Optimization of heavy metals chlorides resistance by Staphylococcus aureus and its ability to remove them

Shamim Naser Radhi
Department of Biology,College of Science ,University of Baghdad,Baghdad,Iraq.
Shamam_bio@yahoo.com.

Abstract

Three *Staphylococcus aureus* isolates were selected after screening on nutrient agar media 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}) from those isolates one *S. aureus* (S3) isolate was selected depending on its resistance to all heavy metals chloride. Minimum inhibitory concentration (MIC) for this isolate was 1000µg/ml for all tested metals chlorides except Hg^{+2} (300µg/ml). Growth of *S. aureus* (S3) was not affected in presence of $pbCl_2$ and $AlCl_2$ for 72hrs; however, it was affected by $ZnCl_2$ and $FeCl_2$ during incubation period while mercury causes no bacterial growth. In response to various temperatures bacterial isolate had clear growth in presence of heavy metals $ZnCl_2$, $FeCl_2$, $AlCl_2$, $PbCl_2$at 28 ºC and 37 ºC and the growth was inhibited at 50 ºC in presence of $FeCl_2$. At different pH values; 4, 7 and 9 the growth of *S. aureus* (S3) isolate was affected at pH4 in presence of the four heavy metals chlorides Al^{+2}, Fe^{+2}, $Pb^{+2}$and Zn^{+2}. S. aureus (S3) isolate showed the highest Zn^{+2} removal ratio 43% while Pb^{+2} has the lowest removal ratio 7%.

Keywords: Staphylococcus aureus,Heavy metals,MIC, Removal.

توصيف الظروف المثلى لمقاومة بكتريا Staphylococcus aureus لكلوريدات العناصر الثقيلة ومعرفة قدرتها على ازالتها

شمم ناصر راضي

قسم علوم الحياة/ كلية العلوم /جامعة بغداد،بغداد–العراق

الخلاصة

اختيرت ثلاث عزلات نعود لجنس ال Staphylococcus aureus على وسط الاكار المغذي الحاوي على تركيز ١٠٠مايكروغرام /مل من كلوريدات العناصر الثقيلة التالية (الحديد، الالمنيوم، الخارصين،الرصاص و الزئبق) من بين هذه العزلات اختيرت العزلة(S3) *S* aureus اعتمادا على مقاومتها لجميع كلوريدات العناصر الثقيلة. كان التركيز المثبط الادنى لهذه العزلة هو ١٠٠٠مايكروغرام/مل لجميع كلوريدات العناصر ماعدا الزئبق حيث كان ٣٠٠مايكروغرام/مل. لم يتاثر نمو العزلة (S3) *S* aureus بعد ٧٢ ساعة حضن بدرجة ٣٧م° بوجود كلوريدات الرصاص والالمنيوم بينما تاثر بوجود كلوريدي الحديد والخارصين في حين سبب وجود الزئبق تثبيطا كاملا للنموا.اظهرت العزلة (S3) *S* aureus نموا واضحا عند درجتي الحرارة ٢٨ و٣٧ م° بوجود كلوريدات العناصر الثقيلة بينما ثبط النمو بوجود الحديد عند درجة ٥٠م°. كان للرقم الهيدروجيني الحامضي pH 4 تاثيرا واضحا على نمو البكتريا بوجود كلوريدات الفلزات. اظهرت العزلة (S3) *S aureus* اعلى نسبة ازالة للخارصين والتي بلغت ٤٣% بينما كانت ازالة الرصاص الاقل والتي بلغت ٧%

Introduction

Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up metal-contaminated [1]. Heavy metals are often defined as a group of metals whose atomic density is greater than 5 g cm^{-3} [2 and 3]. Metals play a vital role in the metabolic processes of the biota. Some of the heavy metals are essential and are required by the organisms as micro nutrients (cobalt, chromium, nickel, iron, manganese and zinc etc.) and are known as 'trace elements'. They are involved in redox processes, in order to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions, and regulating the osmotic balance [3 and 5]. On the other hand some other heavy metals have no biological role and are detrimental to the organisms even at very low concentration (cadmium, mercury, lead etc.). However,
at high levels both of the essential and non essential metals become toxic to the organisms. These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity [6].

In high concentrations, heavy metal ions react to form toxic compounds in bacterial cells as a mechanism of bacterial tolerance to heavy metals. To have a toxic effect, however, heavy metal ions must first enter the cell.
Because some heavy metals are necessary for enzymatic functions and bacterial growth, uptake mechanisms exist that allow for the entrance of metal ions into the cell. 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 [3]. Heavy metals differ from other toxic substance in that they are not metabolically degradable and their accumulation in living tissues can cause serious health threats or death in some cases [7].

Materials and methods

Collection of samples

A total of eight samples of soil and water were collected from different locations in Baghdad. Soil samples were collected in sterilized nylon sacs while water samples were collected in sterile bottles and transported to the laboratory

Isolation and identification of heavy metal resistant Staphylococcus aureus

For the selective isolation of heavy metals resistant *S. aureus* isolates. Stock solutions of Aluminum Al^{+2}, Iron Fe^{+2}, Lead Pb^{+2}, Mercury Hg^{+2} and Zinc Zn^{+2}were prepared by dissolving the respective chloride salts in distilled water in a concentration of10000 µg/ml. Nutrient agar media were prepared and sterilized by autoclaving at 121 °C for 15 min. and incorporated with heavy metals chlorides: Al^{+2}, Fe^{+2}, Zn^{+2}, Pb^{+2}, and Hg^{+2} at 100 µg/ml. Soil samples were diluted from 10^{-1}-10^{-3} while water samples were directly streaked on these media and incubated at 37°C for 24-48 hrs. 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 resistant to one heavy metal were tested for their resistance to the rest of the heavy metals at a concentration of100 µg/ml [8]. The pure cultures which had highest resistance to the majority of heavy metals chlorides were identified on the basis of their morphology and biochemical characters for any *S. aureus* isolates [9]. Pure culture of *S. aureus* isolates had been maintained by subcultured on nutrient agar slant and stored at 4°C.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the heavy metal resistant *S. aureus* isolate grown on heavy metals incorporated media, against respective heavy metal was determined by gradually increasing the concentration of the heavy metal 100 µg/ml each time on nutrient agar plate. The starting concentration used was 200µg/ml. The culture growing on the last concentration was transferred to the higher concentration by streaking on the plate. MIC was noted when the isolates failed to grow on plates even after 10 days of incubation [10].

Effect of heavy metals on S. aureus growth

The heavy metal resistant *S. aureus* isolate (OD 0.2 at 600nm)was inoculated into 50ml of nutrient broth incorporated with MIC of heavy metals chlorides (1000µg/ml) for Al^{+2}, Fe^{+2}, Zn^{+2}, Pb^{+2} and (300µg/ml) for Hg^{+2}; incubated at 37ºC for 3 days. Medium without metal but with

bacterial inoculum was considered as bacterial growth control [11]. Bacterial number was counted every 24 hours for 3 days using dilution to extinction method.

Effect of temperature on *S. aureus* resistance isolate to heavy metals

The heavy metal resistant *S. aureus* isolate was inoculated into 50ml of nutrient broth incorporated with different heavy metals chlorides as mentioned above and incubated at different temperature; (28, 37 and 50) ºC for24 hrs. At the end of incubation period, bacterial number was counted using dilution to extinction method.

Effect of pH on *S. aureus* resistance isolate to heavy metals

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

Removal of heavy metals ions by S. aureus

S. aureus 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 each 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 then 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 [12].

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) [13].

Results and discussions

Isolation and identification of heavy metals resistant S. aureus isolate

In the present study fifteen isolates were selected depending on their resistance to the majority of heavy metals chlorides that used in this study: Al^{+2}, Fe^{+2}, Zn^{+2}, Pb^{+2}, and Hg^{+2} at a concentration of 100µg/ml from these isolates, three isolates were identified as *S. aureus* depending on morphological and biochemical characteristics [9]. *S. aureus* isolates varied in their resistance to heavy metals chloride in respect of the type of metals *S. aureus* (S3) isolate (isolated from soil) had highest resistance to all heavy metals chlorides **(Table-1).**

(Table-1) Growth of *S. aureus* isolates on nutrient agar containing100 µg/ml of metal chloride

Isolate code	Heavy metals chlorides 100 µg/ml				
	$ZnCl_2$	$FeCl_2$	$AlCl_2$	$PbCl_2$	$HgCl_2$
Staph1	-	+	+	+	-
Staph2	+	-	+	+	-
Staph3	+	+	+	+	+

(+) growth (-) no growth

Determination of MIC

S. aureus (S3) isolate which had the highest resistance to all heavy metals chloride 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 MIC. Results showed that the Minimum inhibitory concentration for all heavy metals was 1000 µg/ml except for mercury was 300µg/ml as it shown in **(Table-2)**. The microbial resistance to heavy metal is attributed to a variety of detoxifying mechanism developed by resistant microorganisms such as complexation by exopolysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another [1]. Bacterial cell walls possess many charged groups. 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 [14]. The carboxyl groups of

peptidoglycan were primarily responsible for the interactions between cell walls and cations [15].

(Table-2) Minimum inhibitory concentrations of *S. aureus* isolate (S3) to different heavy metals chlorides

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

Effect of heavy metals on *S. aureus* growth

For the determination of heavy metals impact on bacterial growth *S. aureus* (S3) isolate was grown in nutrient broth incorporated with the maximum tolerable concentration of heavy metals chloride that prepared separately for 3 days. This isolate exhibited different growth patterns in the presence of different heavy metals.It was observed that growth of S3 isolate has not affected by presence of $pbCl_2$ and $AlCl_2$ in growth media but it was affected by $ZnCl_2$ and $FeCl_2$ during incubation period compared to the control without metal amendment while mercury causes no bacterial growth **(Figure-1).**

In studying the effect of heavy metals on growth of *S. aureus* bacteria it was found that the lower optical density values of *S. aureus* bacteria revealed that the bacterial growth was affected due to the presence of heavy metals in the growth medium [11]. Microbes apply various types of resistance mechanisms in response to heavy metals [16]. These mechanisms may be encoded by chromosomal genes, but more usually loci conferring resistance are located on plasmids [17]. So *S. aureus* resist $HgCl_2$ in a concentration of 300µg/ml at a first time but this resistance may disappear because it's located on plasmids. Mercury-resistance determinants have been found in a wide range of Gram-positive bacteria isolated from different environments. These resistance determinants vary in the number and identities of genes involved and are encoded by the *mer* operon located on plasmids [18].

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 [15]. It binds to sulfhydryl groups of enzymes, thereby inactivating vital cellular functions [9].

Cell age is considered as an important microbial factor that affects metal accumulation. Maximum heavy metal uptake by bacterial strains occurred after three days incubation these results are in conformity with the findings. This is possibly due to the presence of many highly active enzymes at this growth phase, during which cells are at their most metabolically active stage [19].

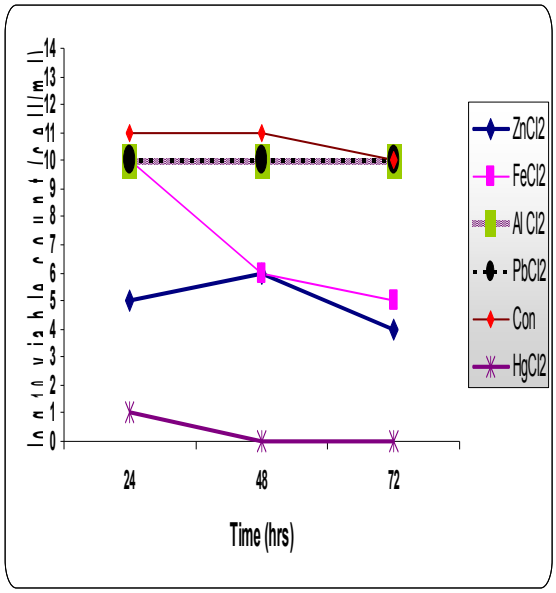


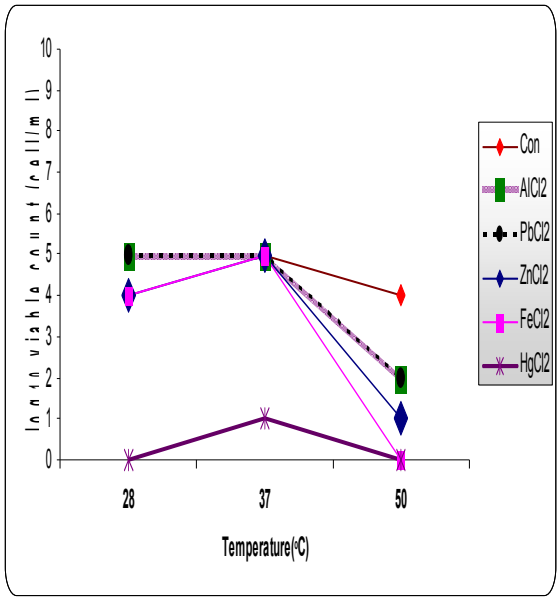
Figure-1) Effect of different heavy metals chlorides on the growth of *S. aureus* (S3) isolate after incubation at 37 ºC for different period.

Heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity [6].Microbial survival depends on intrinsic biochemical and structural properties, physiological, or genetic adaptation including morphological, changes of cells, as well as environmental modifications of metal speciation [18].

Effects of temperature on *S. aureus* (S3) isolate resistance to heavy metals

In studying the effect of temperature on the ability of *S. aureus* (S3) to grow in presence of certain concentrations of heavy metals results

showed that *S. aureus* (S3) isolate had clear growth in presence of heavy metals $ZnCl_2$, $FeCl_2$, $AlCl_2$, $PbCl_2$at temperatures 28 and 37 ºC and decreased at50 ºC . What's more, the growth was inhibited at 50 ºC in presence of $FeCl_2$, while there are no growth in the presence of HgCl2 at 28 ºC and 50 ºC **(Figure -2).** Atlas *et al*. (1995) [20] mentioned that optimum temperature for *S. aureus* growth is 35-37 ºC with a minimum temperature 6-7 ºC and maximum 45-48 ºC.



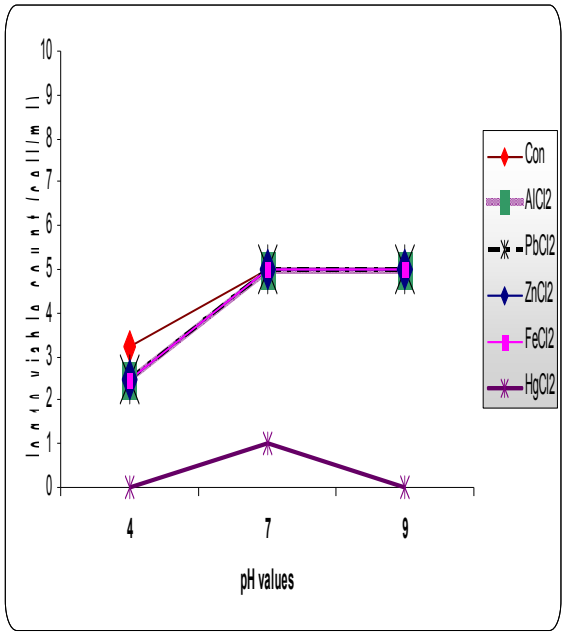
(Figure-2) Effect of different temperatures on the growth (cell/ml) of S. *aureus* (S3) in presence of different metal chloride for 24hrs.

The influence (or not) of temperature on the metal uptake (and thus metal resistance) determines whether metabolic energy is required or not for the uptake [21]. The temperature of the adsorption medium could be important for initial metal adsorption by microbial cells; energy dependent mechanisms may be affected by the temperature of the adsorption medium. Temperature can affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. The binding sites on the isolated bacterial species might be simultaneously affected by these factors and may cause reduction in metal removal [22]. Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature. Mostly adsorption is an exothermic process, whereas, some examples of endothermic adsorption have also been reported [23]. Physical damage to the biosorbent can be expected at high temperatures. Due to the exothermic nature of some adsorption processes, an increase in temperature has been found to reduce the biosorption capacity to biomass [13; 25] this indicates that the dynamic adsorption process of metals are of a passive energy independent process [24] and the high temperature was influential in the growth rate of bacterial isolates, but didn't affect or has a minor effect in the components of the cell [25].

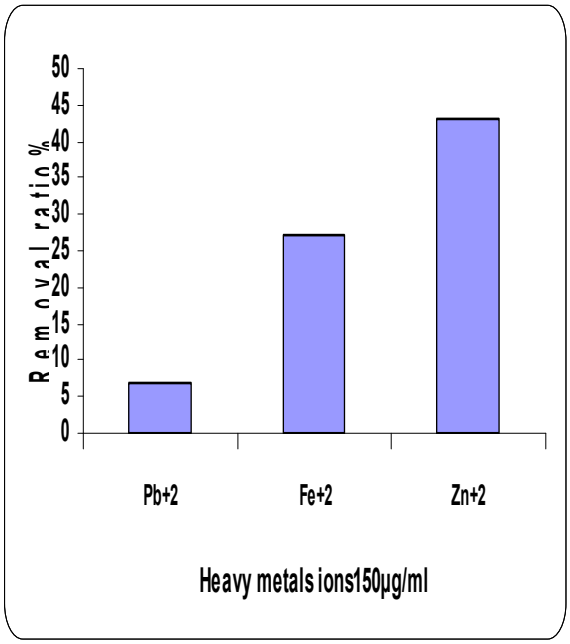
Effect of different pH on S. aureus (S3) resistance to heavy metals

Results showed that at pH values; 7 and 9 the growth of *S. aureus* (S3) 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 but bacterial growth was affected at pH 4**(Figure-3)**. pH affects metal toxicity because many metal ions form complexes with various medium or buffer components or may be precipitated by phosphates, especially at pH near neutrality or higher [21]. Tynecka *et al.* (1981) [26] studied the effect of pH in accumulation of metal ions in bacterial cells they reported that the choice of pH also affects metal ions binding, which generally decreases as the pH falls, probably because of competition for binding sites by hydrogen ions.

The low bioaccumulation capacity at pH values below five is attributed to the competition of hydrogen ion with metal ion on the sorption site. Thus, at lower pH, due to the protonation of binding site resulting from high concentration of proton, negative charge intensity on the site is reduced which results in the reduction or inhibition for the binding of metal ion. Most of the microbial surfaces are negatively charged due to the ionization of functional group, thereby contributing to metal binding. At low pH, some of the functional groups will be positive charged and may not interact with metal ions. At higher alkaline pH values (8 and above), a reduction in the solubility of metals contributes to lower uptake rates [27].



(Figure-3) Effect of different pH values on the growth of S. *aureus* (S3) isolate in presence of different metal chlorides at 37°C for24 hrs.



(Figure-4) Adsorption of heavy metal chlorides by *S .aureus* (S3) isolate

Most of the microbial surfaces are negatively charged due to the ionization of the functional groups, thereby contributing to metal binding. The pH of the biosorption medium affects the solubility of the metal ions and the ionization state of the functional groups [22]. Extremes of pH are detrimental to microorganism because they cause changes in the charged groups of proteins such as amino acids and carboxylic acids groups ultimately protein coagulation and denaturation results [9].

Removal of heavy metals ions by S. aureus

S. aureus (S3) isolate showed the highest Zn^{+2} removal ratio 43%, then Fe^{+2} 27% while Pb^{+2} has the lowest removal ratio 7% **(Figure-4).** The results, given in **(Figure-1)** and **(Figure-4)** 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 [28].

Some basic points about the surface structures of Gram-positive bacteria should be briefly presented. A characteristic component of Gram-positive cells are teichoic acids and acids associated to the cell wall, whose phosphate groups are key components for the uptake of

metals. The literature reports several studies on the interaction of heavy metals with bacterial surfaces, but just a few works consider these interactions at the molecular level [4;31]. Thus, a detailed investigation of the chemical structures of bacterial cells and the understanding of the mechanism involved in the interaction is still missing in the study of the bioaccumulation process. 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. Microbial biomass offers an economical option for removing heavy metals by the phenomenon of biosorption [29].

Conclusions

The findings in this study that the isolate strain of *S. aureus* bacteria can tolerate some of heavy metals and the differentiation in temperatures can slightly effect on bacterial growth while the different pH values hadn't any clear effect also this isolate had an ability of removing heavy metals from water and can applicable this at industrial level for big scale

treatment of waste water before discharge in the environment.

References

[1]. Silver, S. **1996**. Bacterial resistances to toxic metal ions-a review. *Gene*. **179**: 9-19.

[2]. Gadd, G.M. **1992**.Metals and microorganisms: A problem definition. *FEMS Microbiology Letters* **100**: 197-204.

[3]. Nies, D.H. **1999**.Microbial heavy metal resistance. *Applied microbiology and biotechnology*. **51**:730-750.

[4]. Bruins, M.R, Kapil,S. , Oehme, F.W. **2000**. Microbial resistance to metals in the environment.

[5]. Hussein, H. Farag, S., Kandil, K. and Moawad, H. **2005**.Tolerance and uptake of heavy metals by pseudomonads. *Process Biochemistry*. **40**: 955-961

Ecotoxicology and Environmental Safety. **45**:198-207

Roane,T.M.Pepper,I.L.**2000**. Microbial responses to environmentally toxic cadmium. *Microbial Ecology***. 38**: 358-364

[7]. Hetzer,A,Daughngy,C.J.and Morgan, H.W. **2006**.Cadmium ion biosorption by thermophilic bacteria *Geobacillus stearothermophilus* and *G thermocatenulatus*. *Appl. Environ. Microbiol*.**72**: 4020-4027.

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

[9]. 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.

[10]. Shakoori, A.R.; Zaidi, K.S. and Haq, R. **1998**.Cadmium resistant *Enterobacter clacae* and *Klebsiella* sp isolated from industrial effluents and their possible role in cadmium detoxification. *World Journal of Microbiology and biotechnology*.**15**: 249-254.

[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]. 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.

[13]. 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

[14]. 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.

[15]. Beveridge, T. J., and R. G. E. Murray. **1979**. How thick is the *Bacillus subtilis* cell wall. *Curr. Microbiol*. **2**:1-4.

[16]. Nies, D.H. **2003**.Efflux-mediated heavy metal resistance in prokaryotes. *FEMES Microbiol. Rev*. **27**: 313–339.

[17]. Wuertz, S., Mergeay, M. **1997**.The impact of heavy metals on soil microbial communities and their activities. In:van Elsas,J.D, Wellington, E.M.H.,Trevors,J.T.(Eds.),*Modern Soil Microbiology*. Marcel Decker,NY .pp. 1–20.

[18]. Radstrom, P.; Skold, O., Swedberg, G.; Flensburg, J.; Roy, P.H and Sundstrom, L. **1994**.Transposon Tn*5090* of the plasmid R751, which carries integron, is related to Tn7, Mu, and the retroelements. *Journal of Bacteriology*. **176**: 3257-3268.

[19]. Arti Suri Hakeem and Smita Bhatnagar.**2010**.Heavy Metal Reduction of Mill Effluent by Indigenous Microbes. *ASIAN J.EXP.BIOL.SCI*. **1**:1.

[20]. Atlas, R. M.; Brown, A. E. and Parks, L. C. **1995**. *Laboratory Manual of Experimental Microbiology*. 1st ed. Mosby-Year Book. Inc. St Louis.

[21]. Shoeb, Erum .**2006**.Genetic basis of heavy metal tolerance in bacteria. PhD thesis, Department of Genetics University of Karachi, Karachi, Pakistan. pp.40

[22]. Rajeshkumar, R.; Shankar, C. and K. Thamaraiselvi **2011**.Evaluation of isolated fungal strain from waste recycling facility for effective sorption of toxic heavy metal Pb (II) ions and fungal protein molecular characterization- a mycoremediation approach. *Asian J. Exp. Biol. Sci. 2***:** 342-347

[23]. Rajeshkumar, R. and Kartic**,** N. **2011**. Removal of Cu^{2+} Ions from Aqueous Solutions Using Copper Resistant Bacteria. *Our Nature*.**9**:49-54.

[24]. Sarhan,O.A.**2004**.Study of biosorption of some heavy metals by local isolates of Zoogloea ramigera. MSc. Thesis, College of Science, University of Baghdad.

[25]. Khafaji, Z. M. **1987**. Vital bacterial activities. Faculty of agriculture/ Department of food industries/ University of Baghdad/ Abu Ghraib. No. deposit in the National library in Baghdad 1254.

[26]. Tynecka, Z., Gas, Z., and Zajac, J. **1981**.Reduced cadmium transport determined by a resistance plasmid in *Staphylococcus aureus*. *Journal of Bacteriology*. **147**(2): 305-312.

[27]. Congeevaram, Sh.; Dhanarani, S; Park J.; Dexilin, M. and Thamaraiselvi, K. **2007**.Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *Journal of Hazardous Materials*. **146**:270–277.

[28]. 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.

[29]. Gupta R. and Mahapatra H, .**2003**. Microbial biomass: An economical alternative for removal of heavy metals from waste water. *Ind Jou Exp Biol*. **41**: 945-966