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Biosorption of Pb and Ni From Aqueous Solution by *Staphylococcus aureus*, *Pantoea* and *Pseudomonas aeruginosa*

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Abstract

The presence of heavy metal in environment associated with several health problems. The clean up environment from lead (Pb) and Nickel (Ni) represent major challenges. In his study, planktonic and immobilized bacteria were used to purify the water from Pb and Ni in Lab. In the present study, three bacterial isolates of *Staphylococcus aureus* (isolated from wound swaps), *Pseudomonas aeruginosa* (isolated from wound swaps) and *Pantoea* (isolated from urine samples) and identified using biochemical methods to check their ability to biosorb Pb and Ni. Ten PPM of Pb and Ni were added to the deionized distilled water and 107 c.f.u. of planktonic bacteria were used to biosorpe Pb and Ni. Similar experiment was repeated but in this case, the immobilized bacteria (*S. aureus*, *Pantoea*, and *P. aeruginosa*) on silica gel and eggshells were used. It was found that *S. aureus* decreased the level of Pb and Ni significantly ($P<0.05$) in planktonic and immobilized form. *Pantoea* decreases the level of Ni only in planktonic form. This bacteria decreased the level of Pb and Ni significantly when it immobilized on silica gel and eggshells ($P<0.05$). *P. aeruginosa* could not decrease the level of Pb and Ni when it was in planktonic form but it can decrease the level of heavy metals in the immobilized form on silica and eggshells ($P<0.05$). It can be concluded that the studied bacteria can purify water from heavy metals in immobilized status more efficiently than planktonic form.

Keywords: Biosorption, Pb, Ni, *Staphylococcus aureus*, *Pantoea* and *Pseudomonas aeruginosa*

ألأمتزاز الحيوي لعنصري الرصاص والنيكل من محلول المائي بواسطة العنقوديات الذهبية والبنتويا و الزائفة الزنجارية

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الخلاصة

أرتبط وجود المعادن الثقيلة في البيئة بالعديد من المشاكل الصحية. يمثل تنظيف البيئة من الرصاص والنيكل من التحديات الرئيسة. هنا استخدمنا البكتيريا الحرة والمقيدة لتنقية المياه من الرصاص والنيكل في المختبر. في الدراسة الحالية ، تم عزل ثلاث عزلات بكتيرية (العنقوديات الذهبية والبنتويا و الزائفة الزنجارية) وتشخيصها باستخدام طرق الكيمياوية الحيوية. تم إضافة عشرة أجزاء من المليون من الرصاص والنيكل إلى الماء المقطر منزوع الأيونات و 107 وحدة مكونة للمستعمرة من البكتريا الحرة وذلك للأمتصاص الحيوي

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للرصاص والنيكل. تم تكرار التجربة ولكن في هذه الحالة تم استخدام البكتيريا المقيدة على هلام السيليكا وقشور البيض. وقد وجد أن بكتيريا العنقوديات الذهبية خفضت مستوى الرصاص والنيكل بدرجة كبيرة (P >0.05)في شكلها الحر والمقيد. عند استخدام بكتريا البنتويا وبشكلها الحر وجد انها تقلل من مستوى النيكل فقط. هذه البكتيريا خفضت من مستوى الرصاص والنيكل بشكل كبير ونوعي عند استخدامها بشكلها المقيد على هلام السيليكا وقشور البيض (P <0.05). لم تستطع بكتريا الزائفة الزنجارية بشكلها الحر من خفض مستوى الرصاص والنيكل. وعند استخدام هذه البكتريا بشكلها المقيد على السلكا و قشور البيض فقد وجد انها تخفض من مستوى الرصاص والنيكل وبشكل نوعي .(P <0.05) يمكن الاستنتاج أن العزلات البكتيرية التي شملتها الدراسة الحالية يمكنها تنقية المياه من المعادن الثقيلة في حالة تقييدها بشكل أكثر كفاءة من شكل الحر الغير مقيد.

Introduction

Heavy metal pollution is one of the most important ecological troubles. In recent years, biotechnology was used to control and eradicate metal pollution. It becomes a very important field of metal pollution control because of its possible application. Lead (Pb) is one of the most toxic of heavy metals [1]. Pb is emitted into the environment can be taken by the respiratory system, or it can be ingested. It is absorbed very fast into the bloodstream and affects negatively on the central nervous system, immune system and cardiovascular system [2].

Nickel (Ni) is a toxic metal and carcinogen [3]. It is one of the metals that commonly have high toxicity against DNA and protein and is often present in blood with several problems. The problem of Ni is looking like zinc. Zinc deficiency is very common in people eating in Western diets, so if the body required zinc and it is not present in food, the body will take look-alike Ni instead. But Ni does not do the duty and, indeed, doing the way of normal biochemistry. Ni sensitivity is very common in these days and often diagnosed from rashes from jewelry, zips, watches etc. [4].

Metals that enter the environment system will generate different problems that required practical solutions. Conventional methods of remediation such as precipitation, filtration or ion exchange technique are costly and at times ineffective [5] and associated with several practical problems, while biological methods or biosorpents, including bacteria, algae, fungi and yeast are cheaper and effective, especially when metal concentrations are little [5]. From these groups of microorganisms, bacteria may play a critical role in bioremediation. Some strains of Pseudomonas, Bacillus and Streptomyces have successfully been applied as biosorbents [6]. Most of these microorganisms studied for metal bioremediation were obtained from metal-polluted areas [6]. These microorganisms metal resistance might be due to their ability to avoid metal absorption and/or increase through some metal-excluding mechanism [7]. In this study, three bacterial species were adapted to high external concentrations of Ni and Pb, in order to evaluate their ability to remove one or both of these metals by biosorption.

Materials and Methods

Isolation and Identification of Bacterial Isolates

The standard method of Subhi et al. (2016) [8] was followed to isolate *P. aeruginosa* from wound swabs. The *S. aureus* was isolated from wound swaps according to the method of Gandara et al. (2006) [9]. The method of Xie et al. (2019) [10] was followed to isolate *Pantoea* from a urine sample. Bacteria were preserved by freezing in glycerol and were routinely cultured at 37 ^{o}C on Luria Bertani agar plates and stored at 4 oC, Subcultures were made every week [11-14] .

Pb and Ni solutions

The sterile deionized water was used to prepare the working solutions of Pb and Ni. 10 part per million (PPM) of Pb was prepared in deionized distilled water similar concentration of Ni was prepared. The solutions were sterilized by subjecting them to pressurized saturated steam at 121 °C (249 °F) for 15 minutes (autoclave, thermo scientific) [15, 16].

Biosorption of Pb and Ni by planktonic bacterial cells

In the present study, four sets of tubes were used (2 tests and 2 controls). The first test of tubes (three tubes) contains 10^7 c.f.u. of *P. aeruginosa* in 10 ml of DI water containing 10 PPM of Pb the second test tubes (tree tubes) contain 107 c.f.u. of *P. aeruginosa* in 10 ml of DI water containing 10

PPM of Ni. The first control tubes (three tubes) contain 10 ml of DI water containing 10 PPM of Pb, the second control tubes (tree tubes) contain 10 ml of DI water containing 10 PPM of Ni. Similar experiment was repeated once by using *S. aureus* and another by using *Pantoea*. After incubation the tubes for 24 hours, the tubes were centrifuged at 10000 rpm for 15 minutes. The concentration of Pb and Ni were measured in supernatants by flame atomic absorption spectrometer.

Biosorption of Pb and Ni by immobilized bacterial cells

The 10^7 c.f.u. of bacterial cells (*P. aeruginosa, S. aureus* and *Pantoea*) were fixed on Silica, eggs shells, aluminum full. The standard method of Alvarez et al. (2007) [17] was followed to immobilize the bacterial isolates into silica gel. The method of immobilizing bacterial isolates on eggshell was described in this paper. Briefly, 1 gm of eggshells was homogenized by mortar and washed three times with DI water and re-suspended to 2 ml by DI water and sterilized by subjecting them to pressurized saturated steam at 121 °C (249 °F) for 15 minutes (autoclave, thermo scientific). The sterile 2 ml of eggshell were mixed with 1 ml of DI water contains 10^7 of bacterial cells (*P. aeruginosa, S. aureus* and *Pantoea*, each bacterial was immobilized separately), the suspensions were incubated at 37 ^{o}C for 24 hours and washed three times with deionized distilled water. The free bacteria were discarded by centrifugation. The ability of immobilized bacteria was checked by mixing with either 10 PPM of Pb or 10 PPM of Ni. The efficiency of biosorbtion was evaluated by measuring the concentration of Pb and Ni flame atomic absorption spectrometer.

Statistical analysis

All values have been used to give a mean value and the standard deviation (s.d.) were calculated. The differences were analyzed by using Student's t-test employing original version 8.0 software. A value of P<0.05 was considered to be statistically significant [18].

Results

Biosorption of Pb and Ni by planktonic bacterial cells

The current study showed that the significant decrease (P< 0.05) in the levels of Pb and Ni in the test tubes (tubes contain 10 PPM of Pb or Ni and *S. aureus*) as compared with control tubes (tubes contain 10 PPM Pb or Ni). The significant decrease was seen in the test tubes of Ni treated with *Pantoea* (P<0.05) but no significant decrease was observed in test tubes of Pb that contain *Pantoea*. No significant different was observed in the level of Pb and Ni in the test tubes (tubes contain 10 PPM of Pb or Ni and *P. aeruginosa*) as compared with control tubes (tubes contain 10 PPM Pb or Ni) (P> 0.05). The present study proved that *S. aureus* has the highest ability to eradicate Pb and Ni from fresh water (Figure-1). *Pantoea* can eradicate the only Ni from water but it does not have an ability to clean up the water from Pb. In this study, the planktonic cells of *P. aeruginosa* do not have an ability to remove the heavy metals (Pb and Ni) from water in Lab .

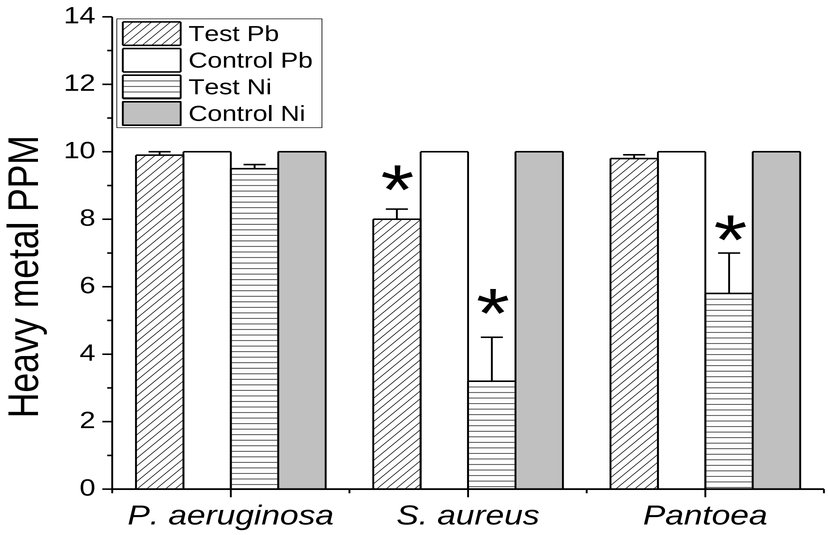

Figure 1-Pb and Ni biosorption post treated with different planktonic cells (*P. aeruginosa*, *S. aureus* and *Pantoea*). The values represent the amount of heavy metal that measured in tubes post treated with bacteria. Test, bacteria + 10 PPM of Pb or Ni; Control, only 10 PPm of Pb or Ni. Asterisks indicate a significant difference from control tubes (*P<0.05*).

Biosorption of Pb and Ni by immobilized bacterial cells on silica matrices

Figure-2 showed a significant decrease in the level of Pb and Ni in tubes post mixing with immobilized bacteria (*P. aeruginosa, S. aureus* and *Pantoea*), (P<0.05). The highest ability of eradicating heavy metals was seen in the case of *S. aureus*. The current study proves that the immobilizing bacteria increase the ability of bacteria to eradicate the heavy metals from water in Lab .

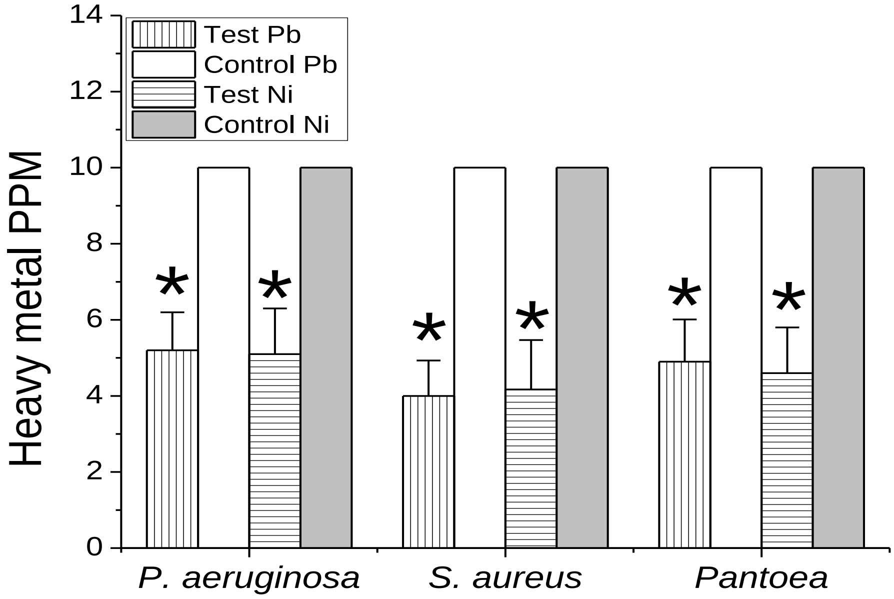

Figure 2-Pb and Ni biosorption post treated with immobilized bacterial (*P. aeruginosa*, *S. aureus* and *Pantoea*) on silica matrices. The values represent the amount of heavy metal that measured in tubes post treated with immobilized bacteria. Test, immobilized bacteria into silica + 10 PPM of Pb or Ni; Control, only 10 PPM of Pb or Ni. Asterisks indicate a significant difference from control tubes (P<0.05).

Biosorption of Pb and Ni by immobilized bacterial cells on eggshell Figure-3 showed significant decrease in the level of Pb and Ni in tubes post mixing with immobilized bacteria (*P. aeruginosa*, *S. aureus* and *Pantoea*) on eggshells (P<0.05). The highest ability to eradicate heavy metals was seen in the case of immobilized *S. aureus*. The current study proves that the immobilizing bacteria increase the ability of bacteria to eradicate the heavy metals from water in Lab .

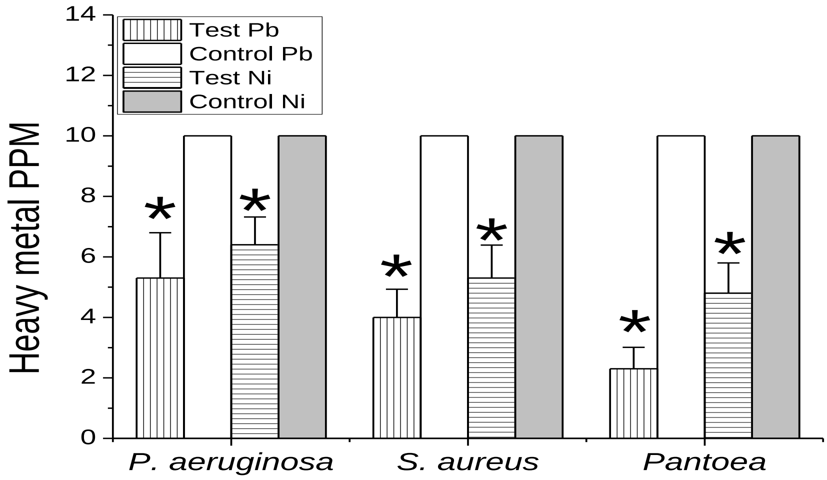

Figure 3-Pb and Ni biosorption post treated with immobilized bacterial (*P. aeruginosa*, *S. aureus* and *Pantoea*) onto eggshells. The values represent the amount of heavy metal that measured in tubes post treated with immobilized bacteria. Test, immobilized bacteria onto eggshells + 10 PPM of Pb or Ni; Control, only 10 PPm of Pb or Ni. Asterisks indicate a significant difference from control tubes (P<0.05).

Discussion

In the present study, three isolated bacteria of *P. aeruginosa, S. aureus* and *Pantoea* were used in planktonic and immobilizing form. We found that *S. aureus* is the best planktonic bacteria from three studied isolates to decrease the heavy metals. Here we found that immobilizing three isolates on either silica or eggshell increase the ability of these bacteria to pull the heavy metals from water.

There is a lot of methods can be used to clean up the water from heavy metals but the biological method represents the best cheaper and safe method as compared with chemical or other methods [5]. Several previous studies focused on using microorganisms for biosorption of heavy metals from water [19-21] .

The presence of functional groups in the cell wall and the chemical configuration of the metal play an important role in biosorption of heavy metals. The cell wall contains amines, amides, and carboxylic acid functional groups that are either protonated or deprotonated, depending on the pH of the aqueous medium. The negative charge at the surface of the cells are helping in biosorption of Pb and Ni and another factor is competition between cations and H+ for the binding sites on bacteria [19]. It may be living cells uptake a part of Pb and Ni through intracellular accumulation since it is possible to remove metals from cell surfaces after biosorption but not bioaccumulation [19].

The current study showed that the immobilization of bacteria increases the ability of bacteria to eradicate the heavy metal from water solution. The attractive results were seen in the case on *Pantoea* after immobilizing on eggshells, in this case, the maximum decrease in the level of Pb was seen.

Immobilization of microorganism on the innate surface increases the ability of the microorganism to adsorb heavy metal [22]. There is no previous study focused on the ability of bacteria that attached on eggshells to adsorped heavy metals, this needs more investigation that will investigate in a new project in the future.

From that, it can be concluded that the best method for eradicating the heavy metal is that using the immobilized *Pantoea* onto eggshell. The working is going on in our laboratory to use different carriers to immobilize bacteria on, to find whether the fixation of *Pantoea* on eggshell is the best or there is another method is the best to biosorption of Pb and Ni from the water .

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