ISSN 1996-0700

Asian Journal of Biotechnology

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Asian Journal of Biotechnology 2 (2): 99-109, 2010
ISSN 1996-0700

Investigation of Heavy Metals Biosorption on *Pseudomonas aeruginosa* Strain MCCB 102 Isolated from the Persian Gulf

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Abstract: In the present study, uptake of heavy metal ions by *Pseudomonas aeruginosa* strain MCCB 102 isolated from the Persian Gulf was investigated in two single and multi-mix forms. The highest adsorption was observed for Cu, Zn, Cd and Pb, respectively. Removal of metals was maximized when the metal ions was in a single form. Scanning Electron Microscope (SEM) analysis showed *Pseudomonas aeruginosa* strain MCCB 102 accumulated heavy metals in the cell wall and along the external cell surfaces. This suggested that heavy metals uptake involves both surface phenomena and diffusion. EDX and SEM studies emerged as the best approach to monitor heavy metal adsorption on the bacteria cells and as an alternative method for measuring heavy metals in the bacteria. Energy Dispersive X-ray (EDX) analysis was less sensitive and less reliable compared to atomic absorption analysis, but was rapid and easier to direct heavy metals in the bacteria.

Key words: EDX, SEM, cell modification, accumulation, biosorption

INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today. In non-marine environment, heavy metals are discharged into the environment through mining, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and application, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation etc. Thus, metal as a kind of resource is becoming shortage and also brings about serious environmental pollution, threatening human health and ecosystem (Wang and Chen, 2009).

The Persian Gulf is one of the most contaminated areas in the world its environment has been stressed by several sources that include wars, oil spills, fishing, port activities, tanker transportation, polluted rivers and effluents of industrials waste water (ROPME, 1991). It is

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the busiest tanker route in the world, about 850 ships pass to the Persian Gulf through the Hormuz strait each day. This busy traffic has meant that oil transportation, shipping, port activities and industries (i.e., power plants, refinery, boilers, mining and electroplating) will discharge aqueous effluents containing relatively high levels of heavy metals (cadmium, lead, zinc and copper) into the Persian Gulf.

Three-dimensional structure of the colonies and inter-relationships of the constituent organisms can be ideally studied using the Scanning Electron Microscope (SEM). This technique can examine the surface modification more quickly, accurately and directly (Goodhew *et al*., 2001). However, SEM not only has the advantage of continuously variable useful magnification, it also has a considerably greater depth of field than the optical microscope.

The surface morphology of individual organisms could be studied under SEM by a variety of techniques (Kuo, 2007). SEM and Electron Microprobe Analysis (EMPA) have proved useful tools in localising uranium in macrolichens (Trembley *et al*., 1997; Haas *et al*., 1998). Experimental studies have identified the hydrophilic matrix of cell walls in lichens as important uranium binding sites (Purvis *et al*., 2004). The techniques were identified uranium and P-bearing acicular nanocrystals along cell walls and within the extracellular gelatinous mucopolysaccharide matrix and within proteinaceous organelles, concentric bodies, in *Peltigera membranacea* (Suzuki and Banfield, 1999).

The mineralogical characterization of the mineral precipitate was performed by X-ray powder diffraction (XRD) with a diffractometer (Philips; model PW 1710) with CuKa radiation. The mineral was determined to be crystalline hydrozincite [$Zn_5 (CO_3)_2 (OH)_6$]. Minor minerals, such as quartz and calcite, were also observed. XRD spectra also showed a significant background noise, which was due to the presence of organic matter (Podda *et al*., 2000).

The effects of heavy metals in environment would be very well visualized by Scanning Electron Microscopy (SEM). They can also be analyzed by Energy-Dispersive X-ray analysis (EDX) for confirmation and analysis of the heavy metals (Goodhew *et al*., 2001). The use of SEM and EDX is rather suitable to recognize of the heavy metals modification on the bacteria cells to direct (SEM) an indirect (EDX) assay of the heavy metals consequence. The study of heavy metals effects on the bacteria cells during biosorption is important to understand the fate of uptake and bacteria modification. Several principal sites of heavy metals complex formation in biological systems have been indicated, including accumulation in the cell wall, carbohydrate or protein polyphosphate-uranium complexation, complexing with the carboxyl group of the peptidoglycans in the cell wall, or entering into cells via an energy-dependent mechanism (Silver, 1991).

The surfaces of bacteria cells are functional groups that act as sorption sites for special components, including heavy metals in the marine environment. An understanding of the surface modification and adsorptive properties of the bacteria cells are necessary to predict how bacteria cells contribute to the environment alterations. Because of their high sorption capacities and low production costs, the application of bacterial biomasses has also attracted the attention of specialists in the field of water treatment (Chubar *et al*., 2008).

The SEM and EDX analysis are important for detection of heavy metals modification on the bacterial isolates during biosorption. On the other hand, the study provides excellent photographic evidence that demonstrate bacteria could bind and remove heavy metals from the environment. The implication of such achieves on the bacteria itself could be researched. This study was designed to identify the modification of heavy metal removal bacteria cells, that isolated from the Persian Gulf upon metal absorption and to study X-ray diffraction (EDX) system for detection of heavy metals on the bacteria cells.

MATERIAL AND METHODS

Pseudomonas aeruginosa strain MCCB 102, already identified as *Pseudomonas aeroginosa* ATCC 27853, was most frequent among isolated bacteria from the Persian Gulf in the summer of 2001 (Zolgharnein *et al*,. 2007). The *P. aeruginosa* strain MCCB 102 was chosen for the full scanning of heavy metals on the surface of the bacteria by using SEM and EDX.

Stationary-phase cells of *P. aeruginosa* strain MCCB 102 were inoculated into separate minimal salt solution media which had already been supplemented with $CuSO_4$, $ZnSO_4$, $Cd(NO_3)_2$, $PbNO_3$ and the control sample containing no heavy metals. The final metal concentrations were 0.5, 1, 1.5, 2, 0 mM, respectively. Each concentration was run in M.S.S media. The cultures were incubated at 28°C and agitated at 200 rpm. The growth rate was measured by optical density. The culture turbidity was measured using a spectrophotometer upon calibration of the Optical Density (OD) in a 1 cm cell at a 600 nm wavelength. These analysis were repeated twice for each organism. Growth rate experiments were performed in batch cultures of M.S.S medium (100 mL medium in a 500 mL flask). The growth was monitored at OD_{600} every 60 min.

Pseudomonas aeruginosa strain MCCB 102 was grown in 500 mL flasks containing Minimal Salt Solution (MSS) media that comprised 0.03% glucose 0.03% yeast extract as carbon and nitrogen sources respectively and mineral salt medium (4 g of K_2HPO_4, 10 g of KH_2PO_4, 6 g of $(NH_4)\ SO_4$, 0.2 g of NaCl, 0.026 g of $CaCl_2\ 2H_20$, 0.9 g of $MgSO_47H_2O$, 0.004 g of $MnSO_47H_2O$, 0.04 g of $FeSO_47H_2O$. The media were supplemented with 1mM of heavy metals, separately and 1mM of the multi-mix heavy metals, together. About 0.1 mM of fresh bacterial culture inoculated into the minimal salt solution and then incubated at 28°C over night.

Bacteria cells were harvested by centrifugation at 9000x g for 20 min at 4°C minimums washed twice with de-ionized water. The cells were suspended in de-ionized water to a final concentration of 2.5 mg dry weight mL^{-1}. Forty milliliter of the suspension were added to 160 m; of selected concentrations of $CuSO_4$, $ZnSO_4$, $Cd(NO)_3$ and $Pb(NO)_3$ separately. The suspensions were incubated at room temperature on a shaker with a speed of 100 rpm for 1 h then centrifuged at 9000x g for 20 min. The bacterial cells were heated to 105°C overnight in an oven. After heating, the dry weight was measured.

Two milliliter of nitric acid were added into 25 mL plastic bottles that were previously rinsed with nitric acid and washed with de-ionized water. Sixteen mg of dried bacterial cells were added into the concentrated nitric acid separately and incubated in a water bath at 100°C for 1 h. The mixture was later cooled to 25°C. The volume of the mixture was raised to 20 mL with distilled water and the concentration of heavy metals was measured by flow injection atomic absorption spectrophotometry (Massadeh *et al*., 2005). Determination of copper, zinc, cadmium and lead was done by using a special lamp for each metal at a specific wavelength.

All data were presented as mean values of three replicates. Two way ANOVA analysis were performed with the statistical program SPSS 13, to fit the data obtained for the heavy metal toxicity experiments.

Pseudomonas aeruginosa strain MCCB 102 was grown in 500 mL flasks containing M.S.S media to which 1 mM Zn, 1 mM Cu, 1 mM Cd and 1 mM of Pb was added separately. Bacteria cultures were incubated at 28°C overnight.

Cultures were transferred to typical laboratory tubes (in a 15 mL glass tube). The bacteria cells were then collected through a 3 min centrifugation at a speed less than 2500 rpm.

After centrifugation the supernatant was carefully removed by using disposable pipettes and was then added to a buffer containing 0.1 M of sodium cacodylate buffer. Before washing with sodium cacodylate, 3 changes occurred.

Two milliliter of 1% osmium tetroxide was added to the sample for a duration of 90 min. Then the sample was washed with 0.1 M sodium cacodylate buffer, resulting in three changes, The tube was left in the fume hood for 30 min before it was covered with parafilm and briefly centrifuged at 2500 rpm again. The osmium tetroxide solution was removed with a disposable pipette.

The sample was dehydrated by re-suspending it in a series of acetone solutions increasing in concentration. Solutions of 35, 50, 75, 95 and 100% acetone were used. This was accomplished by gently centrifuging and re-suspending the sample as described in the washing procedure. The sample remained a minimum of 10 min in the first four acetone concentrations. It remained in the 100% acetone solution for a sum of 15 min in three intervals. The sample was centrifuged and the final 100% acetone supernatant was discarded.

After dehydration, samples were transferred onto aluminum foil where the specimens were basked and then placed into the critical dryer for 30 min.

After drying, the specimen was carefully stuck onto a stub using sticker a stud. Then, it was stored in a closed container.

The sample was coated with a very thin layer of gold alloy to minimize charging during the observation of the bacteria using the SEM. Therefore, the specimen was coated by very thin gold layer. The SEM and EDX specimens were analyzed with Jeol JSM-6400 with a Link EDX system (operating voltage 15 kV).

RESULTS

The metal response experiments were carried out in a minimal salt solution, which mimic the sea water in the Persian Gulf. *P. aeruginosa* strain MCCB 102 exhibited different growth patterns in the presence of different heavy metals. Examined bacteria increased its growth and reached its maximum growth at 13 h. A decrease in growth (measured in terms of optical density) was observed upon increasing metal concentration at any given time interval in comparison to the control without metal amendment ($p<0.05$).

Pseudomonas aeruginosa strain MCCB 102 was observed to have the highest accumulation of Cu around 70.4 mg g^{-1} dry wt. of cells, when it was exposed in 1 mM of concentration. While the lowest percentage was showed for Pb about 4.5 mg g^{-1} dry wt. of cells, when it exposed in 0.5 mM of concentration at Multi-mix (multi heavy metal) condition.

The growth curve of *P. aeruginosa* strain MCCB 102 in the different concentrations of heavy metals and untreated media (without heavy metals) as a control sample were exhibited in Fig. 1a-d. The growth of *P. aeruginosa* strain MCCB 102 in the presence of 0.5 mM Cu, Zn, Cd and Pb illustrated a lag phase as well as in the control sample. There was no difference in growth between the control sample and heavy metals containing media. In the presence of 1 mM of Zn and 1 mM of Cd, there was no difference between the growth of *P. aeruginosa* strain MCCB 102 and the control sample. When it grew in the presence of 1 mM of Cu and 1 mM of Pb, the growth of bacterium decreased. The concentration of Cu and Pb was increased to 1.5 and 2 mM, respectively. As a result, the growth of *P. aeruginosa* strain MCCB 102 was decreased by Pb and was completely inhibited by Cu.

The accumulation of lead, Cadmium, Zinc and Copper in 0.5 and 1 mM concentration are shown in Table 1. It was found that the amount of heavy metals taken up by the bacteria cell slightly increased, when the concentration of heavy metals was increased from 05 to 1 mM. The growth of *P. aeruginosa* strain MCCB 102 cells in untreated media (without heavy metals) as a control sample was shown in Fig. 2a and b.

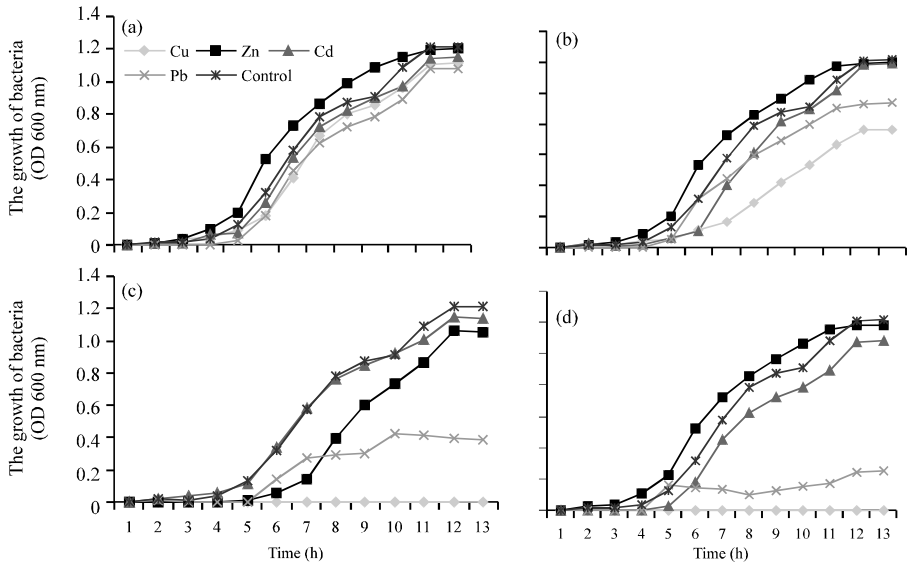

Fig. 1: The growth of *P. aeruginosa* strain MCCB 102 in (a) 0.5 mM of heavy metals, (b) 1 mM of heavy metals, (c) 1.5 mM of heavy metals and (d) 2 mM of heavy metals of Cu, Zn, Cd, Pb and without heavy metals as the control

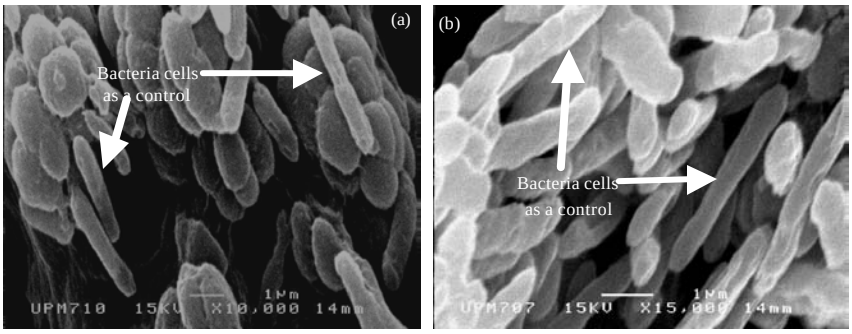

Fig. 2: SEM photomicrograph of *P. aeruginosa* strain MCCB 102 cells when grew in untreated media (without heavy metals) as a control sample for (a) over night and (b) after four days

Table 1: Heavy metals uptake by isolates bacteria at 0.5 mM and 1mM of concentration when exposed single and multi-mix conditions

	Uptake of heavy metals by bacteria			
Treatment concentrations	Cu mg g^{-1} dry weight of cells	Zn mg g^{-1} dry weight of cells g	Cd mg g^{-1} dry weight of cells	Pb mg g^{-1} dry weight of cells
0.5 mM of Multi-mix	9.05	5.58	6.8	4.50
1 mM of Multi-mix	8.30	8.60	6.8	7.30
0.5 mM of Single	60.12	49.23	41.2	54.16
1 mM of Single	70.40	65.90	58.5	42.80

The effect of copper on bacterium cells is illustrated in (Fig. 3a, b). Bacterium cells showed some modification when grown in the presence of 1 mM of copper for 12 h. The cells

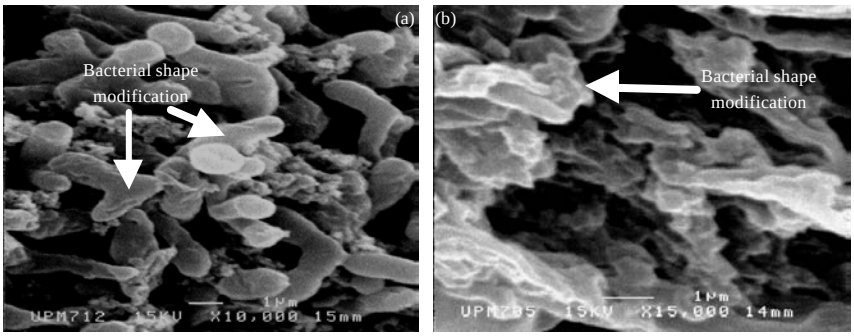

Fig. 3: SEM photomicrograph of *P. aeruginosa* strain MCCB 102 cells when exposed to 1 mM copper for (a) over night and (b) four days indicate bacteria shape modification and copper aggregates associated with the cells

Table 2: EDX analysis of Cu, Zn, Cd and Pb in the *P. æruginosa* strain MCCB 102

Metal treated	a	b	c	Mean
Cu	2.32	2.45	1.96	2.24
Zn	0.69	1.90	1.48	1.35
Cd	10.41	10.41	10.41	10.41
Pb	6.50	6.50	6.50	6.50

appeared clumped together. EDX investigation revealed that the aggregates were copper elements, with maximum and minimum copper in the bacterium colonies at 2.48 and 1.96 %, respectively (Table 2). SEM demonstrated two different shapes of *P. aeruginosa* strain MCCB 102, when it was exposed to copper overnight and later, for four days (Fig. 3a, b). Figure 3a shows the shape of bacterium cells that had slightly changed when *P. aeruginosa* strain MCCB 102 grew in the presence of copper overnight, as some bacterium cells became attached to others. The accumulation of heavy metals on the surface of bacterium cells formed cell bunches and the shape of bacterium cells changed completely, when the incubation time was increased to four days (Fig. 3b).

Scanning Electron Microscopy (SEM) was used to examine bacterium cells after an overnight and a four-day exposure to 1 mM of zinc (Fig. 4a, b). It was illustrated that zinc elements did not change the surface of bacterium cells, as observed in copper. In fact, it did not show any change on the surface of the bacterium. However, the four-day exposure brought changes to the surface of the bacterium, making them appear wrinkled. EDX analysis showed that the amount of zinc on the bacterium cells was a maximum of 1.9 and a minimum of 0.69%, respectively (Table 2).

Following exposure to 1 mM of cadmium on the cell surface showed different modifications (Fig. 5a, b). Some clumps of materials were observed attached to the bacterium cells, but this was not demonstrated upon exposure to 1 mM of cadmium overnight. EDX analysis showed the amount of cadmium at high levels of 10.41% of bacterium component (Table 2). Although, EDX randomly examined two areas on bacterium colonies, it detected similar amounts of cadmium at two different places of bacterium colonies.

Exposure to 1 mM of lead overnight and, later, during a four-day period, showed clear changes on the bacterium surface (Fig. 6a, b). The lead precipitated on the bacterium cells, where it completely covered the surface of the bacterium cells. The use of EDX for the analysis of lead on the bacterium cells confirmed the presence of lead. The two areas selected for EDX analysis revealed a similar rate of lead, 6.5% of the total components (Table 2).

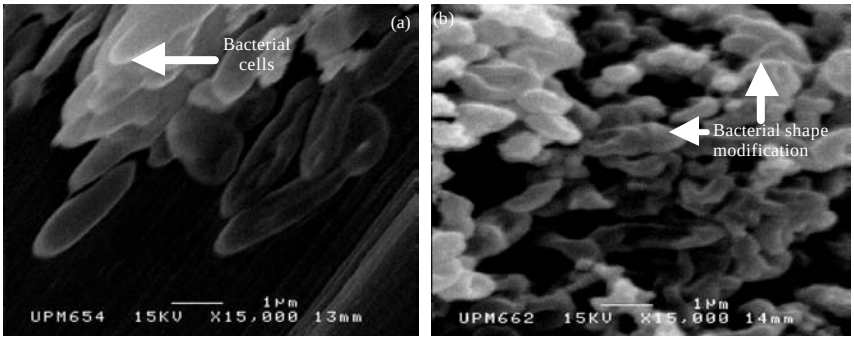

Fig. 4: SEM photomicrograph of *P. aeruginosa* strain MCCB 102 cells when exposed to 1 mM zinc for (a) over night and (b) four days indicates bacteria shape modification and zinc aggregates associated with the cells

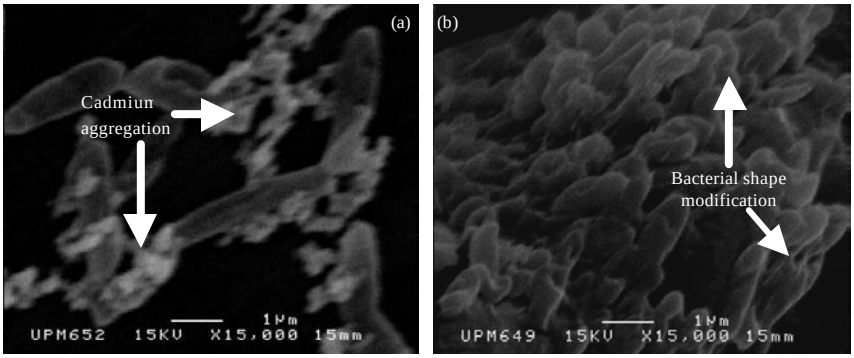

Fig. 5: SEM photomicrograph of *P. aeruginosa* strain MCCB 102 cells when exposed to 1 mM cadmium for (a) over night and (b) four days indicates cadmium aggregation and bacteria shape modification

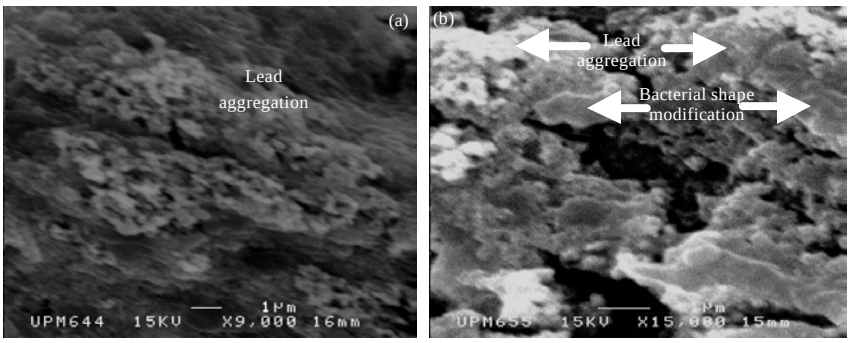

Fig. 6: SEM photomicrograph of *P. aeruginosa* strain MCCB 102 cells when exposed to 1 mM lead for (a) over night and (b) four days indicate bacteria shape modification and lead aggregates associated with the cells

DISCUSSION

From a biological point of view, heavy metals can be divided into two categories; essential and non-essential. However, high concentrations of essential heavy metals have also been reported to be toxic. Many of these metals have a direct influence on various physiological and biochemical processes, including reduction in growth, or inhibition of enzyme activities (Abalde *et al*., 1995). Meanwhile, some heavy metals, such as manganese, iron and zinc are essential micronutrients and are frequently referred to as trace metals. They may limit microbial growth if their concentrations are too low, but they can be toxic at high concentrations.

It is well known that the growth inhibition of some *P. aeruginosa* strains at up to 1 mM concentration (Nies, 1999; Spain, 2003). Copper showed more toxicity than other heavy metals (Teitzel and Parsek, 2003). This study showed the effects of heavy metal toxicity to *P. aeruginosa* was Cu > Pb > Cd > Zn, when the tolerance of the three metals were compared with each other, it was evident that the examined bacterium was more sensitive to Cu than the other metals. It was also revealed that *P. aeruginosa* strain MCCB 102 can tolerate high concentration levels of zinc (2 mM). This result is in agreement with the findings of Abskharon *et al.* (2008) who studied the heavy metal resistance of *E. coli* isolated from wastewater sites in Assiut, Egypt.

In the present study the growth of *P. aeruginosa* strain MCCB 102 significantly decreased when it was exposed to more than 0.5 mM of heavy metal concentrations in the treated media. In the lower concentration (0.5 mM), inhibition of bacterium growth was insignificant, which suggests that low concentrations of heavy metals are not toxic to the tested bacterium.

It was found that the amount of heavy metals taken up by *P. aeruginosa* strain MCCB 102 slightly increased with increase in concentration from 05 mM to 1 mM. This result is agreement with the findings of DaCosta and Duta (2001) which reported an increase in uptake with higher concentration.

Many microorganisms, such as bacteria, yeast and algae can take up dissolved metals from their surroundings onto their bodies and can be used for removing heavy metal ions successfully (Asku *et al*., 1991). The first mechanism for removal of heavy metals from environment involves extra-cellular binding. Cationic heavy metals attach to some anionic compounds on the bacteria surface. So binding of heavy metals to the surface of bacterium cells causes changes in the shape of the bacterium.

It is well recognized that microorganisms which, include bacterium, fungi and algae have a high affinity for metals and can accumulate both heavy and toxic metals by a variety of mechanisms highly effective in sequestering heavy metals (Wong *et al*., 1993). The absorption of heavy metals, include Cd and Pb through the extracellular polymeric of bacteria revealed by the Scanning Electron Microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). These bacteria removed over 70% of Cd and 98% of Pb within 72 and 96 h, respectively from the growth medium that had initial metal concentrations of 100 ppm (DeJaysankar and Vardanyan, 2008). The detoxification efficiency for Cd and Pb indicates good potential for their application in bioremediation of toxic heavy metals. In the present study, isolated bacterium from the Persian Gulf showed that they accumulated lead and cadmium at high concentrations. The capabilities of *P. aeruginosa strain MCCB 102* for removing lead was around 54.16 mg g^{-1} of dry weight when it was grown in the presence of a Minimal Salt Solution (MSS).

The modification of bacterium cells and precipitation of heavy metals on the cells was revealed by the SEM and EDX study, respectively. In the present study, the effect of heavy metals on bacterium cells was studied using a Scanning Electron Microscope (SEM) associated with an X-ray system (EDX). This experiment showed that the shape of bacterium cells was modified after the adsorption of the heavy metals (Zn, Cu, Cd and Pb). The SEM observation illustrated the accumulation of heavy metals on the surface of the bacterium cells. These results were in agreement with Hafez *et al.* (1997) which found that uranium and thorium accumulated extracellularly on the surface of *Aspergillus flavus*. Uranium and thorium were accumulated in a dense layer around the surface of *Aspergillus flavus* cells.

The first mechanism for the removal of heavy metals by bacterium cells is the binding of cationic heavy metals on the surface of bacterium cells (Clarke *et al.*, 1987). These complex forms are generally not readily transported into the cell because of their structure and complexity. Secondly, cells can increase the excretion rate of certain metal ions using energy-driven efflux pumps (Ybarra and Webb, 1999). The third resistance mechanism is one of the most important mechanisms by which bacterium combat heavy metal exposure and subsequent accumulation. In *Cyanobacteria*, metal ion sequestration inside the cell is performed by the Class II metallothioneins. Class II metallothioneins are thiol containing, cysteine-rich, metal-binding proteins that sequester metal, thus preventing accumulation of potentially toxic-free metal ions within the cell (Zhou and Goldsborough, 1994). Metal ion binding occurs through the interactions of the ions with the thiol groups of cysteine residues.

He *et al.* (2004) observed the adsorption of Ag/Al_2O_3 on yeast cells by using Scanning Electron Microscopy (SEM) images and showed that the cell surfaces were densely covered with nanograde granules. Some cells collapsed and led to the release of inclusions. These SEM images showed that yeast cell surfaces were dramatically damaged by the Ag/Al_2O_3, which indicates that the inactivation is caused by chemical reaction and decomposition. Generally, in the present study, Scanning Electron Microscopy (SEM) was used to illustrate how some heavy metals were accumulated on and thoroughly covered bacterium cells forming aggregates and clumps on the surface of the bacterium cells. Some heavy metals seem to form dense layers on the bacterium cells. Although, different heavy metals appeared in dissimilar forms after accumulation, all of them changed the shape of bacterium cells when compared with the control bacterium cells. The outer envelope of the bacterium cells may change when grown in the presence of heavy metals (Konstantinidis *et al.*, 2003).

EDX analysis was used in the present study to provide evidence for the accumulation of four heavy metals; copper, zinc, cadmium and lead in *P. aeruginosa* strain MCCB 102. The appearance of surface attachment of heavy metals was investigated by SEM analysis. Both experiments were performed with *P. aeruginosa* strain MCCB 102 growing in a nutrient broth containing four heavy metals (Cu, Zn, Cd and Pb), in an overnight and 4-day stage. The SEM analysis revealed heavy metal accumulation on the surface of the bacterium cells that was confirmed by EDX analysis. This showed that the aggregates consisted of cadmium, lead, copper and zinc. Heavy metal aggregates were observed irregularly on the bacterium cells. There was a significant relationship between the rate of heavy metals on the surface of the bacterium cells and the modification of the bacterium shapes.

Lead was accumulated by isolates at high concentrations when it was analyzed by atomic absorption in the presence of the minimal salt solution, which was even more than cadmium. In the present study, EDX analysis detected high precipitate of cadmium on the bacterium cells. It probably originated from the processing of fixation and the location of accumulation and/or cultural media. However, zinc appeared as low precipitates on the

bacterium cells. When results were compared with untreated bacterium cells as a control group, it was revealed that there were fewer differences between the shapes of the bacterium cells which were grown in the presence of zinc and the controlled bacterium cells. Therefore, the relationship between the accumulation of heavy metals inside bacterium cells and surface attachment were not obtained.

However, when the EDX analysis was compared with that of the atomic absorption results, there were clearly some differences. This difference may be as a result to media preparation for bacterium cell growth, equipment and the conditions of analysis. The fixation of processing in the two mentioned methods of analysis was entirely different. The sensitivity of measurement by atomic absorption is very high, but SEM and EDX are very suitable for the direct observation of heavy metal effects on bacterium cells, but a great problem with this method may be the washing procedure using fixation solutions that remove some heavy metals from the media.

Halttunen *et al.* (2008) established the binding of lead on the surface of *Lactobacillus fermentum* ME3 and *Bifidobacterium longum* 46 using TEM. Transmission Electron Micrographs of *Lactobacillus fermentum* ME3 and *Bifidobacterium longum* 46 taken before and after the lead binding experiments clearly established the presence of lead deposits on the bacteria surface. In the present study, the binding of heavy metals on the surface of *P. aeruginosa* strain MCCB 102 was examined using SEM. The results showed that heavy metals attached to the surface of bacterium and significantly altered the shape of the bacterium compared to the control group.

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