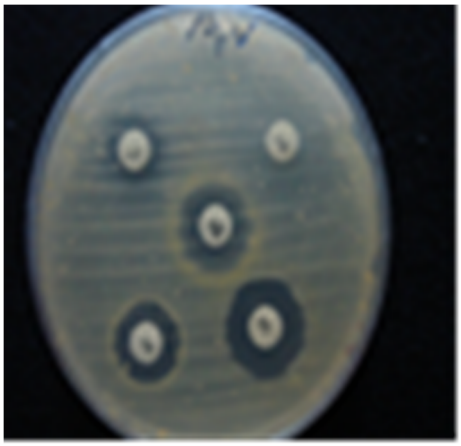

**Figure1.** Antibiotic susceptibility testing *Mso*$_2$

The best conditions of growth for $Mso_2$ was 6, 37°C and 200rpm in the presence of 100mgl$^{-1}$lead in a total of five levels of acidity, four levels of temperature and three levels of shaking rate respectively. Elimination of lead by *Mso*$_2$ against a control has been shown in figure2. This strain, showed 50% of lead elimination in the aqueous solution under optimal conditions.

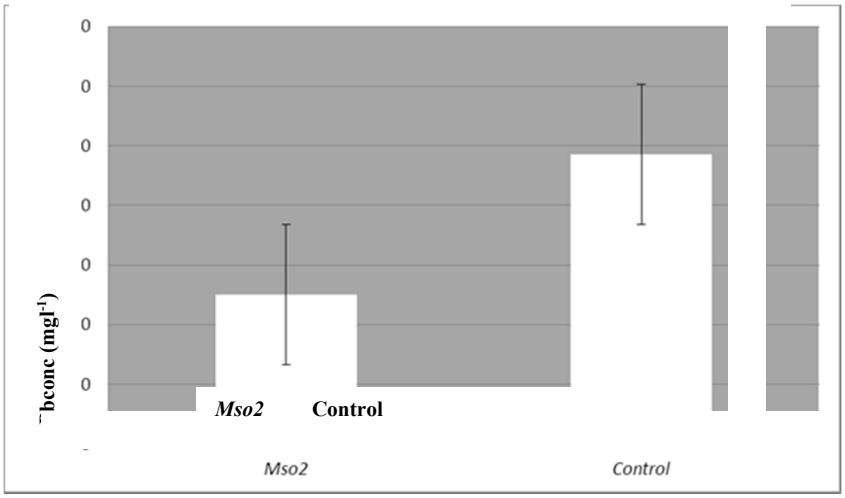


**Figure2.** The lead content in supernatant of culture,$Mso_2$ compared with control

To check the results, an independent sample T test (Table2) was carried out which was significant at $P<0.05$. The t value indicates that the second group (control)has removed lead from the aqueous solution much less than the first group ($Mso_2$).

**Table2.** Statistical Independent sample T test to study of confidence between two groups (control strain $Mso_2$)

| T test for equality of means | | | | |
|---|---|---|---|---|
| ***P*** | **df** | **t** | | |
| 0.000 | 4 | -57.563 | Equal variances assumed | Lead |
| 0.000 | 4.000 | -57.563 | Equal variances not assumed | |

## DISCUSSION

Biosorption of heavy metals is one of the best new technologies for removing the toxic metals from wastewaters. Pérez and Albus studied on the absorption of chromium, copper, manganese and zinc with concentrations of 50, 49, 60 and 70mgl$^{-1}$respectively, by isolated *Pseudomonasaeruginosa* AT18 from oil sites. In this study, pH of solution and ionic strength were important factors in metal removal and absorption capacity of this strain. According to the results, this bacterium exists in oil soils that have capacity of metal absorbing. In current study, *Pseudomonas* strains isolated from oily soils and it was identified that, they can absorb metals [4].Edward Raja and colleagues were believed that, heavy metals can be removed well by *Pseudomonas* spp. They evaluated the minimum inhibitory concentration for metals such as chromium, cadmium and nickel and lead 100 to 800mgl$^{-1}$which lead was tolerated up to 800mgl$^{-1}$by strain BC15.In this study, the resistance was observed up to 2000 for $Mso_2$.*Pseudomonas*, BC15, which was studied by Raja, was able to remove the lead from liquid solution up to 65% while in the presented study, isolated *Pseudomonas* strain eliminated it more than 50% from the liquid medium [1].Another study which was conducted by Chang and colleagues about absorbing of lead, copper and cadmium in live biomass of *Pseudomonasaeruginosa* Pu21, effects of environmental conditions and bacterial growth on absorbing these metals was investigated. The results showed that this strain has attracted 110mgg$^{-1}$dry weight of bacterial cell mass in pH level of 5.5.In the present study; the bacterium had the best growth in pH 6 in the presence of 100mgl$^{-1}$of lead. In Chang study, inactive cells were able to absorb the mentioned metal as much as 70mgg$^{-1}$ of cell dries weight. These results showed that, living cells have more abilities in absorbing heavy metals. Thus, in this study, living cells was used for absorbing process. *Pseudomonasaeruginosa* Pu21 biomass among heavy metal elements has absorbed lead more than cadmium and copper ions [13].The Strain examined in this study in compliance with Xiao et al., research[14] was resistance against erythromycin and chloramphenicol. Since the resistance genes to antibiotics and heavy metals can be placed on a same plasmid, the correlation of these kinds of resistance can be observed in result of this research [14].In conclusion, it must be said that, according to the results, $Mso_2$ strain may be a good candidate for studies of removing and detoxifying the environment.

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