Effect of some environmental factors on the tolerance of *Bacillus subtilis* to heavy metals

شمم ناصر راضي،سحر قاسم، سناء برهان الدين
قسم علوم الحياة/ احياء مجهرية/ كلية العلوم جامعة بغداد

Abstract

Twelve isolates of bacteria were obtained from samples of different soils and water amended with 100µg/ml of five heavy metals chlorides (i.e: Aluminum Al^{+2}, Iron Fe^{+2}, Lead Pb^{+2}, Mercury Hg^{+2} and Zinc Zn^{+2}). Four isolates were identified as *Bacillus subtilis* and *B. subtilis* (B2) isolate was selected for this study according to their resistance to all five heavy metals chlorides. The ability of *B. subtilis* (B2) isolate for growing in different concentration of heavy metals chlorides ranging from 200-1200 µg/ml was tested. The highest conc. that *B. subtilis* (B2) isolate tolerate was 1000 µg/ml for Al^{+2}, Fe^{+2}, Pb^{+2}, and $Zn^{+2}$and 300 µg/ml for Hg^{+2} for 24hour.The effect of heavy metals chlorides on bacterial growth for 72 hrs was studied. *B. subtilis* (B2) isolate had not affected by presence of $pbCl_2$ $FeCl_2$and $AlCl_2$ in growth media. However, it was slightly affected by $ZnCl_2$ during incubation period while mercury causes no bacterial growth. The effect of temperature on bacterial growth in media with heavy metals was studied. *B. subtilis* (B2) isolate showed growth in temp. range (30 - 50) ºC with the present of the four heavy metals chlorides Al^{+2}, Fe^{+2}, $Pb^{+2}$and Zn^{+2} while there are no growth in the presence of Hg^{+2} at all temperatures. The effect of different pH values on growth of *B. subtilis* (B2) isolate in media with heavy metals was studied.
Results showed the ability of B2 isolate for growing in different pH values 4,7,9 with presence of the four heavy metals chlorides Al^{+2}, Fe^{+2}, $Pb^{+2}$and Zn^{+2} in all pH values at conc. 1000 µg/ml and unability for growing in media with 300 µg/ml Hg^{+2}. The ability of *B. subtilis* (B2) isolate for removal of heavy metals chlorides was studied and showed that the highest Zn^{+2} removal ratios 75% then Fe^{+2}removal ratio 71% while Pb^{+2} has the lowest removal ratio 37%.

Introduction

Heavy metal pollution of soil and wastewater is a significant environmental problem [1]. Wastewaters from the industries and sewage sludge applications have permanent toxic effects to human and the environment [2]. There is a lot of heavy metal in our environment: cadmium, chromium, cobalt, copper, lead, mercury, etc. Interestingly, small amounts of these elements are common in our environment and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity. In small quantities, certain heavy metals are nutritionally essential for a healthy life but they become toxic when they are not metabolized by the body and accumulate in the soft tissues [3]. Removal of excesses of heavy metal ions from wastewaters is essential due to their extreme toxicity towards aquatic life and humans [4]. Toxicity of these heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Also, toxicity can occur as a result of alterations in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance [5].

Several methods are proposed for the removal and recovery of heavy metal ions from water. It includes evaporation, electro-deposition, ion-exchange, precipitation, flocculation, sorption, etc. These methods have a number of disadvantages like higher operational cost, high energy consumption, additional requirement of chemicals and complicated residual metal sludge disposal techniques. Due to the towering costs involved in these processes, the uses of micro-organisms have received considerable attention. In recent years, bacteria, fungi and algae are generally used as biomass [6]. Different genus of *Bacillus, Aspergillus, Pseudomonas*, etc., has been reported as efficient heavy metals reducers. The bacterial biomass is capable of removing heavy metals with the active participation of several anionic functional groups present in the bacteria [7].

The objectives of this study are to determine maximum tolerable concentration (MTC) of some heavy metals of *Bacillus subtilis*, growth studies, and investigation the biosorption of metal ion (Pb^{+2}, Fe^{+2}, Zn^{+2}) from aqueous solution onto *B. subtilis*.

Materials and methods

Media & solutions

***Heavy metals stock solution*:** stock solutions were prepared in a concentration of10000 µg/ml by dissolving the following heavy metals chlorides: (i.e: Aluminum Al^{+2}, Iron Fe^{+2}, Lead Pb^{+2}, Mercury Hg^{+2} and Zinc Zn^{+2}) in distilled water.

***Nutrient agar and broth*:** It was prepared and sterilized by autoclaving at 121 °C for 15 min. Different concentrations (200-1200 µg/ml) of the heavy metals were supplied into the medium after cooling to 50°C.

Samples collection

A total of ten samples of soil and water were collected in sterile plastic bags and bottles.

Isolation and identification of heavy metal resistant Bacillus subtilis

For the selective isolation of heavy metals resistant *B. subtilis*, heavy metals incorporated media were used. Basal media nutrient agar incorporated with heavy metals like Al^{+2}, Fe^{+2}, Zn^{+2}, Pb^{+2}, and Hg^{+2} were prepared separately. The concentration of each heavy metal was maintained at 100 µg/ml of the medium. Soil samples were sterilized and then diluted from 10^{-1}-10^{-3} while water samples were directly streaked on these media after sterilizing in order to kill vegetative cells and incubated at 37°C for 24-48 hours. After the incubation period the plates were observed for any kind of growth on the media. The isolated and distinct colonies on these selective media were subcultured separately on the same media for identify there resistance to all heavy metals. The pure culture was identified on the basis of their morphology and biochemical characters [8].

Preservation of B. subtilis

Pure culture of *B. subtilis* isolates were stabbed into nutrient agar then incubated at 37°C and stored in dark place at room temperature [9].

Determination of Maximum Tolerable Concentration (MTC)

MTC of the heavy metal resistant *B. subtilis* isolate grown on heavy metals incorporated media, against respective heavy metal was determined by gradually increasing the concentration of the heavy metal salts 100 µg/ml each time on nutrient agar plate. The starting concentration used was 100µg/ml. The culture growing on the last concentration was transferred to the higher concentration by streaking on the plate. The maximum concentration of metal in the medium which support the growth was taken as MTC [10].

Effect of heavy metals on B. subtilis growth

The heavy metal resistant *B. subtilis* isolate (OD 0.2 at 600nm)was inoculated into 50ml of nutrient broth incorporated with MTC of heavy metals chloride (1000µg/ml) for Al^{+2}, Fe^{+2}, Zn^{+2}, Pb^{+2} and (300µg/ml) for Hg^{+2} and incubated at 37ºC for 3 days. Medium without metal but with bacterial inoculum (bacterial growth control) and medium with metal but without bacteria (abiotic control) [11]. Bacterial number was counted every 24 hours using dilution to extinction method [12benson]; also bacterial growth was measured in terms of optical density at 600 nm using Spectrophotometer every 24 hrs for 3 days

Effect of temperature on B. subtilis resistance isolate to heavy metals

The heavy metal resistant *B. subtilis* isolate was inoculated into 50ml of nutrient broth incorporated with different heavy metals chloride as mentioned above and incubated at different temperature; (30, 37 and 50 ºC) for24 hrs. At the end of incubation period bacterial number was measured using dilution to extinction method [12benson]

Effect of pH on B. subtilis resistance isolate to heavy metals

The heavy metal resistant *B. subtilis* isolate was inoculated into 50ml of nutrient broth incorporated with different heavy metals chloride prepared at different pH values (4, 7 and 9); and incubated at 37 ºC for24 hrs. Bacterial number was measured using dilution to extinction method [12benson].

Removal of heavy metals ions by B. subtilis

B. subtilis isolate was grown in nutrient broth medium for 24hrs. Cells were separated by centrifugation at 6000rpm for 15min and washed three times in normal saline. 100ml from heavy metal solution at a concentration of (150 µg/ml) like Fe^{+2}, Zn^{+2} and Pb^{+2} that prepared separately was taken in 250ml flasks. Harvested cells were transferred to the metal solutions and incubated for 2 h at 37 ºC. Solutions were centrifuged at 6000rpm for 15min, the concentrations of three heavy metals Fe^{+2}, Zn^{+2} and Pb^{+2} were measured by atomic absorption spectrophotometer [13].
Removal of ions with bacterial cells was calculated as ratio of ions removal %.

$R\ (\%) = (C_0 - C_1) / C_0 \times 100$

Where R = Removal Ratio (%); C_0= concentration of heavy metals ions in the original solution (µg/ml) and C_1 = concentration of heavy metals ions in the treated solution (µg/ml) [14].

Results and discussions

***Isolation and identification of heavy metals resistant B. subtilis* isolate**

Twelve isolates were selected after direct isolating on nutrient agar amended with 100µg/ml of heavy metals chlorides Al^{+2}, Fe^{+2}, Zn^{+2}, Pb^{+2}, and Hg^{+2} depending on their highly resistance to the majority of heavy metals chlorides from these isolates, four isolates were identified as *B. subtilis* depending on morphological and biochemical characteristics [15] (Table 1).

Table1: Biochemical tests of *B. subtilis*

Test	Result
Gram stain	+
Cell shape	Rod
Catalase	+
Voges-Proskauer	+
Indole	-
Starch hydrolysis	+
Nitrate reduction	+
Motility	+
Citrate utilization	+
Lecithinase	-
Glucose fermentation	+
Lactose fermentation	-
Mannitol fermentation	+

(+) positive result (–) negative result

B. subtilis isolates varied in their resistance to heavy metals chloride in respect of the type of metals (Table 2).

Table2: Effect of metal chlorides on *B. subtilis*.

Isolate code	Heavy metals chloride 100 µg/ml				
	$ZnCl_2$	$FeCl_2$	$AlCl_2$	$PbCl_2$	$HgCl_2$
B1	-	+	+	+	-
B2	+	+	+	+	+
B3	+	+	+	-	-
B4	+	+	+	+	-

(B) *B. subtilis* (+) growth (-) no growth

The ability of microbial stains to grow in the presence of heavy metals would be helpful in the waste water treatment where microorganisms are directly involved in the decomposition of organic matter in biological processes for waste water treatment, because often the inhibitory effect of heavy metals is a common phenomenon that occurs in the biological treatment of waste water [16].

Determination of MTC

B. subtilis B2 isolate (isolated from soil) which have the highest resistance to all heavy metals chlorides that used in this study at a concentration of 100 µg/ml was grown in heavy metals incorporated media at different concentrations from 200-1200 µg/ml to determine the MTC. Results showed that the Maximum tolerable concentration for all heavy metals was 1000 µg/ml except for mercury was

300µg/ml as it shown in (Table3). *Bacillus* represents the common soil bacteria and has been reported as soil inhabitants [17]. Bacterial cell walls possess many charged groups such as peptidoglycan can contribute both carboxyl and amino groups. In many gram-positive bacteria, teichoic acids provide highly charged anionic clusters due to the presence of repeating phosphodiester residues [18]. For *B. subtilis* walls, (Beveride and Murrey, 1979) [19] have predicted two steps mechanisms for resistance to heavy metals ions. The first step is the stoichiometric interaction of metal with relative chemical groups which reside primarily in the peptidoglycan. After complexation these same sites nucleate the deposition of more metal by chemical precipitation. Those sites constrained within the interstics of the wall can develop only small grain precipitates since sites have no such constraints and with time and enough metal, very large-grain precipitate can develop.

Table 3: Maximum tolerable concentrations of *B. subtilis* isolate B2 to different heavy metals chlorides.

Heavy metals chloride	MTC (µg/ml)
$ZnCl_2$	1000
$FeCl_2$	1000
$AlCl_2$	1000
$PbCl_2$	1000
$HgCl_2$	300

Effect of heavy metals on B. subtilis growth

For the determination of heavy metals impact on bacterial growth *B. subtilis* B2 isolate was grown in nutrient broth incorporated with the maximum tolerable concentration of heavy metals chlorides that prepared separately for 3 days. Results showed that growth of *B. subtilis* B2 isolate has not affected by presence of $pbCl_2$, $FeCl_2$ and $AlCl_2$ in growth media but affected slightly by $ZnCl_2$ during incubation time while no growth showed with $HgCl_2$ (Table 4).

Microbes apply various types of resistance mechanisms in response to heavy metals [20]. Silver (1996) [21] summarized the mechanisms of bacterial resistance to heavy metals as follows: bacteria have specific genes for resistance to toxic ion; metal ion resistance have been found in plasmids as well as chromosome of all eubacterial group; the mechanism of resistance are generally efflux pumping and enzymatic detoxification. So *B. subtilis* B2 resist $HgCl_2$ in a concentration of 300µg/ml at a first time but this resistance may disappear because it's located on plasmids

Table 4: Effect of different heavy metal chlorides on the growth (cell/ml) of *B. subtilis* B2 isolate incubated at different times

Heavy metal chloride and its conc. (µg/ml)	Contact time in hours		
	24h	48h	72h
	viable count (cell/ml)	viable count (cell/ml)	viable count (cell/ml)
$ZnCl_2$ 1000	10^7	10^6	10^5

$FeCl_2$ 1000	10^{10}	10^{10}	10^{10}
$AlCl_2$ 1000	10^{10}	10^{10}	10^{10}
$PbCl_2$ 1000	10^{10}	10^{10}	10^{10}
$HgCl_2$ 300	10^{1}	0	0
*control	10^{10}	10^{10}	10^{10}

*control= bacteria without heavy metal solution

Figure 1 showed that *B. subtilis* B2with $pbCl_2$, $FeCl_2$ and $AlCl_2$ exhibit growth similar to the bacterial growth (control) over the experimental time 72hrs while decrease in growth was observed in the presence of $ZnCl_2$ comparing with control.

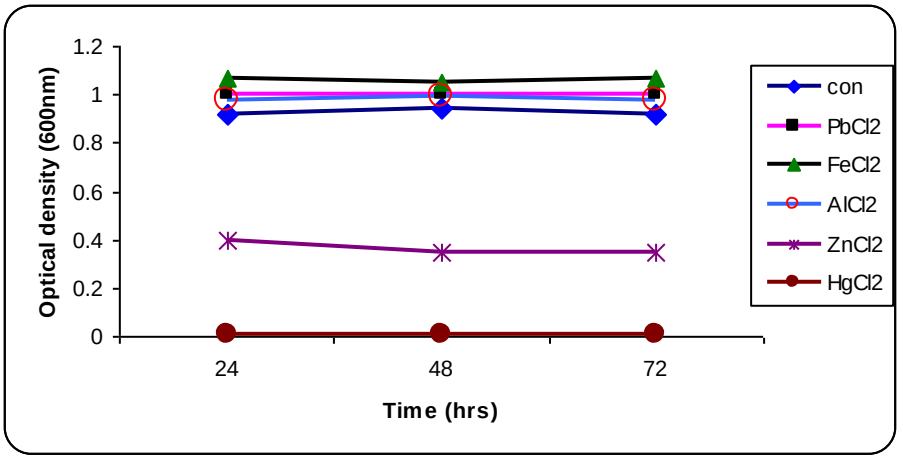

Figure 1: Growth of *Bacillus subtilis* in nutrient broth with different heavy metals chlorides incubation at 37 °C for different time.

This result is similar to other results found that levels of Zn^{2+} tolerance among *B. subtilis* reduction of their growth to 50 % [5]. In contrary to other results reported that the growth of *B. subtilis* was not affected in the presence of Zn^{2+} [22]. For lead the same result was reported that *B. subtilis* was not significantly affected by lead [23].

Mercury is one of the most toxic elements tested affinity of the mercury for thiol groups is stronger than the affinity of cadmium for sulfide [24]. It binds to sulfhydryl groups of enzymes, thereby inactivating vital cellular functions [25].

Effect of temperature on B. subtilis resistance to heavy metals

Results showed that temperature change doesn't affect in absorption or adsorption of metal ions and then in growth and inhibition of bacterial isolate.

Table 5 showed that *B. subtilis* B2 isolate had clear growth in presence of heavy metals $ZnCl_2$, $FeCl_2$, $AlCl_2$, $PbCl_2$at temperatures from 30-50 ºC, while there are no growth in the presence of $HgCl_2$ at all temperatures. This indicates that the dynamic adsorption process of metals is of a passive energy independent process [26].

Every type of bacteria has an optimum- minimum and maximum growth temperature. Temperatures below the optimum for growth depress the rate of metabolism of bacterial cells. Above the optimal temperature, the growth rate decreases and thermal death may occur **[**27**].**

The removal of heavy metal ions from aqueous solution by bacteria is effected not only by the surface properties of the organism but also on biosorption time, pH, temperature and shaking speed using aerobic batch suspension culture of the mesophilic bacteria *B. stibtilis* [28].

Table 5: Effect of different temperatures on the growth (cell/ml) of *B. subtilis* (B2) in presence of different metal chloride

Heavy metal chloride and its conc. (µg/ml)	Bacterial viable count (cell/ml), incubation temperatures		
	30 ºC	37 ºC	50 ºC
$ZnCl_2$ 1000	10^5	10^5	10^5
$FeCl_2$ 1000	10^5	10^5	10^5
$AlCl_2$ 1000	10^5	10^5	10^5
$PbCl_2$ 1000	10^5	10^5	10^5
$HgCl_2$ 300	0	10^1	0
*control	10^5	10^5	10^5

*control= bacteria without heavy metal chlorides

Effect of different pH on B. subtilis resistance to heavy metals

At different pH values; 4, 7 and 9 the growth of *B. subtilis* B2 has not affected by the presence of heavy metals chloride such as $ZnCl_2$, $FeCl_2$, $AlCl_2$ and $PbCl_2$ compared to the control without metal (Table 6).

Microorganisms generally have specific range of pH suitable for their growth and performance of vital functions. Out of that range, microorganisms are either killed or inhibited [29].

In principle at very low pH value the presence of proton promote competition with metal cations for the essentially negatively charged binding sites (carboxyl, phosphate etc) on cell wall surface, while at higher pH, hydroxyl group dominate in solution and complex metal cations, preventing attachment to ligands on cell wall surface [30]. Variation in pH of the medium result in changes in the activity of the bacteria and hence the bacterial growth as well as the biosorption rate. Bacteria are very active over a certain pH range. When pH differs from the optimal value, the maintenance energy requirements increase [26].

Table 6: Effect of different pH values on the growth of *B. subtilis* B2 isolate in presence of different metal chlorides

Heavy metal chloride and its conc. (µg/ml)	pH4	pH7	pH9
	Bacterial viable	Bacterial viable	Bacterial viable

	count (cell/ml)	count (cell/ml)	count (cell/ml)
$ZnCl_2$ 1000	10^5	10^5	10^5
$FeCl_2$ 1000	10^5	10^5	10^5
$AlCl_2$ 1000	10^5	10^5	10^5
$PbCl_2$ 1000	10^5	10^5	10^5
$HgCl_2$ 300	0	10^1	0
*control	10^5	10^5	10^5

*control= bacteria without heavy metal solution

Removal of heavy metals ions by B. subtilis

B. subtilis B2 isolate showed the highest Zn^{+2} removal ratio 75% while Pb^{+2} has the lowest removal ratio 37% (Figure 2). The results, given in table 3 and figure2 indicated that the bacterial isolate with the highest Zn^{+2} bioremoval ratios showed the lower tolerance level and vice versa. The same result has been demonstrated in literatures as well; which established an inverse relationship between tolerance and metal uptake that is the microorganism accumulates more metal if it less tolerant and accumulate less metal if it is more tolerant [31].

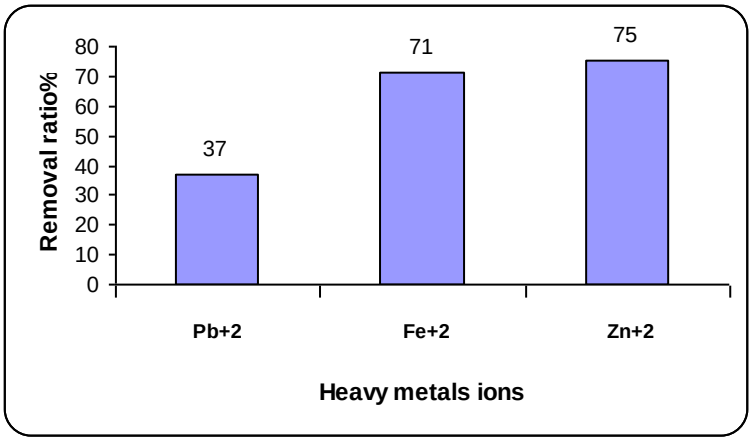

Figure 2: Adsorption of heavy metal chlorides by *B. subtilis* B2 isolate

Different *Bacillus* strain tested presented distinct uptake capacities for zinc, copper and lead the best results were obtained for *B. subtilis* and *B. cereus* [32]

The rationale for using *Bacillus* cells to study the uptake of heavy metal elements is that Gram-positive cells accumulate a much higher amount of heavy metals than Gram-negative cells. Carboxyl groups are the main agents in the uptake of heavy metals. The sources of these carboxyl groups are the teichoic acids, associated to the peptidoglycan layers of the cell wall [33].

To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state [34].

Conclusions

Based on the obtained data we can conclude that the isolate strain of bacteria of the genus *Bacillus subtilis* is suitable organism for accumulate a large amount of Zn^{+2}, Pb^{+2} and Fe^{+2} from contaminated water.

References

1. Cheng, S. (2003). Heavy metal pollution in China: origin, pattern and control. Environmental Sciences and Pollution Research 10, 192-198.

2. Rehman, A Zahoor, A. Muneer, B. and Hasnain, S. (2008). Chromium tolerance and reduction potential of a *Bacillus* sp.ev3 isolated from metal contaminated wastewater. Bulletin of Environmental and Contamination Toxicology 81, 25-29.

3. Sa'idi, M. (2010). Experimental studies on effect of Heavy Metals presence in Industrial Wastewater on Biological Treatment. International journal of environmental sciences. 1(4):666-676.

4. Volesky, B. (1990). Biosorption of Heavy Metals, p. 408, Boca Raton, FL: CRC Press Inc, ISBN 0-84934917-6.

5. Rathnayake, I. V. N.; Megharaj,M.; Bolan, N. and Naidu, R. (2009). Tolerance of heavy metals by Gram positive soil bacteria. World academy of science, engineering and technology. 53: 1185-1189.

6. Martins, B.L.; Cruz, C.C.V.; Luna, A.S.; Henriques, C.A. (2006). Sorption and desorption of Pb^{2+} ions by dead *Sargassum* sp. biomass, Biochem. Eng, J. 27:310–314.

7. Sethuraman Pandiyan and Dharmendirakumar Mahendradas. (2011). Application of Bacteria to Remove Ni(II) Ions from Aqueous Solution. European Journal of Scientific Research. 52(3):345-358

8. Rajbanshi, A.(2008). Study on heavy metal resistant bacteria in Guheswori sewage treatment plant. Our Nature .6: 52-57.

9. Vandepitte, J.; Verhaegen, J.; Engbaek, K; Rohnor, P.; Piot, P. and Heuck, C. C. (2003). Basic laboratory procedures in clinical bacteriology, 2ed edition. World health Organization.

10. Sundar, K.; Vidya, R.; Amitava Mukherjee and Chandrasekaran, N. (2010). High chromium tolerant bacterial strains from Palar River Basin: Impact of tannery pollution. Research Journal of Environmental and Earth Sciences. 2(2): 112-117

11. Abdelatey, L.M., Khalil, W. K. B., Ali, T, H. and Mahrous , K F.. (2011). Heavy metal resistance and gene expression analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Egyptian soils. Journal of Applied Sciences in Environmental Sanitation, 6 (2): 201-211.

12. Benson.(2001). Microbial applications Laboratory in General Microbiology. 8th ed. pp: 93-98. McGraw−Hill Companies.

13. Philip, L., Lyengar, L. and Venkobachar, C. (2000). Biosorption of U, La, Pr, Nd, Eu and Dy by *Pseudomonas aeruginosa*. Journal of Industrial Microbiology & Biotechnology. 25: 1-7.

14. Qin, Y.; Shi, B. and Liu, J. (2006). Application of chitosan and alginate in treating waste water containing heavy metal ions. Indian Journal of Chemical Technology.13:464-469.

15. Atlas, R. M.; Brown, A. E. and Parks, L.C. (1995). Laboratory manual of experimental microbiology. Mosby company,Year book, Inc., St. Louis.
16. Filali, B.K. Taoufik, J. Zeroual, Y. Dzairi, F.Z. Talbi, M. and Blaghen, M. (2000). Waste water bacteria resistant to heavy metals and antibiotics. Current Microbiology 41, 151-156.
17. Yilmaz,E.I. (2003). Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1", Research in Microbiology, vol. 154, pp. 409-415,.
18. Doyle, R. J.; Matthews, T. H. and Streips, U. N. (1980). Chemical basis for selectivity of metal ions by the *Bacillus subtilis* cell wall. Journal of Bacteriology. 143: 471-480.
19. Beveridge, T. J., and R. G. E. Murray. (1979). How thick is the *Bacillus subtilis* cell wall. Curr. Microbiol. 2:1-4.
20. Nies, D.H., (2003). Efflux-mediated heavy metal resistance in prokaryotes. FEMES Microbiol. Rev. 27, 313–339.
21. Silver, S. (1996). Bacterial resistances to toxic metal ions-a review. Gene 179: 9
22. Hassen,A.; Saidi, N.; Cherif,M. and Boudabous,A. (1998). Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. Bioresource Technology. 65: 73-82.
23. fosso-Kankeu, E.; Mulaba-Bafubiandi, A.F.; Mamba, B.B. ; Marjanovic, L. and Barnard, T.G. (2009). Optimising the removal by *B subtilis* and *B bacterium* of metal found around mining aeeas: evaluation of the effect of physical and physiological parameters. In: Water institute of Southern Africa and international mine water association: Proceedings International mine water conference. P. 392-400.
24. Nies, D.H. (1999). Microbial heavy metal resistance. Applied microbiology and biotechnology. 51:730-750.
25. Wagner-Döbler, I., Lünsdorf, H., Lübbehüsen, T., Von Canstein, H.F and Li, Y. (2000). Structure and species composition of mercury-reducing biofilms. Applied and Environmental Microbiology. 66: 4559-4569.
26. Sarhan, O.A.(2004). Study of biosorption of some heavy metals by local isolates of Zoogloea ramigera. MSc. Thesis, College of Science, Baghdad.
27. Pclczar, M. J. Chan, E. C. S.; Kring, N. R. 2004. Microbiology-5th edn. Tata McGraw-Hill Publishing Co. Ltd. New Delhi India.
28. Hossain, S. M. and Anantharamanb, N. (2006). Studies on bacterial growth and lead(IV) biosorption using *Bacillus subtilis*. Indian Journal of Chemical Technology. 13: 591-596.
29. Prescott LM, Harley JP and Klein DA. (2002). Microbiology, Fifth Edition. New York, US. McGraw-Hill Higher Education. 95-112.
30. Wang J and Chen C. (2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. Biotechnology Advances. 24, 427-451.
31. Burke, B.E. and Pfister, R.M. (1986). Cadmium transport by a Cd^{+2}- sensitive and a Cd^{+2}– resistant strain of Bacillus subtilis. Can. J. Microbial.32: 539-542.
32. Augusto, A. C. and Duta, F. P. (2001). Bioaccumulation of copper, zinc, cadmium and lead by *Bacillus* spp. *Bacillus cerus, Bacillus* sphericus and *Bacillus subtilis*. Barazilian journal of microbiology. 32(1).

33. Bilos, C.; Colombo, J. C.; Skorupka,C.N.; Rodriguez Presa, M.J.. (2001). Source, distribution and variability of airborne trace metals in La Plata City area, Argentina. Environ Pollut. 111(1):149–158.
34. Spain, A. (2003). Implications of microbial heavy metal tolerance in the environment, Reviews in Undergraduate Research. 2: 1-6.

تاثير بعض العوامل البيئية على تحمل بكتريا *Bacillus subtilis* للمعادن الثقيلة

شمم ناصر راضي،سحر قاسم، سناء برهان الدين
قسم علوم الحياة كلية العلوم جامعة بغداد

الخلاصة

عزلت 12 عزلة بكتيرية من نماذج مختلفة من التربة والمياه استطاعت النمو في وسط يحتوي على 100 مايكروغرام / مل من كلوريدات الفلزات الثقيلة شملت: الالمنيومAl^{+2} ، الحديدFe^{+2} ، الرصاصPb^{+2} ، الخارصينZn^{+2} ، الزئبقHg^{+2} . اربعة عزلات من مجموع العزلات شخصت على انها *Bacillus subtilis* وانتخبت العزلة*B. subtilis* B2 الاكثر مقاومة لكل انواع كلوريدات الفلزات . اختبرت قدرة هذه العزلة على النمو في تراكيز تراوحت من 200-1200 مايكروغرام /مل من كلوريدات الفلزات الثقيلة وكان اعلى تركيز نمت فيه بكتريا*B. subtilis* B2 هو 1000 مايكروغرام/مل للAl^{+2}, Fe^{+2}, Pb^{+2}, Zn^{+2} و 300 مايكروغرام/مل للHg^{+2} لمدة 24 ساعة.

درس تاثير كلوريدات الفلزات الثقيلة على نموالعزلة ولمدة 72 ساعة. اظهرت النتائج ان العزلة *B. subtilis* B2 لم يتاثر نموها بوجود كلوريدات العناصرAl^{+2}, Fe^{+2}, Pb^{+2} مع تاثرنموها قليلا بوجود $ZnCl_2$ بينما ادى وجود الزئبق الى تثبيط نمو البكتريا. درس تاثير درجة الحرارة في نمو البكتريا في وسط النمو الحاوي على كلوريد الفلز الثقيل اظهرت النتائج عدم تاثر العزلة *B. subtilis* B2 بدرجات الحرارة من 30 الى 50 درجة مئوية بوجود كلوريدات الفلزات بتركيز 1000مايكروغرام/مل بينما سبب وجود الزئبق عدم نمو البكتريا عند درجات الحرارة المختلفة. كذلك درس تاثير الرقم الهيدروجيني على نمو العزلة *B. subtilis* B2 بوجود كلوريدات الفلزات الثقيلة. اظهرت النتائج عدم تاثر العزلة *B. subtilis* B2 بوجود كلوريدات الفلزات الثقيلة وعند ارقام هيدروجينية 4، 7 و 9 وعند تركيز 1000 مايكروغرام/مل بينما سبب وجود الزئبق عدم نمو البكتريا عند تركيز 300مايكروغرام/مل. لوحظ قدرة العزلة *B. subtilis* B2 على ازالة المعادن الثقيلة وان اعلى نسبة ازالة كانت للZn^{+2} 75 % ويليه الFe^{+2} 71% ثم ال Pb^{+2} 37%.