Removal of Lead and Chromium From Industrial Wastewater by Locally*Citrobacter spp*.Isolates

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Abstract

Biosorption is an effective method to remove toxic metals from wastewaters. In this study biosorption of lead and chromium ions from solution was studied using *Citrobacter freundii* and *Citrobacter kosari* isolated from industrial wastewater. The experimental results showed that optimum grwoth temperature for both bacteria is 30°C and the optimum pH is 7 &6 for *C. freundii* and *C. kosari* respectively. While the optimum incubation period to remove Pb and Cr for *C. freundii* and *C. kosari* is 4 days and 3days respectively. Also the biosorption of Pb and Cr in mixed culture of bacteria and mixed culture of Pb and Cr was investigated. Result indicate that uptake of Cr and Pb for *C.freundii, C. kosari* and in mixes culture of both bacteria is 58%, 53% and 82% respectively for chromium in solution containing 1000ppm of Cr and 59%, 63% and 78% respectively for lead in solution containing 1000ppm of lead. But the uptake decreases in solution containing both heavy metals lead and chromium.

Keywords: Biosorption, Lead, Chromium, *C. freundii, C. kosari.*

ازالة عنصري الرصاص والكروم من مياه الفضلة الصناعية بوساطة عزلات محلية من بكتريا

Citrobacter spp.

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الخلاصة

الامتزاز عملية فعالة لازالة المعادن السامة من المياه الملوثة. لقد تم دراسة امتزاز كل من عنصري الرصاص و الكروم باستعمال سلالات من بكتريا *Citrobacterfreundii* و*Citrobacterkosari*المعزولة من مياه الفضلة الصناعية. لقد بينت النتائج ان درجة الحرارة ٣٠°م هي الفضلى لنمو كلا العزلتين، والرقم الهيدروجيني الامثل لنمو العزلتين هو ٦ و ٧ لبكتريا *C.freundii*و*C. kosari* على التوالي. اما فترة الحضانة الملائمة لبكتريا*C.freundii* و *C. kosari* فقد كانت ٤ ايام و ٣ ايام على التوالي.وايضا تم دراسة امتزاز عنصري الكروم والرصاص في وسط يحتوي على كلا العزلتين وايضا على وسط يحتوي على خليط من عنصري الرصاص والكروم. لقد بينت النتائج ان النسبة المئوية لازالة عنصري الكروم و الرصاص لبكتريا*C. freundii* و*C. kosari* وخليط العزلتين كانت ٥٨%، ٥٣%، ٨٢% على التوالي بالنسبة لعنصر الكروم في وسط حاوي على ١٠٠٠جزء بالمليون من الكروم اما بالنسبة للرصاص فقد كانت نسب الازالة ٥٩%، ٦٣%، ٧٨% على التوالي في وسط حاوي على

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١٠٠٠جزء بالمليون من الرصاص اما الوسط الحاوي على خليط من الرصاص والكروم فقد كان الامتزاز اقل من
الوسط الحاوي على احد العنصرين.

Introduction

   Lead and chromium are the major toxic pollutants, which entered the water streams through various industrial operations. Chromium is the major pollutant in the industrial wastewaters of many industries such as leather tanning, pigment plants and electroplating [1]. Lead is used as industrial raw material in the manufacture of storage batteries, pigments, leaded glass, fuels, photographic materials, solder and steel products. Lead and chromium are toxic contaminants, even in very low concentrations. The presence of lead at concentration 2-4 mg/l for short time is not harmful, but if it presence for long time is harmful and very dangerous. The lead is toxic at concentration of 8-10 mg/l, 15 mg/l in the same period and may cause death [2]. Lead poisoning in humans causes severe damage to kidney, nervous system, reproductive system, liver and brain [3].Chromium (VI) is carcinogenic to both humans and animals. Chromium causes the sensitivity of the skin, nasal membranes, and a decrease in the respiratory efficiency [4]. According to the United States Environmental Protection Agency (USEPA) the maximum permissible limits in wastewater and potable water are 0.1 mg/ l and 0.015 mg/ l for lead (II) and 1.0 mg/ l and 0.05 mg/ l for chromium(VI), respectively. The conventional methods for treatment of lead (II) and chromium (VI) wastes include: precipitation, adsorption with activated carbon, ion exchange, membrane processes, oxidation and reduction. These methods are expensive and often generate chemical sludge, whose disposal is problematic [5].

   The biosorption is a process that utilizes low-cost biosorbents to sequester toxic heavy metals.Biosorption has distinct advantages over the conventional methods which include: reusability of biomaterial, low operating cost, selectivity for specific metal, short operation time and no chemical sludge [3]. In the recent years many biosorbents materials of agricultural based have been utilized for heavy metal biosorption. Biosorbents for the removal of metals mainly come under the following categories: bacteria, fungi, algae, agricultural wastes, and various polysaccharide materials. These biosorbents can effectively sequester dissolved metal ions from dilute complex solutions [6].
The objective of this study is to investigate the ability of *Citrobacter freundii* and *Citrobacter kosare* to remove lead and chromium from industrial wastewater.

MATERIALS AND METHODS

Sample collection

   Wastewater samples were collected in screw capped sterilized bottles from Al-tabieaa production paints in Baghdad. The concentration of Pb and Cr mg/ml in wastewater was measuredby the use of atomic absorption, Perkin Elmer Analyst 300.

Isolation the heavy metals-resistant bacteria

   Heavy metal resistant bacteria were determined by plate diffusion method. Heavy metal salt solutions were prepared in different concentrations, about 200, 400, 800 and 1000 mg/L. Each plate was spread with overnight cultures of appropriate organisms. To each of the plate 150 μl of appropriate metal salt solutions were added in each wells of 10 mm in diameter and 4 mm in depth. Nutrient agar plates were incubated at 30°C for 24 h. After incubation, the zone of inhibition was measured. A zone size less than 1 mm scored as resistance strain [7].

Identification of resistance bacteria By using VITEK 2 Compact system(bioMérieux, USA)**:**

Sterile test tubes (ID and AST test tubes) used to prepare inoculums were filled with saline water and placed in a cassette. The identification (ID) test tube was used to prepare inoculum from the pure colonies and mixed thoroughly using a vortex until a suspension of McFarland was formed. The McFarland was determined using Densichek (bioMérieux, USA). A volume of inoculum from the ID test tube was pipetted into the antibiotic susceptibility testing (AST) test tube and mixed thoroughly. The Gram negative (GN) ID test cards and AST test cards were inserted in the respective test tubes and loaded into the Vitek instrument. While in the Vitek instrument, the cards were filled, sealed and incubated in the Vitek 2 system incubator until results were generated by the expert advanced system of the Vitek 2 system for the type of organism.

Factors affecting the biosorption of chromium and lead from the aqueous solutions :
Incubation period :
Fifty ml of nutrient broth at pH 7 with 0.5 ml of 1000 ppm heavy metal solutions (Pb or Cr) were added to the flasks. These flasks were inoculated with freshly prepared 0.5 ml (OD = 05) of bacterial isolates individually. The control containing 50 ml of nutrient broth and 0.5 ml of 1000 ppm of lead or chromium. All the flasks were incubated at 30° C in the incubator for 7 days, every day optical density (OD) was observed at 600nm on spectrophotometer against control and viable count technique was counted.

Temperature and pH :
The bacterial isolates (0.5) were inoculated into a series of 100 ml conical flasks containing 50 ml NB and 0.5ml of either 1000mg/ L of chromium or lead. The pH was varied from 4 to 8 (4, 5, 6, 7 and 8). The pH of the medium was adjusted using dilute HCl or NaOH. To simultaneously search for optimal temperature, for each pH the represented cultures were incubated at different temperatures(25, 30, 35, and 40∘C). After 4 days of incubation, heavy metal removal and biomass were measured. Based upon the heavy metal removal and biomass data, the optimal pH and temperature were determined.

Initial lead and Cr concentration:
Optimal culture conditions were used with varying initial heavy metal concentrations. To each freshly prepared 50ml nutrient broth, 0.5ml of chromium and lead were prepared in concentrations ranging from 500, 1000, 1000 mg/ L and inoculated with 0.5 ml of bacterial isolates. After 4 days of incubation, the biomass and heavy metal concentrations were measured.

Removal of lead and Cr by single bacterial isolates :
Fifty ml of nutrient broth at 0.5 ml of 1000 ppm heavy metal solutions (Pb or Cr) were added to the flasks. These flasks were inoculated with freshly prepared 0.5 ml (OD = 05) of bacterial isolates individually to the flasks to determine the removal of heavy metals by single bacterial isolate or inoculated with 0.5 ml of cell suspensions from the two isolates (OD = 0.5).Then incubation at optimum pH and temperature for 4 days. After that heavy metal concentration and biomass were determined.

Removal of lead and chromium in multiple heavy metal solutions:
Nutrient broth (50ml) containing 0.25 ml of 500, 1000 and 1500 ppm concentration of each heavy metals (Pb and Cr) together, inoculating with 0.5 ml of cell suspensions from the two isolates (OD = 0.5). After 4 days of incubation at 30^0 C, optical density was recorded at 600 nm on

spectrophotometer and Solutions were centrifuged and the supernatant was analyzed for the residual concentrations of the metal ions by using flame atomic absorption.

Statistical analysis :
Statistical analysis was conducted by using (F) and (LSD) tests

Results and Discussion

Heavy metal concentration
   The maximum chromium and lead in wastewater content was 10 mg/l and 3 mg/l respectively, recorded in February while the minimum content (4 mg/l and 8 mg/l)were found in February and October for chromium and lead respectively (Figure-1).
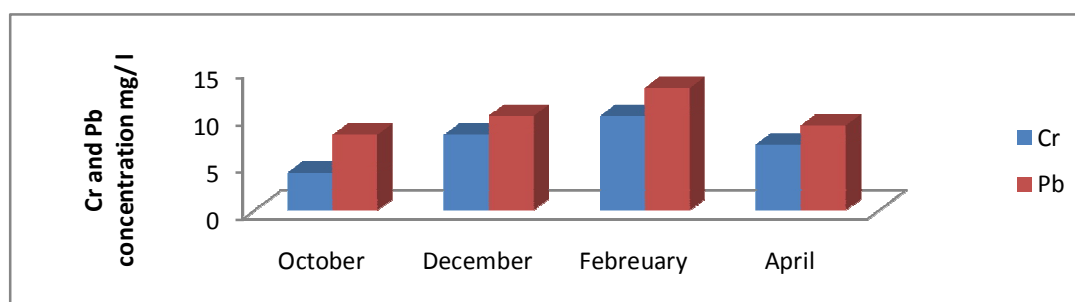

Figure 1 - Cr and Pb concentrations of wastwater collected during study period

The concentration of Cr was above than the standards limit. Presence of Cr (VI) and lead more than the standard limit in the water bodies causes many adverse effects to human beings, animals, plants etc. According to the Iraqi standard rivers conservation system (1967) the limit concentrations of lead and Chromium are 0.1 mg/L [8].

Screening of bacterial isolates forPb and Cr-resistance:
Using the VITEK 2 system (bioMérieux, USA) the results showed that the Pb and Cr-resistant bacterial isolates were found to be:
A: *Citrobacter freundii*   B:*Citrobacter kosari*

Growth factors

Incubation Periods
   Data of mean values of optical density of two examined bacteria incubated for 8 periods of 7 days with chromium and lead are given in (figure-2 and figure- 3). It seems clearly that the best incubation periods are 6 days for both bacteria *C. freundii* and *C. kosari* in case of chromium while for lead, these incubation periods were 6 days for *C. kosari* and 5 days for *C. freundii*.Growth was monitored spectrophotometrically by measuring optical density (OD) at 600 nm and Viable counts of bacteria at different time points of growth.

Citrobacter freundii
   In case of chromium, the highest mean optical value (0.828±0.024) was recorded for bacteria *C. freundii* for 4 days while the lowest mean value (0.096±0.010) was found with zero day incubation

(Figure-2).Regarding lead, highest mean value (0.790±0.038) was obtained after five days incubation while again the lowest mean data (0.113±0.014) was after zero day incubation.
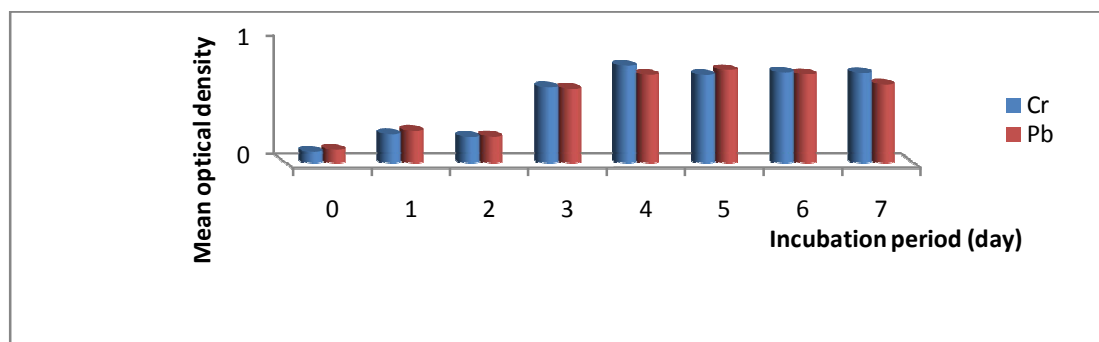

Figure 2- Inocubation of *C. freundii* for different peiods with chromium and lead.

Analysis of variance of these results shows significant (P≤0.001) impacts of incubation periods on mean optical density and also significant (P≤0.001) differences were detected between chromium and lead. Furthermore, least significant test confirms these differences where LSD value was 0.0553.

Table 1- The effect of incubation period on*C. freundii*with chromium and lead

Dilutions	Days															
	0		1		2		3		4		5		6		7	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
10^{-4}	-	-	1	3	7	11	99	117	150	164	64	58	104	113	55	50
10^{-5}	-	-	-	-	1	2	12	15	17	16	17	15	13	15	16	10
10^{-6}	-	-	-	-	-	-	2	3	8	4	3	2	1	3	3	1

R1= Replicate 1, R2= Replicate 2, H= Heavy

The growth of *C. freundii* with the presence of Pb or Cr is shown in (Figure- 2 and table 1) The growth rate reached a maximum when biomass of four days old was used.
The results are in agreement with that obtained by Manukhof *et al*., [9] who reported that the *C. freundii* reach the late (post-stationary) secondary phase of growth after four days of incubation. . Another study [10] showed the growth characteristics of *C. freundii* was examined on selenate containing LB agar plates in experiment to reduce the selenate. *C. freundii* reach the maximum growth after 4 days of incubation The growth or production of biomass increased with incubation period and reached the maximum at equilibrium time and then remained constant.

Citrobacter kosari
    The results of incubation periods of bacteria *C. kosari* with chromium have shown that mean optical density varied from 0.085±0.004 at zero days to 0.779±0.015at six days (table 2 and figure-3) while with lead, these data were found to range from 0.081±0.002 to 0.981±0.017 at zero and three days respectively (Figure- 3).
    Analysis of variance of these data has shown significant (P≤0.001) effects of incubation periods on the mean optical density and also the examined heavy metals had similar significant [P≤0.001] impacts. Furthermore, LSD test had shown significant (P≤0.05) differences between these results (LSD=0.05299).
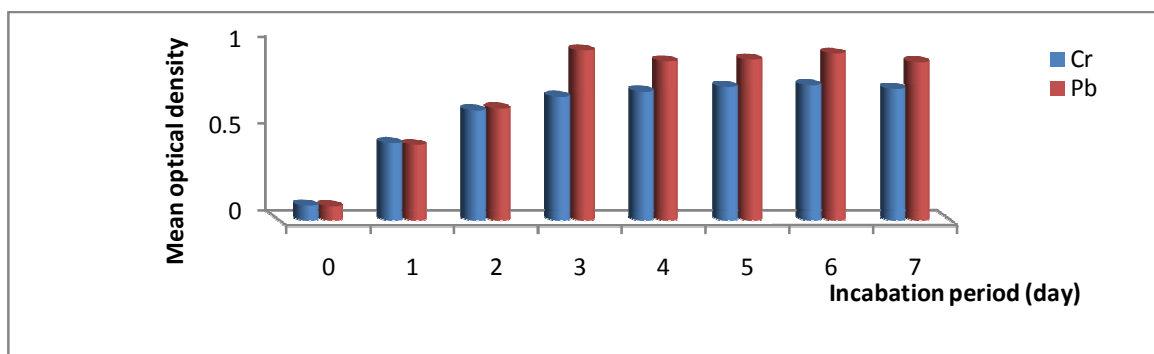
Figure 3- incubation of *C. kosari* for different peiods with chromium and lead.

Table 2-The effect of incubation period on *C. kosari* with chromium and lead

Dilutions	Days															
	0		1		2		3		4		5		6		7	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
10^{-4}	3	5	87	92	H	H	H	H	H	H	190	184	180	168	70	57
10^{-5}	-	-	22	15	58	60	80	79	44	50	18	15	9	14	3	6
10^{-6}	-	-	3	-	3	5	8	6	3	3	-	-	-	-	-	-

Figure-3 and table 2 have shown the effect of the presence of heavy metals on the incubation period of *C.kosari*. The best result was obtained after 3 days of incubation.

The experimental resultsof [11] about biosorption of Zn, Cu, Cd and Pb by *C.kosari* showed that intracellular concentration of heavy metals increases with incubation time. Moreover, it showed that the highest heavy-metal bioremoval efficiency was observed in case of *C.kosari* after 4 days.

The microbes are able to reduce/ remediate Cr and Pb ions even at higher concentrations though it takes a long time and reach the stationary phase. However, rates of Cr and Pb reduction decreased over time with all Cr and Pb concentrations. It was probably due to heavy metal toxicity towards biological activity. With increasing time interval there is a distinct increase in biomass growth for heavy metal concentration [12].

Temperature

The effect of temperature on different bacteria with chromium and lead at various temperatures are given in Figure-4. It seems clearly that better performances of all examined bacteria were recorded at temperature of 30 C° in case both heavy metals. *C. freundii* with chromium had highest (0.844±0.02) mean optical density followed by that (0.829±0.02) of *C. kosari* at 30 C°. Lowest mean values for *C. freundii* (0.353±0.03), *C. kosari* (0.471±0.03) were at 40 C° (Figure-4).When the bacteria mixed with lead ions the higher mean value of optical density (0.822±0.02) was recorded at 30 C° for *C. kosari*while the lower mean value (0.302±0.03) was found at 40 C° for *C. freundii*. For bacteria *C.freundii* & *C. kosari*, the mean values were 40 C° which were 0.302±0.03 and 0.361±0.02 respectively but for the remaining values at a sequence of 30, 25, and 35 C° for *C. freundii* while was 30,35.25.40 C° for *C. kosari* (Figure-4).
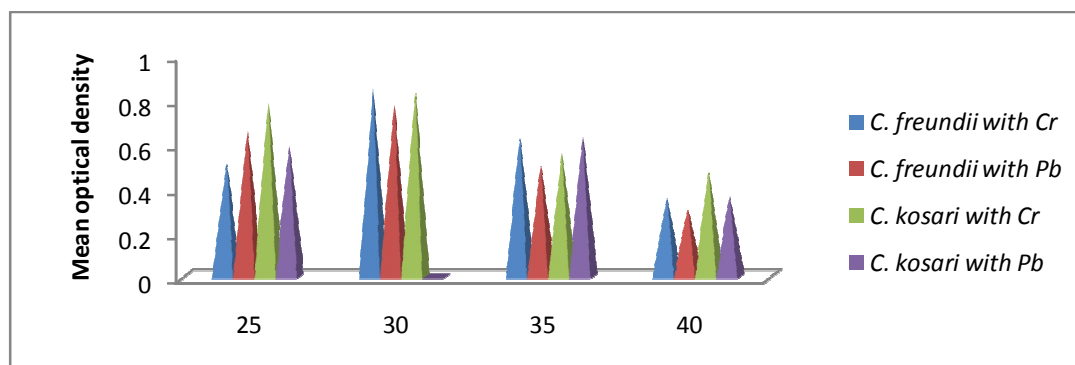
Figure 4- The effect of teperature on different bacterial grwoth with chromium and lead

Analysis of variance of these results gives when the bacteria with chromium significant (P≤0.001) of temperature on optical densities and also, the different examined bacteria have similar significant (P≤0.001) impacts on these values. Furthermore, LSD test shows that the differences between mean optical values of examined bacteria were significant due to the value of this test (0.0017).While the bacteria mixed with lead ions analysis of variance of these data shows significant (P≤0.001) effects of temperature on optical densities and also, the different examined bacteria have similar significant (P≤0.001) impacts on these values. Furthermore, LSD test shows that the differences between mean optical values of examined bacteria were significant due to the value of this test (0.0025).

Table 3- The effect of different temperature on growth of *C. freundii*

Dilutions	Temperature							
	25°C		30°C		35°C		40°C	
	R1	R2	R1	R2	R1	R2	R1	R2
10^{-4}	155	190	heavy	Heavy	heavy	Heavy	66	51
10^{-5}	17	22	heavy	Heavy	47	56	8	6
10^{-6}	3	4	52	44	3	7	_	_

The temperature is an important factor for bacterial growth and microbial reduction. According to our result mentioned in figure- 4 and table 3 the most suitable temperature for growth of *C. freundii* was found to be 30°C. A similar result was observed by [13] when they found out that the optimum growth temperature to *C.freundii* is 30° C. But the result obtain by[14] disagree with this result when they found the optimum temperature for *C. freundii* growth was 37°C.

Table 4- The effect of different temperature on growth of *C. kosari*

Dilutions	Temperature							
	25°C		30°C		35°C		40°C	
	R1	R2	R1	R2	R1	R2	R1	R2
10^{-4}	heavy	Heavy	heavy	Heavy	heavy	Heavy	heavy	Heavy
10^{-5}	77	68	heavy	Heavy	heavy	heavy	20	30
10^{-6}	7	10	57	44	19	33	3	12

The results on the effect of incubation temperatureinFigure-4 and table 4 revealed that the maximum growth of *C. kosari* was observed at 30°C. At 30° *C. kosari* attained its optimum lead and chromium biosorption after 72 h of incubation. The growth of *C. kosari* decreased in the order 30°C>35°C>25°C>40°C.

A study performed by [15] agree with this result when they found that the *C. kosari* were maintained aerobically at 30 C°. Recently [16] show the *C. kosari*grow in 37 °C in the study about bacterial mining.

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 optimum temperature the growth rate decreases and thermaldeath may occur. At high temperature, death rate exceeds the growth rate, which causes a net decreases in the concentration of viable cells [17].

It was reported that optimum Cr (VI) reduction was observed at 30^{0}C and Cr (VI) reduction was severely affected by temperatures above 30^{0}C. It was suggested optimum temperature for Cr (VI) removal as 28^{0}C [18].The maximum removal of lead(II) was achieved at 20 ± 2ºC, where as maximum uptake of chromium(VI) was observed at 40 ± 2ºC [19].

pH

The growth of different bacteria with chromium and lead at various pH values are shown in figure-5. Apparently, the better pH value recorded for all examined bacteria and for both chromium and lead varied from pH6 to pH7 but lowest mean optical values was found at pH4 for all bacteria and both heavy metals.

In the case of chromium the highest mean optical density for *C. freundii* (0.679 ± 0.016) was recorded at pH7 while the lowest mean value (0.143 ± 0.019) was found at pH4. For both *C. kosari*, the higher mean values were 0.722 ± 0.01 at pH6. While the lowest mean value (0.158 ± 0.03) of *C. kosari* was detected at pH4. In general, *C. freundii* had shown higher mean values at all pH values followed by those of *C. kosari*. Regarding lead, all examined bacteria had highest mean optical values at pH6 which were 0.953 ± 0.02 and 0.737 ± 0.02 for *C. kosari* and *C. freundii* respectively, while the lowest mean values were at similar sequence but at pH4 which were 0.419 ± 0.01, and 0.123 ± 0.01 again for *C. kosari*, and *C. freundii*(Figure-5). Obviously, *C. kosari* had the higher mean optical at all examined pH values followed by those of *C. freundii*.
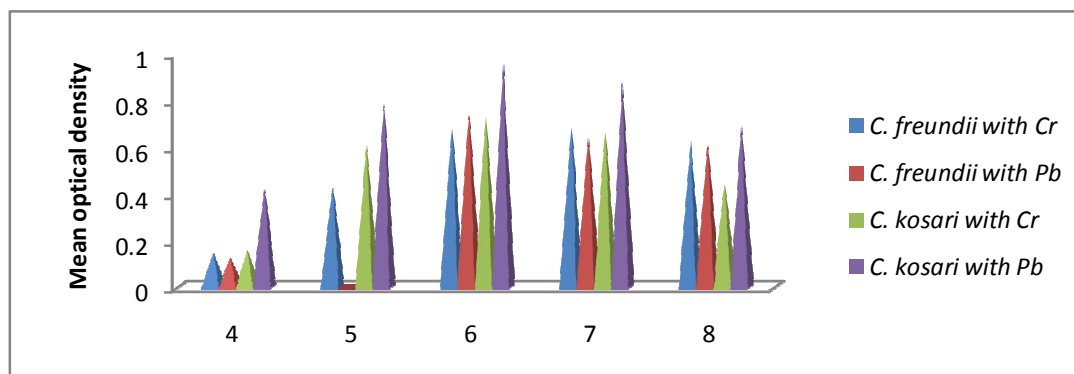

Figure 5- The effect of pH on growth of different bacteria with lead and chromium

Analysis of variance of mean optical values at different pH's when the chromium ion add to the bacteria shows significant (P≤0.001) effects of acidity on these data and the different examined bacteria have similar significant (P≤0.001) impacts on these values. Also, the least significant test (LSD) shows that the differences between mean optical values of examined bacteria were significant (P≤0.05) due to the value of this test (0.0014). But when the lead mixed with the bacteria analysis of variance of these results at different pH's reveals significant (P≤0.001) influences of pH on these data and the different examined bacteria had similar significant (P≤0.001) impacts on these values. Furthermore, the least significant test (LSD) shows that the differences between mean optical values of examined bacteria were significant (P≤0.05) due to the value of this test which was 0.0017.

Table 5- The effect of pH on growth of *C. freundii*

Dilutions	pH									
	4		5		6		7		8	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
10^{-4}	1	1	15	25	68	77	150	149	33	41
10^{-5}	_	-	2	4	12	5	15	18	1	3
10^{-6}	_	-	_	_	1	-	2	3	_	_

The pH is considered as one of the main affecting the growth of bacteria. The medium with initial pH 7 show the maximum growth of *C. freundii.*

In accordance with our results in figure-5 and table 5, the pH 7 is the suitable to the growth of *C. freundii.* It has also been reported that pH 7 was optimum for growth of *C. freundii* in media culture containing Pb ions [20]. While [21] reported optimum pH for growth of *C. freundii* is near neutral. The pH affects the network of negative charges on the surface of the biosorbing cells and the chemistry of the walls, as well as physicochemistry and hydrolysis of the metal.

Table 6- The effect of pH on growth of *C.kosari*

Dilutions	pH									
	4		5		6		7		8	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
10^{-4}	4	7	heavy	Heavy	heavy	Heavy	heavy	Heavy	heavy	Heavy
10^{-5}	-	-	44	53	61	66	58	61	42	32
10^{-6}	-	-	2	6	8	7	7	6	3	3

Figure-5 and table 6 illustrate the growth of *C. kosari* values in different pH. As the solution pH increased, the growth was enhanced. However, the optimal pH for maximal growth for *C. kosari* with the two metals similar (6.0 for lead and chromium). Further increase in solution pH decreased the growth. This result agree with [22] when he found that the growth of *C. kosari* are more suitable at acidic pH. Since [23] found the growth of *C. kosare* was similar at pH 5 and pH 7.

The chromium removal was observed by the growing cells of the selected strain at pH 6.2 [11], and the biosorption of lead and chromium were increased up at pH 5.5, 6, 6.5, 7 respectively [24]. However, since Pb and Cr (VI) reduction is enzyme-mediated, variation in pH will affect the degree of ionization of the enzyme, changing the protein's conformation and affecting the enzyme activity.

Perhaps it can be noticed that microorganisms activity vary with pH , at which the highest average of uptake occurs, which is possibly due to the nature of the functional groups of the cell walls, the charge of cellular surfaces and the type of metal biosorption. The amount of Pb sorption is a function of water pH; sorption increases sharply up to pH 5-6 and reaches a maximum at pH 8, with only slight increases occurring between pH 6 and 8. The increase in Pb sorption at higher pH is attributable to an increase in negative charge, precipitation as hydroxide, and the formation of hydroxyl species that are more strongly retained compared to free metal ion species [25].

Initial concentration and biosorption of Cr and Pb

The higher mean values of optical density were found with Cr concentration of 1000 ppm and a mean of 0.872±0.02 was recorded for *C. freundii* and followed by that of mixed bacteria (0.853±0.04) and then by that of *C. kosari*(0.839±0.02) followed by those values at 750 ppm and then by those data at 1500 ppm.While the lowest mean values were detected for all examined bacteria at 500 ppm but at different sequences (Figure-6). However, it seems clearly that mix bacteria have better performances than the rest examined bacteria. But when bacteria mixed with leadthe higher mean values were recorded in mixed bacteria followed by those of *C. freundii* and followed by those of *C. kosari* (Figure-7). In general, all examined bacteria had mean optical values at 1000, 750, and 500 ppm.

Mixed bacteria had highest mean optical densit (0.980±0.01) at 1000 ppm followed by that (0.908±0.01) of 1500 ppm which was almost similar to that (0.907±0.01) of 750 ppm while the lowest mean value (0.762±0.02) was recorded at 500 ppm.

In case of *C. kosari*, Similar pattern of performance was recorded where highest (0.900±0.03) mean value was at 1000 ppm followed by that (0.799±0.03) of 750 ppm and by that (0.753±0.02) of 1500 ppm while the lowest (0.732±0.03) mean value was found at 500 ppm.

The situation in *C. freundii* was different where highest mean value (0.946±0.02) was recorded at 1000 ppm followed by that (0.905±0.02) at 750 ppm and then by that (0.818±0.02) at 500 ppm while the lowest mean value (0.767±0.03) was found at 1500 ppm (Figure-7).
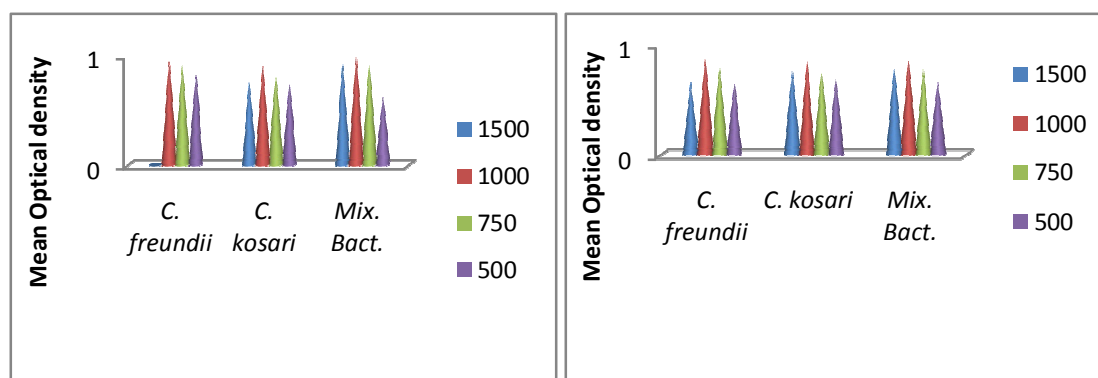

Figure 7 -The effect of initial concentraton of Pb **Figure 6-** The effect of initial conce- ntration of Cr
on growth of bacteria   on growth of bacteria

Analysis of variance of figure- 6 shows significant (P≤0.001) performance of examined bacteria in terms of optical density with chromium ions and also the various concentrations of chromium had similar significant (P≤0.001) impacts. Furthermore, LSD test revealed significant (P≤0.05) differences between mean values of optical densities where LSD value was 0.00219.

Analysis of variance of these figure-7 shows that examined bacteria had given significant (P≤0.001) different mean values of optical density at all lead concentration which in turn had significant (P≤0.001) impacts on these mean values of optical densities. Also, LSD test has detected significant differences between these data since the LSD value was 0.0174.

These results were agrees with the findings of [26] who indicated that the process of biosorption increases with the initial heavy metals increase up to a certain concentration of saturation of free sites from the metal ions.

The rate of Cr and Pb reduction was observed in two phases, an initial phase of faster degradation followed by the phase of slower degradation. The initial faster uptake might be due to the availability of abundant Cr and Pb species and empty metal binding sites of the microbes. The slower phase may be attributed to saturation of metal binding sites. The growth of microbial population reduces with increasing concentration of Cr and Pb. Confirmed that the microbial biomass generation decreased as the concentration of heavy metal increased. The microorganisms release a diverse range of specific and nonspecific metalbinding compounds in response to high levels of toxic metals which can ameliorate the effect of toxic metals and mediate the uptake process [27].

Figure-8 and. 9illustrated Mean residual ion of both chromium and lead concentrations ± standard deviation after growing of different bacteria with various concentrations of both metal while table 7 includes removal percentage of different bacteria at chromium and lead with various metal concentrations.Highest mean values of residual chromium (8.3±0.28) were recorded by *C. kosari* and followed by that of *C. freundii* (7.7±0.25) while the lowest mean value (2.5±0.18) was recorded for mixed

bacteria which were all at 1500 ppm Cr. But at the remaining Cr concentrations, *C. kosari* had the highest mean values followed by those of *C. freundii* and again mixed bacteria had the lowest mean values for the remaining Cr concentrations (Figure-8). However, all these data were much lower than those of control but at similar pattern of sequences.
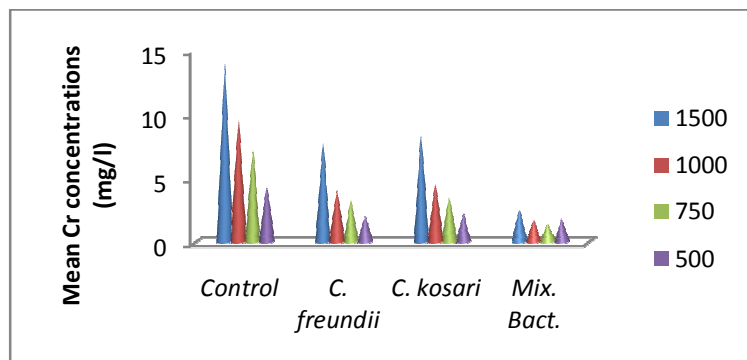

Figure 8- Mean residual Cr concentration after grwoing different bacteria at different Cr concentrations

Analysis of variance of these results shows significant (P≤0.001) performance of examined bacteria in biosorbing chromium ions and also the various concentrations of chromium had similar significant (P≤0.001) impacts. Furthermore, LSD test revealed significant (P≤0.05) differences between mean values where LSD value was 1.0414.

In case of lead the Highest mean value (6.70±0.25) was recorded for *C. freundii* followed by that of *C. kosari* (4.90±0.16) at 1500 ppm while the lowest mean value (3.07±0.29) was found for mixed bacteria. For the remaining Pb concentrations, *C. freundii* had the highest mean values followed by those *C. kosari* while mixed bacteria had the lowest mean values (Figure-9).
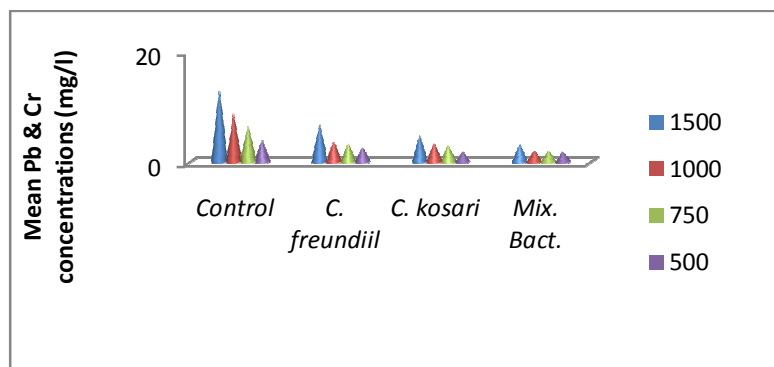

Figure 9- Mean residual Pb concentration after grwoing different bacteria at different Pb concentrations

Analysis of variance of these results shows that examined bacteria had been significantly varied (P≤0.001) in biosorbing lead ions and also the different lead concentrations used in this test had significantly (P≤0.001) affected mean values of biosorbedPb ions. Also, LSD test has detected significant differences where the value was 0.191.

Table 7-Reduction percentage of Cr and Pb By bacteria

Metal con.ppm	Cr٪			Pb٪		
	C. freundii	*C. kosari*	Bacteria mixed	*C. freundii*	*C. kosari*	Bacteria mixed
1500	45	40	82	48	62	76
1000	58	53	82	59	63	77
750	55	51	80	49	55	71
500	54	49	76	36	54	54

Table 7 illustrate that the removal percentage of Cr and Pb by *C. freundii*, *C. kosari* and mixed bacteria is highest in concentration 1000ppm than the other concentrations (58, 53and 82%) for Cr and (59, 63 and 77%) for Pb respectively.

Studies of [27] revealed that the bacterial isolate *Raoultella sp*. completely transformed Cr(VI) to Cr(III) whereas *Bacillus cereus*, *C. freundii* and *C. kosari* showed 94.81%, 95.8% and 95.2% of chromium reduction respectively. *C. freundii* were found to exhibit 100 % resistance to chromium and mercury [28] while, [7] show the *C. kosari* growing in high concentration of Cr and Pb 110mg/l, 400mg/l respectively and selected for Cr and Pb removal studies. A *Citrobacter* spp. strain has been found involved in precipitation of Pb (II) and other heavy metal as phosphates by growing in biofilm.
The removal of Pb (II) ions with growing cells of *Bacillus subtilis* was maximum (36.99%) when initial lead concentration was 74.6 mg/L. While the *Aeromonas hydrophila* 56.32 % lead uptake from 74.4mg/L-1 [29].

The biosorption of chromium and lead in mixed culture of bacteria increased. This result agree with [7] when they observed that the microbial processes in mixed culture of bacteria gave 10% better removal of metals than in single culture. Also [30] according to their result suggested that the Cr reduction efficiency improved when used mixed biofilms containing *A. calocoaceticus,Staphylococcus aureus* and one cyanobacterial strain (*Oscillatoria strain*).

Biosorption in mixed metals

In case of chromium, the higher mean value (3.37±0.181) was recorded for *C. kosari* while the lower mean value (3.17 ±0.255) was recorded for *C. freundii* (Figure- 10). The removal percentage was observed 23% & 27% for *C. kosari* and *C. freundii*.
Regarding lead, similar sequence of residual metal was shown where again *C. kosari* had the higher mean value (3.437±0.172) and the lower mean value (3.383±0.048) was for *C. freundii*(Figure-10). Also, the removal percentage was 24% & 25% for C. *kosari* and *C. freundii*. However, these data of both heavy metals (Cr&Pb) were much lower than those of control which were 4.36± 0.028 and 4.52± 0.048 for Cr and Pb respectively.
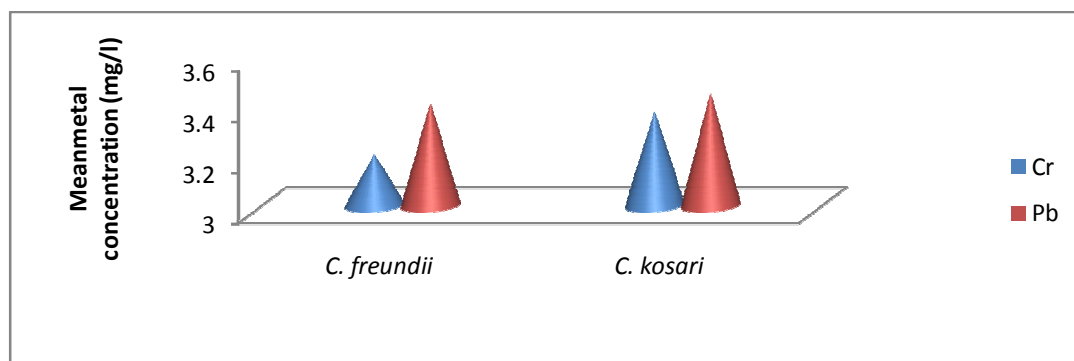
Figure 10 -Mean residual Cr and Pb after grwoing of different bacteria with mixed of Cr & Pb

Analysis of variance of these results has shown only examined bacteria were significantly (P≤0.05) differed in biosorbing both chromium and lead while there were no such differences in case of examined metals and also $LSD_{0.05}$ value (0.0436) confirms such analysis.

The results presented in the previous forms (the impact of metals ions of the chromium and lead biosorption), showed that Cr and Pb ions biosorption decreased in the double system than it was in the singular system, as the study showed the competitive impact of the metals was (antagonistic) according to [31] . Similar result obtain by [9], when he reported that Presence of lead (Pb) was significantly inhibitory for Cr (VI) reduction by *Brevibacteriumcasei*. Also Thebiosorption of three heavy metals was higher in primary solutions than in ternary solutions. Approximately 22 % of Cu, 24% of Cd, and 42.75% of Pb were removed from primary solutions; 16% of Cu, 8% of Cd, and 35% of Pb, were removed from ternary solutions by *Stenotrophomonasmaltophilia*[19].

   The selectivity of biosorption comes from the ability of metal to link with the effective sites, which in turn depends on the extent of ionization.The presence of the metals in the same solution makes them compete with each other on active sites; those with the high ionic force were to biosorb first, if the active sites were not saturated, it was possible that the ions of other metals of the low ionic force to be associated respectively. However, we conclude that each biosorbent organism has a specific preferential ability that differs from other biosorbent organisms. The reason behind the decline of the concentrations of chromium and lead biosorbent in the same solution was due to the competition among the ions on the same active sites and the biomass associated with ions rather than the others, keeping the other ions free in the solution [32].

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