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HEAVY METAL BIOSORPTION BY SOME BACTERIAL SPECIES ISOLATED FROM DRINKING WATER AT DIFFERENT SITES IN SHARQIA GOVERNORATE

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ABSTRACT

The nine most frequent bacterial strains out of 127 were isolated form ten drinking water samples collected from tap water and bottled water in sharqia governorate. The nine isolates were purified and examined for their resistance to increasing concentrations of two heavy metal ions, lead (Pb^{+2}) and iron (Fe^{+3}). Four stains out of the nine isolates encoded I, II, IV, and VIII showed the highest efficiency of both Pb^{+2} and Fe^{+3} uptake from nutrient broth media containing 100 ppm of the heavy metal ions. The four bacteria were preliminary identified and then confirmed by the Biolog examination as *Corynebacterium jeikeium*, *Pseudomonas putida* biotype A, *Acinetobacter calcoaceticus* and *Acidovorax delafieldii*. The increased concentrations of Pb^{+2} ions (from 100 to 500 ppm) in nutrient broth media had deleterious effect on the process of heavy metal uptake (biosorption) by all the four selected isolates. Whereas percentage of Pb^{+2} uptake decreased from 42.9 to 24%; from 72.6 to 42%, from 78.9 to 37% and from 68.8 to 45%, for the four selected isolates, respectively. Meanwhile there was slight decrease change in Fe^{+3} uptakes percentage accompanying the increase in heavy metal ion concentration. Optimization of the cultural conditions releaved maximum uptake op pb^{+2} and Fe^{+3} by the four tested strains in presence of 100 ppm heavy metal concentration when incubated at 25^{o}C except for *Acinetobacter calcoaceticus* at 35^{o}C in case of Pb^{+2} uptake, when pH, was adjusted at 5 under static conditions. Upon addition of 50 ppm Cu^{+2} ions to broth media supplemented with 100 ppm Pb^{+2} ions, the percentage of metal biosorption by the four tested isolated decreased between 41.11% and 48.45% according to type of strain. Similarly presence of Cu^{+2} ions caused decrease in Fe^{+2} uptake by the four isolates ranging between 29.14-45.1%. Percentage of Pb^{+2} ions uptake by the tested bacteria sharply decreased when a sterile tap water sample was used as natural medium for cell-metal contact. The percentage of uptake inhibition of Pb^{+2} ranged between 57-65.23% and between 75.1-84.27% for Fe^{+3} ions. Cell hydrolysate of three tested bacteria appeared to be free from plasmid DNA proving that the genetic character of heavy metal resistance is plasmidless and related to chromosmal DNA in

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case of *Cory. jeikeium*, *P. putida* and *A. delafieldii*. On the other hand, *Acinet. Calcoaceticus* contained plasmid of size 23.130 kb. Examining *Acientobacter calcoaceticus* using transmission electron microscope revealed the accumulation of Pb^{+2} ions on bacterial cell surface and the intracellular absorption of Fe^{+3} ions.

Keywords: Pollution, Heavy metals uptake, Biosorption, Heavy metal accumulation, Plasmid and chromosomal DNA, Transmission electron microscope

INTRODUCTION

The intensified industrial activities, sewage, agriculture and waste water disposals are greatly contributing to the increase of heavy metals levels in the environment, mainly in the aquatic system **(Da Costa *et al* 1996)**.

The presence of heavy metals pollutants in water resources is a threat to humans and other life forms **(Namasivayam and Yamuna 1995)**.

Certain metal ions and their coordination complexes play a variety of fundamental roles in microbial growth and metabolism. Mean while, other metallic spieces are toxic to microbial life. Heavy metals contaminating drinking water represent a threat to public health due to their accumlation through food chain **(Holan and Volesky 1994)**. This aspect coupled with their persistence results in a serious health hazard threatening water supplies and populations depending on them **(Alloways and Ayres 1997)**.

Iron is not considered health hazardous, yet oxidation of iron in water results in settling out of red-brown particles. These sediments are responsible for staining properties of water and cause plugging of water pipes. A frequent problem resulting from increased concentration of iron in water is the occurrence of iron bacteria which form red-brown slime that clog water system **(Dave *et al* 1996)**.

The biologically available concentration of lead is low owing to its high molecular weight and low solubility in water **(Nies, 1999)**. Its toxicity for man and animals is due to its action on central nervous system, kidneys, red blood cells and reproductive system **(Goyer 1993 and Johnson 1998)**.

The removal of heavy metals contaminating water at low concentrations is very difficult by chemical means and often inadequate when applied to large volumes **(Volesky 1987)**. Thus, there is a serious demand to establish new methods for the removal of heavy metals from drinking water.

Biological processes including biosorption of heavy metals and radionuclide by micrbial biomass is relatively rapid and efficient process **(Gadd and White, 1993)**. This phenomenon may be exploited in biotechnology processes concerned with bioremediation of metal bearing effluents and waste water stream. Also, it can be used for metal radionuclid recovery for economic reasons and/or environmental protection **(Wong and Fung, 1997, Andres *et al* 2000, Vals and de-Lorenzo, 2002 and Rawlings, 2002)**.

Application of biosorption technology to the treatment of heavy metals contaminating water has been given significant attention recently by the research community. Biosorption process is based on metal binding capacities of various bio-

logical materials. Biosorption can be defined as the ability to biological materials to accumulate heavy metals through metabolically mediated or physiochemical pathways of uptake **(Fourest and Roux 1992)**.

Uptake of metals by microoragranisms is essentially a biphasic process which can be divided according to the dependence on cell's metabolism into metabolism dependent biosorption and metabolism independent biosorption. It can be also classified according to location of accumulated metal ions into extracellular, cell surface and intracellular accumulation **(Gadd 1990, Bengtsson *et al* 1995, Blackwell *et al* 1995, Volesky and May-Philips, 1995 and Simmons and Singleton, 1996)**.

Algae, fungi, yeast and bacteria have been proved to be potential biosorbents **(Volesky, 1987, Ibeanusi *et al* 1995, Ursa and Macha, 1999, Sag *et al* 2000 and Andres *et al* 2000)**.

The capacity of microbial cells to biosorption process and removal of metal ions from aqueous solutions are significantly influenced by environmental conditions as pH, temperature, biomass concentration and presence of ions **(Chen and Ting, 1995)**.

As a result of metal microorganisms interaction not only microbial population (biomass) was changed but also its molecular structure varied under heavy metal stress **(Yu *et al* 2003)**.

The genetic basis for heavy metal resistance in bacteria had been reported as plasmid-encoded resistance **(Gupta *et al* 1997)** or as plasmid less character **(Li *et al* 2000 and Sharma *et al* 2000)**.

This study aimed to isolate some heavy metal resistant bacterial strains from drinking water at different sites in Sharquia governorate. The capability of isolated and identified bacterial strains to uptake Pb^{+2} and Fe^{+3} was evaluated. The effect of increasing concentration of Pb^{+2} and Fe^{+3} on the efficiency of bacterial strains was examined. Optimization of environmental and cultural conditions for the tested strains to ensure maximum growth and metal uptake was achieved. The plasmid profile of the four tested bacterial isolates was also detected. Ultra structure studies using transmission electron microscope to define the biosorption process and study distribution and compartmentalization of metallic ions in bacterial cells was investigated.

MATERIAL AND METHODS

1. Collection of water samples

Ten drinking water samples six tap water and four bottled water, were collected from different sites in Sharqia governorate as shown in Table (1).

2. Chemical analysis of water

The ten water samples were subjected to chemical analysis using ICP-OES instrument (Inductively Coupled Argon Plasma-Optical Emission Spectrometric "ICP-OES"), to measure the heavy metals content of each sample as mg/L.

The pH values of tested water samples were also were also recorded using pH meter Orion Model 710 A.

3. Isolation of bacteria from water samples using standard membrane filtration method

100 ml of water samples were filtered separately through a flow pore D filter membranes of 0.22 μm pore size. The

bacterial filters were then plated on surface of sterile nutrient agar plates (lablemco powder, 1 gm/l; yeast extract, 2 g/L; peptone, 5 g/L sodium chloride, 5 g/L and agar, 20 g/L) and incubated at 35°C for 48 hrs.

The growing colonies were picked up and purified by streaking on nutrient agar plates and maintained on nutrient agar slants at 4°C until further identification and studies.

4. Effect of increasing concentration of Pb^{+2} and Fe^{+3} ions on growth of tested bacterial isolates

a. Metal salt solutions

Lead acetate Pb $(CH_3COO)_2$. $3H_2O$ and Ferric ammonium citrate ($C_6H_8NO_7Fe$) salts were used as sources of Pb^{+2} & Fe^{+3} ions, respectively.
Stock solutions equivalent to 1000 mg/L Pb^{+2} and Fe^{+3} were separately prepared and filter strerilized.

Different volumes of stock solutions were added to sterile and liquefied nutrient agar medium to achieve the following concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 ppm) of heavy metals.

b. Determination of bacterial growth

Equal volumes (0.1 ml) of serial dilutions of 24 hours old bacteial suspensions were spread on surface of nutrient agar plates containing different concentration of Pb^{+2} and Fe^{+3} ions. Numbers of colony forming units (CFU) were counted after incubating plates at 35°C for 24 hours. % of CFU were calculated compared to initial counts of untreated controls.

5. Identification of bacteria

The four most efficient bacterial stains were identified according to **Sneath *et al* (1986)** and **Holt *et al* (1994)**. Thereafter, identification of bacterial isolates up to species level was confirmed by Biolog examination at Microorganisms Identification and Biological control unit in the Agriculture Research Center, Giza, Egypt.

6. Heavy metal uptake by bacteria

Bacterial suspensions (24 hours old cultures) of each tested strains were adjusted to be approximately 10^7 cells/ml. Thereafter, 1 ml volumes were inoculated in flasks containing 100 ml nutrient broth medium supplemented by 100 ppm of Pb^{+2} and Fe^{+3} ions separately. Inoculated flasks were then incubated at 35°C for 24 hour.

a- Determination of bacterial cells dry weights

Growing bacterial cells were separated by centrifugation at 6000 rpm for 15 minutes. Dry cell weights were then determined after drying at 65°C until constant weights.

b- Determination of residual metal ions in nutrient broth media

Supernatants were used for determining residual metal ions content as mg/l using ICP.

Amounts of metal ions uptake were calculated according to equation of ***Kuycak and Volesky (1988)*** as follows:-

$$\textit{Metal uptake} = V (C_1 - C_F) / W$$

C_1, initial metal concentration (mg/1)
C_F, Final metal concentration (mg/1)
V, Volume of reaction medium (1)
W, Total biomass weight (g)

% Percentage of biosorped heavy metals = (Metals uptake by biomass / Metals concentration in control broth media X 100)/100

7. Effect of different cultural conditions on heavy metal uptake by the most efficient bacterial isolates

a. Effect of shaking on heavy metals uptake

Nutrient broth media adjusted at pH 7 and supplemented by 100 ppm of Pb^{+2} and Fe^{+3} ions separately were inoculated with 1ml volumes of bacterial suspensions (10^7 cells/ ml) of the four most efficient strains.

Inoculated broth media were incubated at 35°C for 24 hour under shaking and static conditions.

b. Effect of increasing concentration of Pb^{+2} and Fe^{+3} ions

100 ml of untrient broth media containing different concentration of Pb^{+2} &Fe^{+3} (100, 200, 300, 400 and 500 ppm) separately were inoculated with 1 ml of 24 hours old cultures (10^7 cells/ml) of tested bacterial strains and incubated for 24 hour at 35°C under static conditions.

c. Effect of incubation temperature

100 ml of nutrient broth media adjusted at pH 7 were supplmented with 100 ppm for Pb^{+2} and Fe^{+3} ions separately. The broth media were inoculated with 1 ml aliquots of tested bacterial suspension (10^7 cells/ml) and incubated for 24 hours at 20, 25, 30, 35 and 40°C under static conditions.

d. Effect of pH values

Equal volumes (1 ml) of 24 hours old bacterial cultures (10^7cells/ml) of tested bacteria were inoculated in 100 ml of nutrient broth supplemented with 100 ppm Pb^{+2} and Fe^{+3} ions separately. The pH values of reaction media were adjusted at 4, 5, 7 and 9 using buffer solutions. The inoculated media were incubated for 24 hour at optimum incubation temperatures under static conditions.

8. Effect of cation addition on heavy metals uptake

In this experiment nutrient broth media were adjusted at pH 5 and supplemented with 100 ppm of Pb^{+2} and Fe^{+3} ions separately in presence of 50 ppm Cu^{+2} ions in the form of copper sulphate ($Cu\ SO_4.\ 2H_2O$).

Inoculated reaction media were incubated for 24 hour at optimum temperatures of each bacterial strain under static conditions.

9. Application of heavy metals uptaking using drinking water as metal cell contact medium

Constant volumes (1 ml) of cell suspension (10^7 cells/ml) of the four tested strains were inoculated into 100 ml of sterilized drinking water samples collected from tap water at Faculty of Science, Zagazig University and supplemented with 100 ppm Pb^{+2} and Fe^{+3} ions separately. Recorded pH value was 8.4.

Inoculated water samples were incoubated under static conditions at 18°C (temperature of water sample at time of collection), for 24 hours.

Percentages of heavy metals uptake were calculated as previously mentioned.

10. Plasmid profile of the four most efficient tested bacterial strains

Detection of plasmid DNA in the four tested strains was done according to **Manniatis *et al* (1982)**.

The bacterial cells hydrolysates stained with ethidium bromide were injected in horizontal agarose gel against λ hind III and 1 kb DNA markers. Electrophoresis was performed and gel was visualized under UV and photographed.

11. Ultra structure studies

The ultra structures of metal treated and untreated cells of *Acinetobacter calcoaceticus* were studied using transmission electron microscope located at NCRRT, Naser city, Cairo. Treated cells were grown in nutrient broth adjusted at pH 5 in presence of 100 ppm Pb^{+2} and Fe^{+3} ions separately and incubated for 24 hour at 35°C under static conditions.

Collected cells after centrifugation were prefixed and prepared for electron microscope investigation according to **Kinner *et al* (1983)**.

RESULTS AND DISCUSSION

The chemical analysis, pH values, nature and localities of the ten drinking water samples collected from Sharqia governorate are recorded in Table (1).

The chemical analysis showed high concentrations of Pb^{+2} in all tested samples ranging from 0.0204 to 0.0671 mg/1 exceeding maximum permissible concentration (0.01 mg/1) approved by **WHO (2005)** in its guide lines for drinking water quality. Concentrations of Fe^{+3} in examined water samples are ranging between 0.0203 and 0.926 mg/1. Although Fe^{+3} ions are not considered toxic for man yet, they cause much troubles in water lines causing plugging and corrosion of water pipes.

127 bacterial strains were isolated from the ten drinking water samples by membrane filter technique. The nine most frequent isolates were purified and maintained on nutrient agar slants until further studies and identifications.

Figures (1&2) show the effect of increasing concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450 & 500 ppm) of Pb^{+2} and Fe^{+3} on growth of the nine tested isolates. Response of the nine tested isolates varied with kind of heavy metal and its concentration. Bacterial isolates coded number I, III, IV & VIII survivied high concentrations of Pb^{+2} (400, 450, 500 & 200 ppm, respectively), and 450, 500 & 500 ppm of F^{+3}, as well.

The varying response of tested bacteria to kind and concentration of heavy metals might be due to differences in cell wall composition of different strains **(Beveridge and Koval, 1981 McLean and Beveridge 1990 and Chang and Huang 1998)**.

The capability of heavy metal uptake (biosorption) by the tested strains was also examined using nutrient broth medium supplemented by 100 ppm Pb^{+2} and Fe^{+3} separately. The same idolates (I, III, IV& VIII) previously recorded high resistance to heavy metals concentrations also showed highest efficiency of Pb^{+2} and Fe^{+3} uptake from their solutions. Data in Table (2) revealed that the amounts of

Fig. 1: Effect of Pb^{+2} on the growth of the nine chosen isolates

Fig. 1 Cont.: Effect of Pb^{+2} on the growth of the nine chosen isolates

Fig. 2. Effect of Fe^{+3} on the growth of the nine chosen isolates

Fig. 2 Cont.: Effect of Fe^{+3} on the growth of the nine chosen isolates

Table 2. Lead uptake by the nine tested strains using 100 ppm Pb^{+2}

Isolate code number	I	II	III	IV	V	VI	VII	VIII	IX
Amount of Removed Pb^{2+} (mg)	40	12	72	77	15	8	14	64	5
%	40.2	12.05	72.2	77.3	15.1	8.03	14.1	64.3	5.02
Pb^{2+}mg/g dry cells	0.070	0.008	0.050	0.090	0.012	0.005	0.012	0.041	0.006

Control % = 99.6

Pb^{+2} uptake by the four isolates are 40, 72, 77 & 64 mg corresponding to 0.07, 0.05, 0.09 & 0.041 mg Pb^{+2} /gm dry cells, respectively. It is clear that there is no correlation between the amount of Pb^{+2} uptake from solution and amount of Pb^{+2} present in dried cells. This result may be attributed to accumulation of Pb^{+2} ions in medium of intercellular spaces between bacterial cells, which had been gotten rid of during drying of the cells. Meanwhile, results in Table (3) showed that amounts of accumulated Fe^{+3} within the bacterial cells of the most efficient strains [1 (0.050 mg/g), III (0.051 mg/g), IV (0.045 mg/g) & VIII (0.052 mg/g)] are directly proportional with the absorbed Fe^{+3} from surrounding medium [1 (71.8 mg), III (68 mg), IV (64 mg) & VIII (71.8 mg)]. This might be due to the transport of Fe^{+3} through cell membranes to be accumulated and/or metabolised inside the cells.

Thereafter, the four most efficient strains were identified according to **Sneath *et al* (1986)** and **Holt *et al* (1994)** and confirmed by Biolog system as *Corynebacterium jeikeium, Pseudomonas putida* biotype A, *Acinetobacter calcoaceticus* and *Acidovorax delafieldii*.

The four selected and identified isolates were inoculated in nutrient broth containing 100 ppm of Pb^{+2} and Fe^{+3} separately, and incubated under both static and shaking conditions for 24 hours at 35^{o}C. Result in Table (4) cleared that shaking lowered the capacities of both Pb^{+2} and Fe^{+3} uptake by the four tested isolates. Uptake inhibition % of (Pb^{+2} and Fe^{+3}) by *C. jeikeium, P. putida, Acient. Calcoaceticus* and *A. delafieldii* were 22.5 & 35.7; 35.8 & 55.4; 17.3 & 50 and 43.6 & 43.3 %, respectively. This result may be attributed to that under shaking condition the soluble heavy metal ions become unavailable to come in contact with active sites to undergo binding on cell surface or to accumulate intracellulrly by active transport system. Shaking created a condition for more availability of nutrients for the microbe and metal uptake process is linked to external metal

Table 3. Iron uptake by the nine tested isolates

Isolate code number	I	II	III	IV	V	VI	VII	VIII	IX
Amount of Removed Pb^{2+} (mg)	71.8	22	68	64	8	14	7	71.8	54.4
%	72.96	22.36	68.1	65.04	8.13	14.23	7.11	72.96	55.28
Pb^{2+} mg/g dry cells	0.50	0.14	0.51	0.45	0.06	0.10	0.05	0.52	0.40

Control % = 98.4

Table 4. Effect of shaking on lead and Iron uptake by the 4 selected isolates

Isolates	Pb^{+2} uptake %			Fe^{+3} uptake %		
	Static	Shaking	Inbibition %	Static	Shaking	Inhibition %
Cory. Jeikeium	41.3	32	22.5	71.5	46	35.7
P. Putida	71.6	46	35.8	68.4	30.5	55.4
Acinet. Calcoaceticus	78.5	64.9	17.3	63.8	31.9	50.0
A. delafieldii	65.1	36.7	43.6	72.7	41.2	43.3

concentration and not to substrate transport across membranes **(Gadd & Mowll 1983 and White & Gadd 1987)**. Environmental redox potential is an important determinant of microbial growth and metabolism. Aeration resulting from shaking increases the redox potential of nutrient medium due to presence of excess free oxidizing radicals undoubtedly interacting with pH causing its shift towards alkalinity **(Silliker *et al* 1980)** and so altering heavy metal uptake which is a pH dependent process.

Examining the effect of increasing concentrations of Pb^{+2} and Fe^{+3} on metal biosorption process by the four selected strains (Table 5) revealed that the capacity of Pb^{+2} uptake generally decreased as the concentration of lead increased in the broth medium. At low concentrations there are few metal ions compared with large cell surface, thus all the present cations could be adsorbed on the active sites on cell surface. On the contrary, at high concentrations competition is created between cations toward active sites

Table 5. Effect of increasing concentration of Pb^{+2} and Fe^{+3} on biosorption process by 4 selected isolates

Isolates / Con. In ppm	Pb^{+2} uptake %					Fe^{3+} uptake %				
	100	200	300	400	500	100	200	300	400	500
Cory. Jeikeium	42.9	40.2	39	31	24	72.4	65.4	64	57	54
P. Putida	72.6	70	68	59	42	68	64.3	60	54	54
Acinet. Calcoaceticus	78.9	76	52	46	37	64	58	56	50	50
A. delafieldii	68.6	66.4	62.2	54	45	73.1	70	66	64	61

of surface adsorption. Thereby, not all metal ions could be adsorbed leaving a residual amounts increasing by further increase of Pb^{+2} concentration **(Pons and Fuste 1993)**. In a similar manner **Chu *et al* (1999)** reported that uptake capacity of lead by *Anabaena cylindrical* and *Chlorella sp*. decreased as concentration of lead increased over 100 ppm.

100 ppm of both Pb^{+2} and Fe^{+3} is the best concentration at which maximum biosorption accompanied by heavy metals resistance occurred so it was chosen to complete further studies.

Studying the effect of incubation temperatures on heavy metal uptake Figure (3) indicated that optimum temperature achieving maximum Pb^{+2} uptake under static condition was 25°C for *C. jeikeium, P. putida* and *A. delafieldii* and 35°C for *Acinet. Calcoaceticus*. Meanwhile, 25°C was the preferred temperature for the four selected strains to uptake highest amounts of Fe^{+3} from solutions (Figure 4).

Figure (5&6) revealed that maximum amounts of Pb^{+2} and Fe^{+3} uptake were attained at pH 5 for the selected and examined strains. This proves that heavy metals biosorption process is a pH dependent process. Divalent positively charged ions are solubilized completely in the solution at acidic pH **(Chang and Huang 1998 and Sharma *et al* 2000)**. In the acidic range the heavy metal ions become available to bind the negatively charged active site or passively diffuse through cell membrane.

It could be said that optimum environmental and cultural conditions for efficient metallic ions uptake differ from group to group. **Aksu *et al* (1991)** and **Sag and Kutsal (1995)** reported that incubation temperature 25°C to 45°C and pH 4.5 to 5 achieved maximum biosorption of Pb^{+2}, Ni^{+2} and Cu^{+2} by different bacterial strains.

Results in Table (6) show the inhibitory effect of added Cu^{+2} (50 ppm) on biosorption process of both Pb^{+2} and Fe^{+3}.

Effect of incubation temperature on heavy metals uptake by the four selected strains

Effect of pH values on heavy metals uptake by the four selected strains

Table 6. Effect of addition of Cu^{2+} cations (50 ppm) on Pb^{2+} & Fe^{3+} uptake %

Isolates	Pb^{+2} uptake %			Fe^{+3} uptake %		
	100 ppm Pb^{2+}	100 ppm Pb^{2+} + 50 ppm Cu^{2+}	Inhibition %	100 ppm Fe^{3+}	100 ppm Fe^{3+} + 50 ppm Cu^{2+}	Inhibition %
Cory. jeikeium	69.9	40	42.8	76.5	42	45.1
P. putida	80.5	41.5	48.5	72.4	51.3	29.1
Acinet. calcoaceticus	82.3	52	41.10	78.3	45.3	42.1
A. delafieldii	72.4	40.3	44.3	76.3	53.7	29.6

It is clear that the inhibition % of Pb^{+2} uptake by *C. jeikeium, P. putida, Acient. calcoaceticus* and *A. delafieldii* are 42.8, 48.5 , 41.1 and 44.3%, respectively. Similarly, the inhibition percentage of Fe^{+3} biosorption by the four tested strains are 45.1, 29.1, 42.1 and 29.6%, respectively. This may be attributed to competition between Cu^{+2} and those of Pb^{+2} and Fe^{+3} for the adsorption sites on outer cell surface and thus prevent the accumulation of Pb^{+2} or delay to an extent the absorption of Fe^{+3}. Results reported by **(Blackwell *et al* 1995; Mogollon *et al* 1998 and Chang & Huang 1998)** are in agreement with the present findings.

Tap water sample was re-sterilized by autoclaving and supplemented with 100 ppm Pb^{+2} and Fe^{+3} to be used as cell-metal contact medium. Chemical and physical characters are recorded in Table (7). 100 ml of prepared water were transferred to conical flasks then inoculated with about 10^7 cells/ml of the our bacterial strains, separately. The removal efficiency of the two metal ions by the four tested strains is recorded in Table (8). It seemed that biosorption processes are greatly affected in tap water compared with results obtained when nutrient broth was used as metal-cell contact medium. Compared to maximum heavy metals uptake under optimum conditions, inhibition percentage of Pb^{+2} uptake in tap water sample by *C. jeikeium, P. putida, Acinet. calcoaceticus* and *A. delafieldii* are 64.2, 57, 65.2 and 60.7%, respectively. Concerning Fe^{+3}, biosorption process was much more inhibited recording inhibition percentages 77.6, 79.8, 75.1 and 84.3% for the four tested strains, respectively. Thus, it can be hypothesized that Fe^{+3} uptake is a metabolism dependent process since water is not a nutrient medium and so bacterial cells would be either in dormant stage or extended lag phase. Despite the observed decrease in Pb^{+2} up take in the drinking water medium yet it is still more than in case of Fe^{+3} giving the possibility that it is a metabolism independent process. Previous reports had recorded the accelerated biosorption of heavy metals in enriched nutrient media over that in water

Table 7. Heavy metal analysis of the drinking water sample used as a medium for metal-cell contact

Parameter	Concentration (mg/l)
Ph value	8.4
Cadmium (0.005 mg/1)	0.0021
Zinc (0.005 MG/1)	0.0028
Cobalt (0.00 mg/1)	0.0042
Chromium (0.05 mg/1)	0.0324
Copper (0.02 mg/1)	0.0361
Iron (0.03 mg/1)	0.0535
Manganes (0.1 mg/1)	0.1241
Nickel (0.005 mg/1)	0.0048
Molybdenum (0.01 mg/1)	0.0155
Lead (0.05 mg/1)	0.0543
Selenium (0.01 mg/1)	0.0132
Sodium (0.1 mg/1)	0.5121
Aluminum (0.01 mg/1)	0.00244
Arsenic (0.01 mg/1)	0.0027
Barium (0.005 mg/1)	0.00546

Table 8. Heavy metals up take from drinking water as metal-cell contact medium by 4 tested isolates

Ioslates	Pb^{2+}		Fe^{+3}	
	Uptake %	Inhibition %	Uptake %	Inhibition %
Cory. Jeikeium	25.22	64.2	17.11	77.6
P. Putida	34.61	57	14.66	79.8
Acinet. Calcoaceticus	30.7	65.2	19.5	75.1
A. delafieldii	28.3	60.7	12	84.3

(Panchanadikar & Das 1994; Pradhan & Levine 1995; Zouboulis *et al* 1997 and Gulay *et al* 2002).

Also, the above result could be due to the competition between metallic ions already present in the drinking water sample (as shown in Table 7) and the added ions (Pb^{+2} and Fe^{+3}) for binding to active sites on cellular membranes **(Cotoras *et al* 1992 and Chang & Huang, 1998)**.

The cell hydrolysates of the four tested strains were prepared for plasmid profile test; stained with ethidium and injected in a horizontal agarose gel in lanes 2,3,4 and 5 against two markers, namely Hind III and 1 kb DNA in Lanes 1 and 6. Photo (1) showed the bands reusulting from the electrophoresis and comparing their Rf values with those of markers, the hydrolysates of *C. jeikeium*, *P.Putida* and *A. delafieldii* appeared to be free from plasmid DNA. It could be concluded that the genetic character of heavy metal resistance in their case is encoded chromosomal. Several reports proved that many bacterial strains showing heavy metal resistance were plasmidless and metal resistance character was conferred by genetic elements of chromosomal DNA **(Surowitz *et al* 1948 and Witte *et al* 1986)**. On the contrary, *Acinet. Calcoaceticus* contained plasmid and the band resolved on agarose gel marked at Rf matching with size 23. 130 kb. In agreement to this result several authors proved the presence of plasmid in some heavy metals resistant bacteria. ***Simon (1996)*** reported that bacterial plasmids encoded resistance systems for several toxic metal ions inculding Ag^{2+}, As^{2+} Cd^{2+} Co^{2+}, Cr^{2+}, Hg^{2+}, Pb^{2+}, Sb^{2+}, Te^{2+}, T^{+} and Zn^{2+}. **Ramussen and Sorensen (1998)** found that plasmids were present in 62% of isolated bacteria from mercury polluted sediment.

Acinet. Calcoaceticus was chosen for electron microscope examination being the most efficient strain in Pb^{+2} and Fe^{+3} biosorption recording 88.3% and 78.3 % uptake under optimum conditions, respectively. The cells were grown for 24 hours at 35^{o}C in presence of 100ppm Pb^{+2} and at 25^{o} C in presence of 100 ppm Fe^{+3} under static condition, Electron photographs of treated cells with Pb^{+2} (photo no. 3) showed surface accumualtion of the metal ions occurring as dark mesh including black spots on surface of celles and in the intercelluar spaces. Meanwhile, treated cells with Fe^{+3} (photo no.4) showed normal cell appearance accompanied by even distribution of metal ions in the cytoplasmic area occurring as dense black patches accumulating at cell membrane boundaries.

Thus, it could be said that Pb^{+2} biosorpotion is a metabolism independent process resulting in surface accumulation of the heavy metal ions. This kind of biosorption is relatively rapid and reversible, explaining this process **Brierly (1990); McLean & Beveridge (1990) and Aksu *et al* (1992)** suggested that surface accumulation of metal ions is due to chemical complexation with negatively charged functional groups such as hydroxyl; sulphdryl and carboxyl groups. **Kuyucak and Volesky, (1988)** reported that divalent ions might exchange with counter positive ions of the extracellular polysacchrides forming salts on outer cell surface. **Aksu *et al* (1992)** also hypothesized that physical adsorption occurred by the help of Vander Waal's forces due to electrostatic interactions between metal ions in solutin and cell walls of microbial cells. Several authors reported the surface

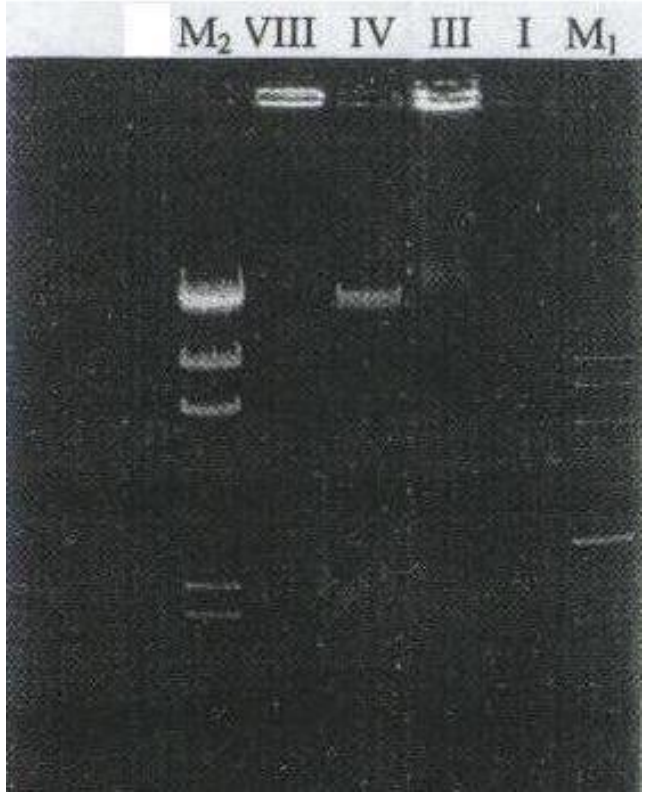
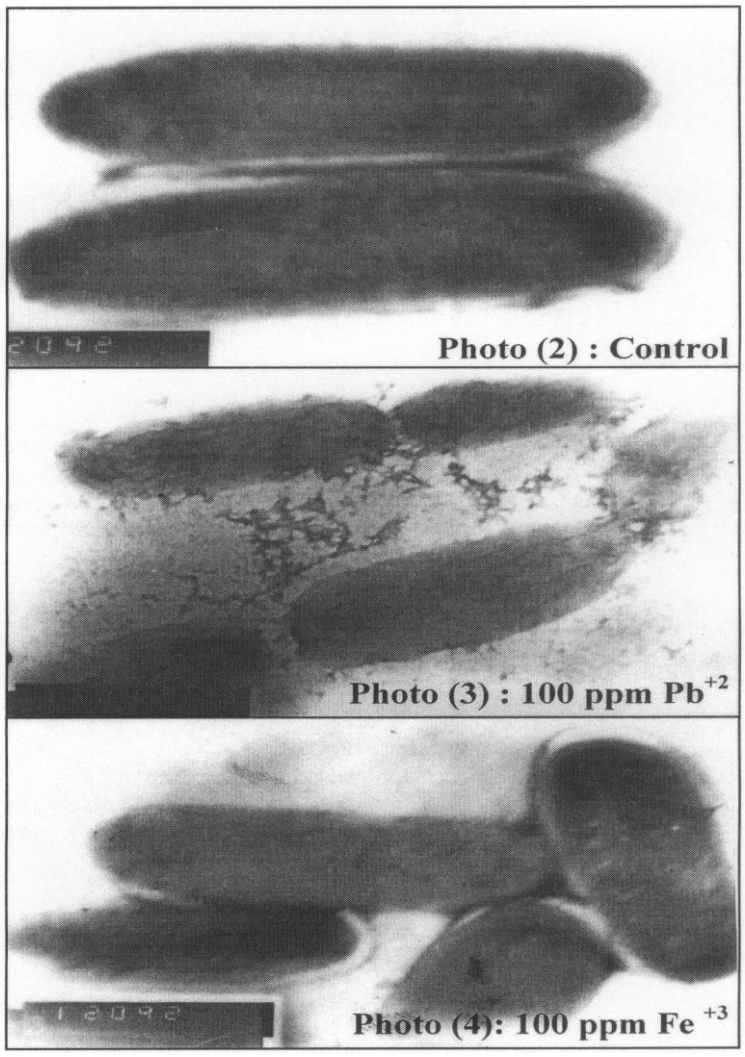


Photo 1. Agarose gel electrophoresis for plasmid profile of the four selected strains

Lane 1 : X Hind III marker
Lane 2 : *A. delafeldii*
Lane 3 : *Acinet. calcoaceticus*
Lane 4 : *P. putida*
Lane 5 : *C. jeikieum*
Lane 6 : 1 kb DNA marker

Ultrastructure of *Acinet. calcoaceticus*



accumulation of lead by *Anabaena cylindrica, Bacillus subtilus* and *Bacillus licheniformis* **(Swift and Forcinii, 1996). Sigg (1987)** explained the intracellular accumulation of metal ions with bacterial cells as a result of interaction with suface ligands followed by slow transport into the cell. Also, **Singletion (1997)** reported that ions may be transported by means of a periplasmic substrate binding proteins that binds the ions and pass them to protein complexes in cytoplasmic membrane which in turn transfer them into the cytoplasm.

As a conclusion in could be said that bacteria naturally inhabiting water may be used as a potential alternative in bioremdiation of drinking water contaminated with lead causing health hazard and iron causing severe problems in drinking water lines and pipoes.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ،١٤(١) ، ١٤٧-١٧٢ ، ٢٠٠٦

الامتصاص الحيوى للمعادن الثقيلة بواسطة بعض السلالات البكتيرية المعزولة من مياه الشرب من أماكن مختلفة فى محافظة الشرقية [١٠]

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تم اختيار تسعة سلالات بكتيرية وهى الأكثر تواجدا من ضمن ١٢٧ مستعمرة تم عزلها من عشرة عينات من مياه الشرب تم جمعها من مناطق مختلفة بمحافظة الشرقية اختيرت قدرة السلالات المنقاة على تحمل ومقاومة التركيزات المرتفعة من أيونات معدن الرصاص والحديد وقد وجد أن أربعة سلالات من التسعة ذو الأرقام الكودية I, III, IV and VIII لهم قدرة عالية على الامتصاص الحيوى لأيونات الرصاص والحديد من البيئة المحتوية على ١٠٠جزء فى المليون عرفت السلالات عن طريق التوصيف المورفولوجى والفسيولوجى والأنشطة الكيميائية الحيوية وبالاستعانة بتقنية البيولوج وأتضح أنهم: كورينبكتيريوم شيكيوم وسيدوموناس بيوتيدا وأكينيتوبكتر كالكواسيتيكس واسيد رفوراكس ديلافليدى .

التركيزات المتزايدة من ايونات الرصاص (١٠٠إلى ٥٠٠ جزء فى المليون) كان لها تأثير ضار على عملية الامتصاص الحيوى لايونات الرصاص حيث انخفضت نسبة إزالة الرصاص من ٤٠,٩ إلى ٢٤% ومن ٧١,٦ إلى ٤٢% ومن ٧٧,٨ إلى ٣٧% ومن ٦٥,١ إلى ٤٥% فى حالة السلالات الأربعة السابقة على التوالى فى نفس الوقت لم يكن هناك فروق معنوية فى حالة إزالة الحديد من الوسط فى وجود التركيزات المتزايدة بالنسبة للأربع سلالات.

وبدراسة الظروف المزرعية وجد أن الظروف المثلى لأعلى سعة إزالة لأيونات المعادن المختبرة كانت عند تركيز ١٠٠ جزء فى المليون بالنسبة للعنصرين وعند الأس الأيدروجينى ٥ وتحت ظروف تحضين ساكنة ودرجة حرارة ٢٥°م لكل العزلات بالنسبة للعنصرين ما عدا فى حالة بكتريا أكينيتوبكتيريوم كالكواسيتيكس فهى تحتاج لدرجة حرارة ٣٥°م لأقصى إزالة لعنصر الرصاص .

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