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BIOREMEDIATION ABILITY OF *LACTOBACILLUS* STRAINS TO SOME OF HEAVY METALS

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Abstract

Heavy metals cause various health hazards. Using lactic acid bacteria (LAB), we tested the reduction of heavy metals e.g. chromium (Cr), lead (Pb) and copper (Cu). The metals levels sorption were tested in two bacterial strains (*Lactobacillus plantarum and Lactobacillus rhamnosus*) using atomic absorption spectrophotometery Flame Atomic Absorption Spectrometry (FAAS) by AA-7000 Shimadzu, the cell turbidity was (1×10^6 cells/ml). The results showed decreased in all metals levels (Pb, Cu and Cr) in MRS broth. The Cr concentrations of the media in the pre-Lactic acid bacteria treatment s (25.39) mg/ml at 100 mg/ml concentration were significantly ($p < 0.05$). Lower than after-Cr treatment (18.57, 4.86) mg/ml by *L. plantarum* and L. *rhamnosus* respectively. The lead also decline at 100 mg/ml concentration from (28.66) mg/ml in control to (17.99 and 3.62) mg/ml by *L. plantarum* and *L. rhamnosus* respectively. The results of the copper reducing experiments were combined and the most promising strain*, L. rhamnosus*, which had the highest copper reducing capacity at 100 mg/ml of Cu concentration., the value reached to (7.39 mg/ml) compared with control (11.03 mg/ml). In order to optimize the best bio remediated bacterial strain Lactic acid bacteria were cultured in specific medium (MRS) at different incubation period (12 to 96)hrs, pH (3.0 to 7.0), and temperature (25 to 37 °C) ,and with or without oxygen. It was observed that maximum reduction at 12 h of incubation period, pH 3.0, and temperature 25 °C , in presence of oxygen. LAB specially *L. rhamnosus* bio sorption may aid the detoxification of people exposed to heavy metals.

Keywords : Bioremediation , Heavy metals , *L. plantarum* and L. *rhamnosus*

Introduction

Substantial metals are a one of a kind gathering of normally happening mixes discharged into the earth by different procedures (Camobreco *et al*., 1996). The human mechanical movement, including mining, refining, and engineered compound creation, has prompted an expansion in the measures of overwhelming metals discharged into the a domain (McConnell and Edwards, 2008) Many nations have administrative rules for overwhelming metals introduction just as remediation . Screening of soil and water sources is led habitually to avoid over utilization, however a large number of these projects and were most certainly not promptly accessible in creating countries, where the weight was most noteworthy (Ahsan *et al*., 2000). The net outcome was that individuals around the world were uncovered, and new methodologies are required to discharge the unfavorable aggregation of these mixes (Li *et al*., 2006)

In ecological biological systems, there is a complex cooperation between substantial metal contaminants and local microorganisms, These life forms have created one of a kind opposition instruments which enable them to endure and, evacuate the convergences of contaminants in their environment. One of these individuals are the gut microbiota, for example, lactobacilli utilized in nourishment applications, may possibly be an aide for lessening metal danger in people. This is on the grounds that they have numerous systems which are powerful in forestalling harm to their cells (Sinha, 2011), and they can tie and sequester substantial metals to their cell surfaces, along these lines evacuating them through consequent poo, anyway the capacity of lactobacilli to tie and sequester metals relies upon the strain's opposition instruments (Robinson and Tuovinen , 1984). Microbes which can trade metals out of their phone diminish harm to the life form by bringing down the phone focus. In consistent, such an instrument isn't perfect for detoxification of the gastrointestinal tract, as it results in the cycling of metals. Conceivably the perfect species for detoxification are those which come up short on the qualities encoding metal transporters and in this manner just tie and sequester overwhelming metals (Gorospe and Gerstenberger, 2008)

Since the beginning, lead has been broadly utilized in mechanical ventures; this had brought about the metal being omnipresent in the earth in soils and air (Levin *et al*., 2008). For the greater part of individuals, introduction to lead happens from optional sources. It tends to be breathed in; it might enter the environment through modern sources, through purifying (Saha *et al*., 2010). Lead can likewise enter drinking water through more seasoned lead pipes, some home paints, and sullied soils, with all causing a progressing wellspring of introduction and peril, particularly for youngsters (Pichery *et al*., 2011)

Lead poisonous quality likewise happen through utilization of defiled sustenance or the admission of lead particles, it can bioaccumulate in both the blood and bones. Its half-life in the blood is around 30 days, yet it can stay in the skeletal framework for quite a long time, and hence, lead lethality is a tenacious issue , particularly for kids (National Research Council, 1980), (Somervaille *et al*., 1988). On the other hand, Chromium is a metal that can be found in various composites and salts. It has been utilized mechanically for over a century and can be identified in focuses going from under 0.1 g/m^3 in air to 4 g/kg in soils. "Normally happening" chromium is generally present as Cr(III), Trivalent Cr(III) and hexavalent Cr(VI) shapes were the most imperative for human wellbeing, however they are inadequately consumed through the digestive system (Aitio *et al*., 1984). Studies in mice demonstrated that gut microbiotas gave the primary line of guard to the body by changing over dangerous Cr(VI) to a less-harmful Cr(III). Pseudomonas spp. gotten from the Cr-focused on rodent had the most elevated MIC appreciation, while *Lactobacillus spp*. and *E. coli* had lower values than bacteria from the normal control rats. This indicated that bacterial tolerance in the Cr-stressed animals contributed to the host's defense (Upreti *et al*., 2004) However, a separate study conducted by (Upreti *et al*., 2011), showed that the exposure of lactobacilli to chromium can generate resistant strains able to better tolerate metals. In a similar study, (Horsfall *et al*., 2005) showed that lactobacilli and other gut-associated bacteria, can transform chromium to its less-toxic form, and the use of lactobacilli as a tool to reduce the burden of metal exposure is advantageous, as it can be applied almost immediately; there is no requirement for expensive technology or infrastructure setup, as fermentation capability is either already available or easily set up, therefore lactobacilli were reported to bind heavy metals and thus represent a promising approach for decontamination of heavy metals in food and water and perhaps gastrointestinal tract as well, extending areas of LAB applications in food industry and probiotics. In contrast to conventional physicochemical techniques microbial metal ion binding exhibits fine specificity and is environmentally friendly, of low-cost, and efficient at low metal ion concentrations (Bellinger *et al*., 1978) Heavy metal binding was reported to be strain, temperature, and pH dependent and efficiently at low concentration ranges commonly observed in foods (Berg *et al*., 2005).

The aims of this work were to determine the potential ability to remove heavy metals including chromium, copper and lead from aqueous solutions and culture medium with two Lactobacillus strains, including *L. plantarum* and *L. rhamnosus*. these strains were specifically isolated from probiotics and Investigations the effect of metals concentration, temperature, pH, oxygen and incubation period on microbial growth and metals reduction.

Materials and Methods

Heavy metals (Stock solution)

Chromium solution : Chromium solution was prepared in (100, 200, 300, 400, 500, 600) mg/mL, stock solutions in Milli-Q water were prepared from $CrCl_3.6\ H_2O$ from (Sigma-Aldrich), chrom solutions were added to the bacterial culture medium after sterilized by filtration (0.22 mM).

Lead solution : Lead solution was prepared in (100, 200, 300, 400, 500, 600) mg/mL, stock solutions in Milli-Q water were prepared from $PbSO_4.7\ H_2O$ from (Sigma-Aldrich), lead solutions were added to the bacterial culture medium after sterilized by filtration (0.22 mM).

Copper solution : Copper solution was prepared in (100, 200, 300, 400, 500, 600) mg/mL, stock solutions in Milli-Q water were prepared from $CuSO_4.5\ H_2O$ from (Sigma-Aldrich), copper solutions were added to the bacterial culture medium after sterilized by filtration (0.22 mM).

Bacterial Strains

The following Lactobacillus strains were used in this study: *Lactobacillus plantarum* obtained from college of agriculture (Baghdad University) and *Lactobacillus rhamnosus* GG obtained from PRO Thera Klari LABs dinsion factor 1 U.S.A. Identification of bacteria were described elsewhere (Bhakta *et al*., 2012). Lactobacilli were cultured in de Man, Rogosa, Sharpe (MRS) broth (BD Difco) under microaerophilic conditions at 37°C.

After incubation period, the plates of MRS agar were examined for typical colonies of Lactobacillus spp. The colonies bearing typical Morphology were purified and subcultured on MRS broth and agar, then stored for further assay.

Measurement of heavy metals concentration.

In order to determination the heavy metals concentrations. In this respect, the digestion of culture samples with concentrated (HNO_3) was achieved. Mineralization of samples was performed by using a Berghof MWS-2 microwave digester. Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples (Dauvalter, 1998). The total metal content of samples was performed by AA-7000Shimadzu, Flame Atomic Absorption Spectrometry (FAAS) with autosampler, which provided a good sensitivity (Saygi and Yiğit, 2012) were used. Blank and standard solutions for devices calibration were used as well.

Lead, Chromium and Copper concentrations in the supernatants were measured using AA-7000Shimadzu, Flame Atomic Absorption Spectrometry (atomic absorption spectrometer). Samples of MRS broth spiked with concentration of lead, chromium and copper were used as quality control samples. Metal removal rate was expressed in mg/ml. Changes of metal concentration in the samples were recorded.

Reduction of heavy metals concentration using LAB

To select the best strains with excellent metal (Cu, Cr or Pb) removal efficiency both, identified LAB were employed for metal removing study following the method described by Pazirandeh *et al*. (1998) with some modifications. Freshly cultured LAB were harvested in 2ml Eppendorf tubes, centrifuged at high speed to pallet the cells and washed twice using sterilized MQ water. Pellet of the cell was resuspended in sterilized Cu or Pb or Cr (100, 200, 300, 400, 500, 600) mg/ ml, solutions prepared from previously mentioned stocks and incubated at 37 °C. After 2 h, samples were collected in 2 ml Eppendorf tubes and centrifuged at 10000 rpm for 10 min, and metal contents in supernatant were measured using by AA-7000Shimadzu, Flame Atomic Absorption Spectrometry (FAAS).

Growth Media and Culture Conditions

Lactobacilli were cultured in de Man, Rogosa, Sharpe (MRS) broth (BD Difco) under microaerophilic conditions at 37°C.

Reduction of heavy metals was studied using a Tecan Infinite F200 PRO (Switzerland) microplate reader. Overnight cultures of the lactobacilli were 10 6*1 CFU/ ml with fresh MRS broth containing (100-600)mg/mL Pb or Cr or Cu and loaded into sterile polystyrene 96-well microplates (flat bottom, CellStar Greiner Bio-One). Microplates were incubated at 37°C and measurements of the optical density at 600nm (OD600) were automatically recorded for each with 150s shaking cycles before measurements were started.

The bacteria for characterization of surface properties were cultured in MRS broth for 12h, harvested by centrifugation at 10000 rpm for 10 min, and washed three times with appropriate KNO_3 solution

The supernatant of the bacteria grown for 12h in MRS broth with contration of these metals were used for measurements of Cr., Cu and Pb concentrations with atomic absorption spectrometry

Determination of optimized initial medium pH : Optimized production medium was adjusted with different initial pH values (3, 4, 5, 6, 7) to study their effect on Heavy metals reduction the cultures were inoculated with 10 % ml of optimized inoculum concentration and incubated in shaking incubator for 48 hrs. Centrifuged and assay metals concentration was recorded.

Determination of optimized incubation temperature : Production medium was inoculated by the addition of 10% ml of optimized inoculum and incubated at different temperature (20, 25, 30, 35, 40)°C for 48 hrs. Then centrifuged and assay metals concentration was recorded.

Determination of optimized incubation period : The optimized MRS medium was inoculated with 10% of optimized inoculum concentration 1*10 6 CFU/ ml and incubated at different time period (12, 24, 48, 72, 96), hrs, centrifuged at (10000 rpm) for 10 min, the culture solution obtained was used to assay heavy metals concentration.

Determination effect of Oxygen : The optimized MRS medium was inoculated with 10% of optimized inoculum concentration 1*10 6 CFU/ml and incubated in presence of oxygen and also without oxygen and incubated for 48 hrs, centrifuged at (10000 rpm) for 10 min, the culture solution obtained was used to assay heavy metals concentration.

Result and Discussion

Presence of toxic metals in the environment and their effects on body including carcinogenicity is known for a long time. The recent studies have been the understanding of the capacity of microbes to detoxify and reduction of metals.

1. Chromium:

Chromium (Cr) is a substantial metal which is found in nature ,and is a broadly utilized modern concoction, widely utilized in paints, metal completions, steel including tempered steel, producing, amalgam cast irons, chrome and wood treatment. The major non-word related wellspring of chromium for people is nourishment, for example, vegetables, meat, urban air, and so forth (Odewabi and Ekor , 2016) Non-word related introduction to the metal happens by means of the ingestion of chromium containing sustenance and water, while word related presentation happens through inward breath. What's more, this metal is restricted in the lung, liver, kidney, spleen, adrenals plasma, bone marrow, and red platelet (Agrahari and Gopal, 2009)

The Cr concentrations of the media in the pre-Lactic acid bacteria treatment s (25.39) mg/ml at 100 mg/ml concentration were significantly ($p < 0.05$). lower than after-Cr treatment (18.57, 4.86) mg/ml *by L. plantarum and L. rhamnosus* respectively, the decreasing Cr levels in all treatments (100, 200, 300, 400, 500 and 600) mg/ml were significantly different from the values obtained for the Cr Treatments ($p < 0.05$). On the other hand implies that *L. rhamnosus* is more efficient in metal reduction (18.92) mg/ml at 600 mg/ml of Cr concentration than *L. plantarum* (40.97) mg/ml at the same concentration of Cr (Table -1).

The outcomes demonstrated that Lactobacillus species had the most astounding decrease impact at 100 mg/ml of Cr fixation .Cr (VI) decrease limit (up to 25 mg/ml Cr-focus) showed their capacity to adjustment. This may likewise reflect flat hereditary exchange coming about because of Cr-stretch. Assorted variety of Cr-safe and Cr-decreasing microscopic organisms in the bacterial populace from a chromium polluted media. It has likewise been proposed that the instruments of Cr obstruction and decrease may contrast in microbial network from species to species .Therefore, Cr) opposition and decrease limit could be the common capacities and not a selective normal for a solitary gathering of microorganisms (Kruger ,2009)

Table 1 : Chromium reduction *by Lactobacillus plantarum* and *Lactobacillus rhamnosus*

Chromium concentration (mg/ml)	Cr concentration after treatment (mg/ml)		
	control	L. *plantarum*	*L. rhamnosus*
100	25.39	18.57	4.86
200	29.45	22.34	7.21
300	32.78	29.72	9.45
400	39.14	31.40	11.93
500	42.57	35.71	15.14
600	57.25	40.97	18.92

2. Lead

Lead is a predominant natural contamination with no useful organic job. It unfavorably instigates oxidative pressure harm to the host. Numerous examinations have demonstrated that LAB can sequester Pb, as well as mitigate the oxidative impact (Okoro *et al*., 2015). Screening LAB strains against Pb danger should take various properties into thought. like LAB strains must show high Pb-restricting capacity, empowering them to tie Pb before the intestinal assimilation of Pb by the host. also, LAB strains should display high protection from Pb to stay away from it being harmed. Moreover, to perform Pb evacuation in the gastrointestinal tract, it is vital for the screened strains to stay reasonable in high convergences of bile and stomach acids (Okoro *et al*., 2015).

In the present study, *L. rhamnosus* showed the best binding aptitude *than L. plantarum* (Table-2) and had a remarkable tolerance to Pb when decline the lead concentran at 100 mg/ml from (28.66) mg/ml in control to (17.99 and 3.62) mg/ml by *L. plantarum* and *L. rhamnosus* respectively.

The cell dividers of the gram-positive microbes comprise of peptidoglycans, teichoic acids, proteins and polysaccharides. These substance likewise contain adversely charged useful gatherings, which fill in as the essential destinations of metal particle sorption on a bacterium surface .The phosphate and carboxyl gathering present in peptidoglycans and teichoic acids are the essential locales of metal particle official on the outside of the bacterial cell (Kazy *et al*., 2009) Our examination demonstrated that Lactic corrosive microbes in the low fixation (100) mg/ml had a higher capacity to dcrease of Pb than high concentration (600) mg/ml. Interestingly, LAB strains such as *L. plantarum* and *L. rhamnosus* can improve the absorption and bioavailability of several trace elements in animals. Thus, it is very significant to exploit LAB as a heavy metal removal agent added to food or feed (Klobukowski *et al*., 2009).

Table 2 : Lead reduction by *Lactobacillus plantarum* and *Lactobacillus rhamnosus*

Lead concentration (mg/ml)	Pb concentration after treatment (mg/ml)		
	control	L. *plantarum*	*L. rhamnosus*
100	28.66	17.99	3.62
200	35.57	22.82	7.24
300	57.00	31.50	15.73
400	71.53	40.77	18.81
500	87.14	62.57	36.43
600	94.26	70.31	42.50

3. Copper:

Screening LAB strains against copper danger should take various properties into thought. like LAB strains must show high Cu restricting capacity, empowering them to tie Cu before the intestinal retention of Cu by the host. what's more, LAB strains should show high protection from the copper to maintain a strategic distance from it being harmed. Moreover, to perform copper expulsion in the gastrointestinal tract, it is essential for the screened strains to stay practical in high convergences of bile and stomach acids (Fengwei *et al*., 2015).

The copper biosorption of lactobacilli may identify with physical adsorption and synthetic adsorption, and particle trade superficially the microscopic organisms is likewise prone to be a factor Judging from the analyses in vitro, there was a noteworthy distinction in copper restricting limit among various strains, which may be owing to contrasts in structure, useful gatherings and species.

The results of the copper reducing experiments were combined and the most promising strain, *L. rhamnosus*, which had the highest copper reducing capacity at 100 mg/ml of Cu concentration., the value reached to (7.39 mg/ml) compared with control (11.03 mg/ml), which could indicated the effectively tolerate copper inhibition, (Table-3).

The effects of Lactic acid bacteria on levels of copper in the media, the levels of copper were significantly lower than those in the control ($p < 0.05$). These results indicate that the strain of *L. rhamnosus* could indeed reduce the copper content in the media, which further verified its function of copper limited in the media.

Table 3 : Copper reduction *by Lactobacillus plantarum* and *Lactobacillus rhamnosus*

Copper concentration (mg/ml)	Cu concentration after treatment (mg/ml)		
	control	L. *plantarum*	*L. rhamnosus*
100	11.03	9.39	7.39
200	20.74	12.56	51.23
300	27.15	27.154	51.78
400	35.76	23.75	20.92
500	43.38	31.25	17.69
600	52.67	33.76	16.20

4. Factors affecting bioremediation

In this investigation the most important parameters for bioremediation were include:

(1) Temperature affects biochemical reaction rates and the rates are double for each 10 C rise in temperature; **and (2)** pH-it ranges from 5.5–8.0, which is the optimum range for the growth of microbes and to destroy the metal contaminants, and(**3**) period of incubation, **then (4)** oxygen.

1. Temperature

In this examination, from Table-(4) at temperature of 25 C the sum adsorbed of metals began to diminish, the dimension of Pb was (12.43) mg/ml .,for Cu was(3.44) mg/ml and for Cr was (12.42) mg/ml. in this way, characterizing a level. Subsequently, it appears to be sensible to suggest that for the exploratory conditions utilized, the arrangement of a total monolayer of metal particle covering the biomass surface, implying that immersion of the biomass surface is by all accounts come to at25 C to 45 C and the ideal temperature of adsorption the three metals (Pb, Cu, Cr) could be gotten inside this range, likewise the outcome demonstrated that the measure of the three metals adsorbed diminished when the treatment with *L. rhamnosus* much superior to *L. plantarum* at a similar temperature. This implies the ideal temperature of metal decrease was inside 25 C Therefore, expanding the temperature past this point won't favored the adsorption. This is like the outcomes acquired by different looks into (Vidali, 2001).

Table 4 : Effect of Temperature on the lead ,Chromium and copper bioremediation by *Lactobacillus plantarum* and *Lactobacillus rhamnosus.*

Temperature C	Bacterial species	Heavy metal concentration (mg/ml)		
		Lead	Chromium	Copper
25	*L. plantarum*	12.43	3.44	12.42
30		14.29	7.66	15.05
35		17.80	9.50	18.82
40		16.46	8.82	17.22
45		13.35	7.46	15.46
25	*L. rhamnosus*	1.60	3.92	7.95
30		2.62	4.56	3.96
35		3.05	7.29	4.88
40		3.55	6.99	4.11
45		2.96	4.33	3.59

2. pH

In this investigation a huge connections between physicochemical parameters (for example pH), and overwhelming metal fixations were evaluated (Table-5). From Table – 5, the outcome demonstrated that when the pH esteems diminished in the media (MRS)broth From pH 7 to pH:3, the dimension of the three metals (Pb, Cu and Cr) were diminished. In this manner, the conductivity esteems had a critical positive association with every substantial metal. There were huge contrasts between and Pb, Cu and Cr decrease levels. It can discovered positive connections among pH and these metals. It could be presumed that the progressions of physicochemical parameters (pH) rely upon how seasons influence the dimensions of a few metals (Vidali, 2001), likewise the outcome showed that the measure of the three metals adsorbed diminished when the treatment with *L.rhamnosus* much superior to *L.plantarum* at a similar pH. This implies the ideal pH of metal decrease was inside pH: 3. In this manner, expanding the pH past this point won't favored the adsorption. This is like the outcomes gotten by different inquires about (Vidali, 2001).

Substantial metals levels in conditions rely upon the physicochemical parameters, for example, pH, . It is notable that the dissolvability of lethal metals increments with the pH decline (from surface to profundity, from antacid to acidic).

Table 5 : Effect of pH on the lead, Chromium and copper bioremediation by *Lactobacillus plantarum* and *Lactobacillus rhamnosus.*

pH	Bacterial species	Heavy metal concentration (mg/ml)		
		Lead	Chromium	Copper
3	*L. plantarum*	13.25	16.53	6.40
4		16.57	17.97	8.16
5		16.99	18.00	9.89
6		17.01	17.44	8.73
7		14.92	15.42	6.50
3	*L. rhamnosus*	1.98	1.50	5.76
4		2.50	2.98	6.21
5		3.83	4.88	7.39
6		3.00	3.95	7.25
7		1.76	2.33	4.03

3. Incubation period

Due to global industrialization, heavy metal toxicity in environment is increasing rapidly. Different industries release huge amount of waste containing toxic heavy metals directly into water bodies without processing (Batta *et al.*, 2013) .Mechanical removal of these heavy metals is costly and time consuming. Heavy metal accumulation property in bacteria might be an alternative metal-resistant (lead, cadmium, and nickel) (Hookoom *et al.*, 2013) Lactic acid bacterial strain (*L. plantarum and L. rhamnosus*) were used at different incubation period, given in **Table-6**. the best reduction activity exhibited *by L. rhamnosus* was recorded to be highest at 12 h of incubation .

Table 6 : Effect of incubation period on the lead ,Chromium and copper bioremediation by *Lactobacillus plantarum and Lactobacillus rhamnosus*.

Incubation period (hrs)	Bacterial species	Heavy metal concentration (mg/ml)		
		Lead	Chromium	Copper
12	*L. plantarum*	13.05	5.43	14.35
24		15.13	6.78	16.73
48		17.86	8.98	17.59
96		18.88	9.30	18.01
72		17.98	8.92	17.20
12	*L. rhamnosus*	2.57	5.67	0.98
24		1.15	6.26	2.51
48		4.38	7.87	2.99
96		4.00	7.89	3.82
72		5.89	6.36	2.01

Bacterial development within the sight of substantial metals makes physiological pressure and create ROS which is extremely hazardous for cell. To decrease these oxidative anxieties, microscopic organisms deliver distinctive sorts of defensive chemicals like CAT and SOD. A critical action of cell reinforcement compound SOD, glutathione peroxidase (GSHPx), glutathione reductase (GR), and mercury reductase (MR) in rumen microscopic organisms Streptococcus bovis and *Selenomonas ruminantium* was watched, after introduction to $HgCl_2$ (Lenaèrtovaè *et al.*,1998).

4. Oxygen

Oxygen—it is chiefly utilized for the underlying breakdown of the hydrocarbon in the defiled locales. Likewise, the measure of accessible oxygen will decide if the bioremediation is completed under vigorous or anaerobic condition (Lenaèrtovaè *et al.*, 1998). The reality despite the fact that From Table-7, the outcomes demonstrated that the measure of the three overwhelming metals adsorbed diminished to (17.44, 14.89 and 7.20) mg/ml for Pb, Cu and Cr separately, in the present of oxygen by *L. plantarum*, while the metals decrease were (17.99, 18.57 and 9.39) mg/ml for Pb, Cu and Cr individually without oxygen. On the other hand Pb, Cu and Cr levels were (2.11, 2.79 and 3.50) mg/ml with oxygen and (3.62, 4.85 and 7.39) mg/ml without oxygen.

Table 7 : Effect of oxygen on the lead ,Chromium and copper bioremediation by *Lactobacillus plantarum* and *Lactobacillus rhamnosus*

Oxygen	Bacterial species	Heavy metal concentration (mg/ml)		
		Lead	Chromium	Copper
Present	*L. plantarum*	14.89	7.20	17.44
Absent		17.99	9.39	18.57
Present	*L. rhamnosus*	2.11	3.50	2.79
Absent		3.62	7.39	4.85

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