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Bioremoval of Lead by *Bacillus amyloliquefaciens* MPA 1034 isolated from wastewater textile factories in El-Mahala El-Kobra city, Egypt

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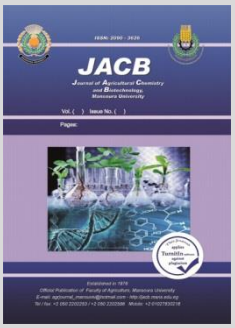

ABSTRACT

Heavy metals are considered serious environmental pollutants. Industrialization activity has put high amounts of toxic effluents, containing toxic metals into the environment. Therefore, there is an urgent need for industrial wastewater treatment. This study aims to lead bioremoval by active Pb-resistance bacterial cells. Only five bacterial isolates were obtained from industrial wastewater samples. Isolate NDSL2 withstand lead concentrations, MIC recorded at 180 µg/ml, and selected as the most resistance isolate. Depending on morphological and molecular characterization, it was identified as *Bacillus amyloliquefaciens* MPA 1034 strain. The optimized conditions for improving Pb-bioremoval efficiency were established. Maximum bioremoval of lead has been revealed by growing and active bacterial cells after 3 days at pH 7, in the presence of lactose, sodium nitrate and yeast extract. SEM, EDX and FTIR analysis confirmed Pd uptake in and/or bounded on bacterial cells. Thus, it could be recommended for using Pb-resistance strain as bioremoval and remediate polluted industrial effluents.

Keywords: bioremediation, Bioremoval, Bacillus, wastewater, lead

INTRODUCTION

Metals in general play important roles in the metabolism of microbial cells through acting as a cofactor for several enzymes. However, lead (Pb) is physiologically non-essential and causes health complication in animals and humans. Elevated lead concentrations can be toxic to microorganisms. Lead has several detrimental effects on microbial cells (Sun *et al*., 2023). Some microbes possess lead detoxification mechanisms that reported by Mitra *et al*., (2021).

Lead is one of the heavy metals which mainly derived from a variety of sources, one of these sources is the textile dyeing industry that has a negative significant impact in the environment. During the dyeing process, various metals are used as mordants e.g. lead white and lead chromate pigments. Dyeing process contributes up to one-fifth of water pollution and the textile dyeing industry is a major culprit. In particular in undeveloped countries, where weak regulations and enforcement prevail, wastewater from dye houses and garment factories is often directly dumped into rivers and streams. This discharge contains a toxic cocktail of carcinogenic chemicals, dyes, and heavy metals. Not only does it harm the environment, but it also contaminates essential drinking water sources (Khattab *et al*., 2020 and Rujido-Santos *et al*., 2022).

Nowadays, the use of microbes as remediators is a solution that is more environmentally compatible compared to chemical and physical approaches. In such polluted areas, microbes often use numerous methods to eliminate and detoxify heavy metals. Moreover, it is cheap, highly effective and not based on high technology. Until now, many microbial agents (bacteria, fungi, and microalgae) have been found as bioremediation approach (Delangiz *et al*., 2020).

Therefore, the present study aims to bioremediate lead through isolate lead-tolerant bacterial strain from industrial' wastewater, improve bioremoval process, investigate the micromorphological and molecular changing to confirm lead adsorption and removal.

MATERIAL AND METHODS

Wastewater collection

Samples of wastewater were obtained from various sites related to the textile industry. Five samples of effluent water were collected from Falcon, and El-Nabrawi factories in El-Mahala El-Kobra city, El-Garbia governorate, and should be mentioned here that these sampling sites are tanneries and spinning and weaving factories (Table 1). Concentrations of metal ions of all wastewater samples were analyzed in µg/ml according to Allen *et al*., (1974). All samples were collected aseptically in sterile plastic containers and stored at 4ºC for further use.

Table 1. Source and description of wastewater samples

No.	Sample description	Code
1.	Source of the sample was from the first tank of yarns dyeing process, El-Nabarawi factory.	NDF
2.	Source of the sample was from the second tank of yarns dyeing process, El-Nabarawi factory.	NDS
3.	Source of the sample was from the first tank of yarns dyeing process, Falcon factory.	FDF
4.	Source of the sample was from the third tank of yarns dyeing process, Falcon factory.	FDT
5.	Source of the sample was from the industrial effluent resulting from the softening step, Falcon factory.	FSS

Screening and isolation of Pb-resistance bacteria

Pour plate method was used for isolation. Under sterile conditions, one milliliter of wastewater samples was mixed with nutrient agar medium containing lead acetate, 100 µg/ml. Petri plates were then incubated at 30°C for 48h. Colonies formed were picked up and purified.

Morphological and molecular identification of Pb-resistance bacterium

The isolates were identified based on some cultural and morphological characteristics given in Bergey's Manual of Determinative Bacteriology (1994). In addition, the molecular

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identification of isolates was carried out by Sigma Scientific Services Company. Where, molecular and phylogenetic characterization of the isolates were performed based on 16S rRNA gene identification. The genomic DNA of the microbial isolates were extracted. The genomic DNA was used as a template for the amplification of 16S rRNA gene using universal primers. The PCR reaction consisted of 25 µL of PCR master mixture, 8 µL genomic DNA, 1 µl of the forward primer, 1 µl of the reverse primer, and 15 µL nuclease free water. The obtained PCR sequences were used to find out the related sequences with known taxonomic information in the GenBank database (http://www.ncbi.nlm.gov/blast).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of lead for isolated bacteria was determined using the pour plate method (Aleem *et al*., 2003; Ansari and Malik 2007). Nutrient agar plates supplemented with different concentrations (ranging from 100 to 350 µg/ml) of lead salt solutions were prepared. The inoculated plates were incubated at 30°C, followed to 72h until obvious growth of bacterial colonies were observed. The lowest concentration of the metal, which inhibits the growth was considered as the MIC of the metal against the tested isolate. Then, the most resistant isolate was selected for subsequent bioremoval experiments.

Preparation of Inoculum

Cell suspensions were prepared from fresh slants (48 h old). One milliliter from cell suspension was inoculated to 250 ml Erlenmeyer flask containing 50 ml sterile nutrient broth and incubated with shaking at 150 rpm at 30°C for 48h. Inoculums of bacterial cultures were adjusted at 10^6cell/ml for further fermentation experiments.

Lead bioremoval in shacking batch culture experiment

Bioremoval of lead was assessed by growing the bacterial isolates interact with Pb(II) in Tryptone yeast extract broth medium at pH 7.2 (Ksheminska et al., 2003). One mL of bacterial' inoculum was inoculated in 250 ml Erlenmeyer flask containing 50 ml of sterile broth medium mixed with lead, 100 µg/ml. Flasks were incubated at 30°C on shaker incubator. After incubation period, the bacterial culture was centrifuged at 6000 rpm for 20 min. The supernatants were collected to determine the amount of the residual or un-adsorbed Pb++ using atomic absorption spectrophotometer (AAS) according to Allen et al., (1974). The bioremoval efficiencies were calculated using the following equation:

$$\% \text{ metals bioremoval} = [(C_i - C_r) \div C_i)] \text{ X } 100$$

where, **C_i is the initial metal concentration in growing medium (µg/ml), and C_r is the residual metal concentration in supernatant (µg/L).**

Optimization of Pb-bioremoval in shacking batch culture

Some parameters were tested to reach the most suitable conditions of the bioremoval process for Pb (II). The performance of metal sorption by living microbial cells is influenced by environmental factors (incubation time and pH values) and essential nutritional factors (carbon and nitrogen sources). Data presented as bioremoval percentage.

Time course

Various incubation periods were tested from 1 to 4 days. One ml of bacterial inoculum (106 cell/ml) was cultured in 250 ml Erlenmeyer flask containing 50 ml of sterile Tryptone yeast extract broth medium mixed with 100 µg metal salt /ml. All inoculated flasks were then incubated at 30°C in shaker incubator to determine the suitable time for metal bioremoval.

Carbon source

Glucose was replaced in tryptone yeast extract broth medium with different carbon sources; lactose, sucrose, maltose, fructose, and starch, at concentration of 2%. Flasks were incubated for 2 and 3 days at 30°C with shaking at 150 rpm. The metal residual of each treatment was determined. Data presented as bioremoval percentage. The best carbon source was used subsequent experiments.

Nitrogen source and their combinations

Different nitrogen sources were examined in tryptone yeast extract broth medium contains the best carbon source and free of nitrogen. Nitrogen sources used were: NH_4Cl_2; $(NH_4)_2SO_4$; $(NH_4)_2PO_4$; NH_4NO_3; NO_2NO_3; yeast; beef extract and peptone at concentration 1%. Flasks were incubated for 2 and 3 days at 30°C with shaking at 150 rpm. The best nitrogen source was used subsequent experiments. As for nitrogen sources combination, mixtures of the best organic and mineral nitrogen sources were: (1) 0.5 g $NaNO_3$; (2) 0.5g beef; (3) 0.25g $NaNO_3$+0.25g beef; (4) 0.125g $NaNO_3$+0.375g beef; (5) 0.375g $NaNO_3$+0.125g beef. Flasks were incubated for 2 and 3 days at 30°C with shaking at 150 rpm. The metal residual of each treatment was determined. Data presented as bioremoval percentage.

Hydrogen ions concentration (pH values)

Various pH values from 5 to 9 were tested for their effect on the bioremoval activity of the tested bacterial cells. One ml of bacterial inoculum (10^6 cell/ml) was cultured in 250 ml Erlenmeyer flask containing 50 ml of sterile Tryptone yeast extract broth medium mixed with lead metal (100 µg /ml). Then, flasks were incubated for 2 and 3 days at 30°C with shaking at 150 rpm.

Characterizations

The bacterial cells grown in optimized tryptone yeast extract broth medium supplemented with lead solution (100 µg/ml) and without (control) were centrifuged and cell pellet used for SEM, EDX and FTIR analysis.

Scanning electron microscopy (SEM)

SEM technique reveal detailed morphological characterization and changes of bacterial cells. The prepared pellets were observed in a Jeol JSM-6510 LV SEM, operating at 30 kV at the Electron Microscopy Unit, Faculty of Agriculture, Mansoura University, Egypt.

Energy dispersive X-ray spectroscopy (EDX)

EDX techniques allow for detailed elemental analysis of bacterial samples. A SEM equipped with EDX was used for analyzing the specimens (Cairns Advanced Analytical Centre, 2017). EDX spectra used to identify the presence of specific elements within the microbial cells and their distribution.

Fourier Transform infrared spectroscopy (FTIR)

FTIR was used to investigate the various functional groups involved in bioremoval of lead and possible metal binding sites of functional groups present in the biomass of the tested isolate. FTIR spectroscopy provides valuable information about the biochemical composition and structural properties of bacterial cells. The prepared sample was placed into the FTIR spectrometer "ThermoFisher Scientific" at the Spectral Analysis Unit, Faculty of Science, Mansoura University, Egypt. Infrared radiation passed through the sample, the resulting infrared spectrum (within the range of 500-4000 cm^{-1}. Spectrum) was recorded. Spectral data were

analyzed using software to identify characteristic peaks and patterns associated with microbial biomolecules such as proteins, lipids, carbohydrates, and nucleic acids.

Heavy metals analysis

The residual heavy metal' ions concentration of the microbial samples was detected in the supernatants and analyzed using a Buck Scientific Accusys 211 series "Atomic Absorption Spectrophotometer" (58 Fort Point St. East Norwalk, CT 06855, USA) in Atomic Absorption Lab, Unit of Genetic Engineering and Biotechnology, at the faculty of Sciences, Mansoura university, by an air/acetylene flame system. The concentration of lead in the samples were determined in µg/ml (Allen *et al*., 1974).

RESULTS AND DISCUSSION

Data represented in Table (2) indicate metal content in wastewater samples thrown from textile and dyeing factories. Among the detected metals, the wastewater samples contain lead with various amounts.

Table 2. Initial concentrations of detected heavy metals (µg/ml) of five wastewater samples from tanneries and spinning and weaving factories.

Metals detected	NDF	NDS	FDF	FDT	ESS
Co^{++}	0.067	0.023	0.059	0.029	0.019
Pb^{++}	0.140	0.009	0.102	0.008	0.025
Ni^{++}	0.728	0.019	0.498	0.019	0.012
Zn^{++}	0.012	0.028	0.048	0.024	0.027
Cd^{++}	0.041	0.014	0.034	0.013	0.023

Isolation and screening of lead resistant bacteria

From wastewater samples, only five bacterial isolates were obtained that able to grow on nutrient agar medium supplemented with lead acetate (100 µg/ml). Then, the effect of several concentrations of $Pb^{++}$from 100 to 200 µg/ml were tested to determine MICs. Data represented in Table (3) show the MICs of metal (Pb^{++}) on the bacterial growth where their response to lead toxicity were varied. Isolates NDFL1 and NDSL2 were high Pb- resistance bacteria. Isolate NDSL2 withstand lead concentrations up to 180 µg/ml, which considered the most Pb-resistant one.

Table 3. Minimum inhibitory concentration (MIC) of lead on isolated bacteria

Metal Concn.	Bacterial isolates				
(µg/ml)	NDFL1	NDSL2	FDTL3	FDFL1	FSSL5
100	+	+	+	+	+
110	+	+	+	+	+
120	+	+	+	+	+
130	+	+	+	+	-
140	+	+	+	+	-
150	+	+	-	+	-
160	+	+	-	-	-
170	+	+	-	-	-
180	-	+	-	-	-
190	-	-	-	-	-
200	-	-	-	-	-
Mic (µg/ml)	170	180	140	150	120

(+) Growth (-) No growth

Many previous researches confirm bacterial tolerance against lead, Al-Ansari *et al* (2021) shows high results compared to the present values of MIC where the MIC of lead for *P. aeruginosa* strain RA-14 was 2800 µg/ml, and Babar *et al*., (2021) also reported that the MIC of Pb^{++} for *Bacillus altitudinis* MT422188 was 5 mM. In addition, Mitra *et al*., (2021) reported that microbes inhabiting Pb-contaminated area are found to have evolved distinctive mechanisms to successfully thrive in the Pb-contaminated environment without exhibiting any negative effects on their growth and metabolism.

Depending on the obtained results, the highest Pb-resistant bacterial isolate was selected for the optimization of metal bioremoval experiments.

Characterization and identification of bacterial isolate

Identification of the most resistance isolate, NDSL2, was depending on morphological and molecular characterization. On nutrient agar medium after 48 h, culture of NDSL2 showed gray smooth colony with irregular shape. Rod cells arranging mostly in single cells and sometime in diploe, cells length between 2-2.4 µm, gram-positive and spore formers were obviously in the microscopic examination. (Fig. 1).

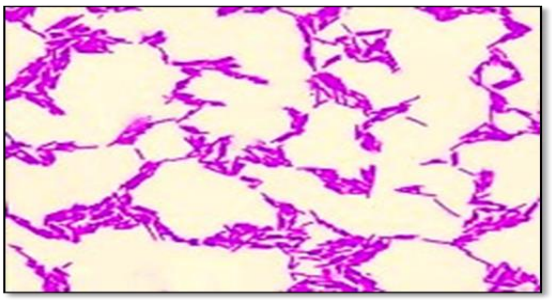

Fig. 1. Microscopic photograph of Pb-resistant isolate, NDSL2, showing morphological characterization

The molecular identification of Pb-resistant isolate, NDSL2, was carried out by Sigma Scientific Services Co. The obtained sequencing of the 16S rRNA gene of the bacterial isolate, NDSL2, was compared with the GenBank' deposited sequences and revealed that the isolate is almost *Bacillus* sp. and identified as 99.88% similarity with *Bacillus amyloliquefaciens* MPA 1034 strain under the accession No. NR117946. The phylogenetic relationship of the identified *Bacillus* sp. and other similar strains can be shown in Fig. (2).

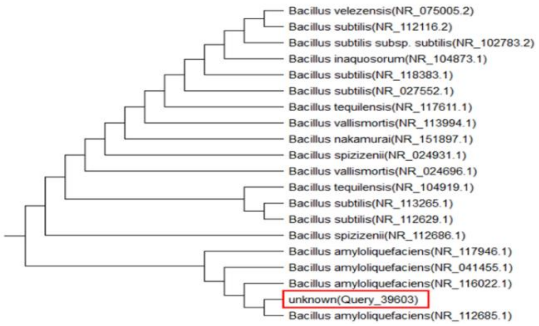

Fig. 2. Phylogenetic tree constructed using the 16S rRNA sequence of *Bacillus amyloliquefaciens* MPA 1034 strain (GenBank accession No. NR117946) and related strains using NCBI BLAST analysis and MEGA6 software.

Optimization of metal bioremoval in shacking batch culture

Metal removal by living cells is growth dependent and may be altered by nutritional and environmental conditions that influence microbe production. Thus, to improve lead removal by the resistant microbial strain, *B. amyloliquefaciens* MPA 1034, some environmental (contact time and pH) and nutritional (carbon and nitrogen) factors were tested.

Time course of metal bioremoval

Incubation period has a significant impact on the bioremoval process as it is directly affecting the microbial growth and their subsequent contact and uptake the metals. Data illustrated in Fig. (3) indicate the bioremoval of Pb^{++} by *B. amyloliquefaciens* MPA 1034 through different incubation periods from 1 to 4 days. It was found the maximum lead removal reached 34.9 and 37.7 % after 2 and 3 days, respectively. These results are in a harmony with those obtained by Salman (2012) who concerning the effect of incubation periods on Pb^{++} biosorption by *Brevundimona svesicularis* (C18), the highest uptake was recorded after 60h, reaching to 30.75% of the initial metal concentration. Also the maximum peak of uptake capacity of Pb^{++} was obtained after 48h of incubation period reaching to 34.68% by *Bacillus circulans* (D21) and 49.82% after 36h by *Klebsiella mobilis* (C19).

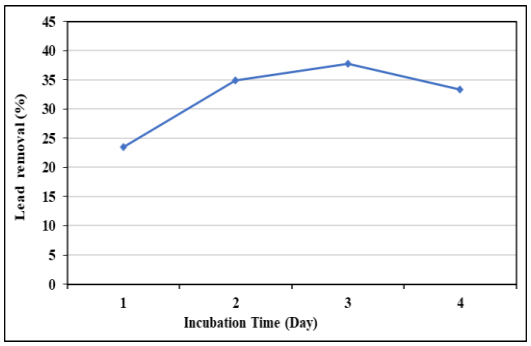

Fig. 3. Effect of incubation time on lead removal by *B. amyloliquefaciens* MPA 1034

Effect of carbon source

Carbon considered as a main nutrient element in bacteria metabolism, which bacteria uses it building up their biomass as anabolic reactions (Rittmann and Mc Carty, 2001). The properties of the organic carbon source play an important role in the operation of biological processes. The assessment has been made mostly by the conventional analyses for proteins and polysaccharides (Li and Yang, 2007; Ye *et al*., 2011; Zhao *et al*., 2018), which affecting the bacterial growth, and reflect on the ability of the microorganisms for heavy metal bioremoval.

Data in Figs. (4) show the effect of carbon sources on the bioremoval of Pb^{++} by *Bacillus amyloliquefaciens* MPA. Replacing glucose with fructose and maltose caused high decreases in lead bioremoval by *B. amyloliquefaciens* MPA, while enrichment of medium with sucrose and lactose resulting in a high bioremoval. Lactose was the best carbon source for lead bioremoval, which achieved 48.9 and 49.3% after 2 and 3 days respectively.

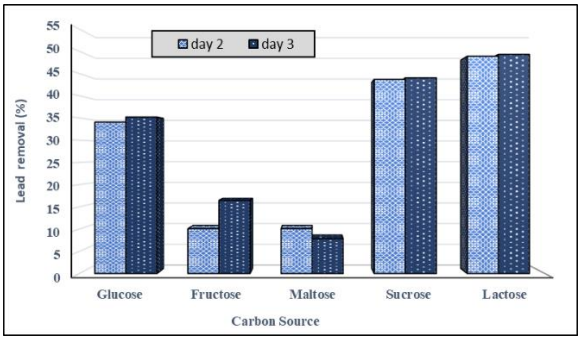

Fig. 4. Effect of carbon sources on lead removal by *B. amyloliquefaciens* MPA

Effect of nitrogen sources

Lead removal by *B. amyloliquefaciens* MPA was affected positively and negatively with nitrogen sources, whereas ammonium sulphate was the best mineral nitrogen source which recorded 50%, while yeast extract was the best organic nitrogen source which recorded 51.3 % after 2 days of incubation (Fig. 5).

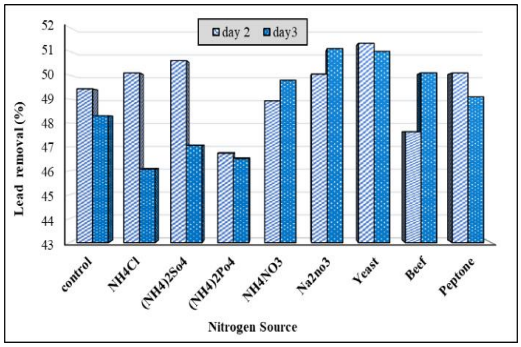

Fig. 5. Effect of nitrogen sources on lead by Bacillus amyloliquefaciens MPA .

Effect of the mix of best mineral and organic nitrogen sources

The best mineral and organic nitrogen source were tested in different proportions (by quarters) to determine the best mixture for *B. amyloliquefaciens* MPA to enhance the bioremoval of the heavy metals. Using mixtures from sodium nitrate and yeast extract caused an increased in the bioremoval of lead by *B. amyloliquefaciens* MPA, which achieved the highest lead bioremoval 67.4 % after 2 days by using mixture of 50% sodium nitrate (mineral nitrogen source) and 50% of yeast extract as organic nitrogen source in Fig. (6).

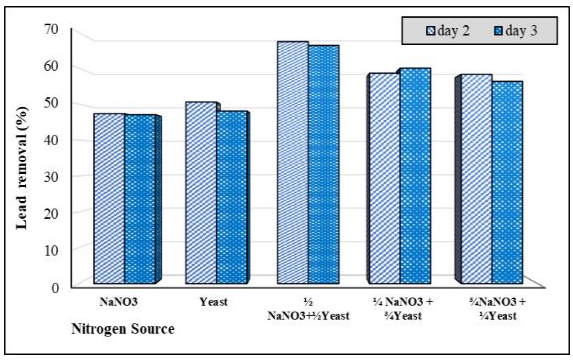

Fig. 6. Effect of mineral and organic nitrogen sources ratios on the bioremoval of lead by *B. amyloliquefaciens* MPA

Effect of pH

One of the important factors to consider in the growth of many microorganisms is the pH, since it plays an important role in how microorganisms will interact with the environment (Mougi 2023). Herein, data illustrated in Fig. (7) revealed the wide range of pH which *Bacillus amyloliquefaciens* MPA could grow and has a high potential of lead bioremoval. Lead bioremoval by *Bacillus amyloliquefaciens* MPA has high efficient in pH range from 6.5 to 8.0, Although the highest lead bioremoval achieved at pH 7 and 7.5, which recorded lead bioremoval 70.3% at pH 7 as well as 70.5 at pH 7.5 after 3 days. Raising initial pH over 8.0 decreased lead bioremoval, which is also happened when lowering pH below 6.

The degree of bioremoval efficiency of microbial cells for heavy metal ions depends upon various external operating factors such as pH, contact time, in addition to aqueous nutritional environment (Aryal and Liakopoulou-Kyriakides 2015).

The pH is a major factor which affects the solution chemistry of metal ions and the surface functional groups of the microbial cell wall, where H+ ions replace some of the positive ions from the biomass surface. Bioremoval of capacity of metal cations also increases with rising in values of pH, that is, may be due to the more negative binding sites exposed on biomass surface. On contrary, at low pH values, the binding sites of the cell wall are blocked and associated with hydrogen ions that prevent the access of metal cations (Singh *et al*. 2022).

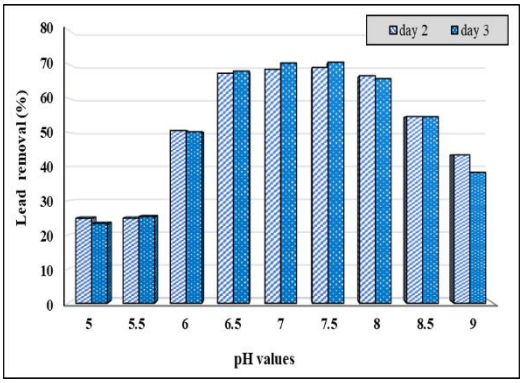

Fig. 7. Effect of initial medium pH on the bioremoval of lead by *B. amyloliquefaciens* MPA

Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDX)

SEM gives a visualization for the microbial cell's morphology at high magnifications, and these images can be used to identify and define changes in the shape, size and surface of the microbial cells after heavy metal exposure. In addition, EDX spectrum perform the elemental mapping of metal loaded by the surface of microbial cells.

Comparing SEM images of untreated *Bacillus amyloliquefaciens* MPA biomass cultured for 48 in optimized tryptone yeast extract broth medium and Pb-treated (100 µg/ml) and SEM images shown in Fig. (8 a&b). It was observed that untreated *B. amyloliquefaciens* MPA' cells showed regular long rods (Fig. 8a). While cells morphology of Pd(II) treated revealed many cells in dissimilar size and deformation of the cells due to wrinkle of cell wall (Fig. 8b). These images were further confirmed by EDX analysis for the elemental mapping of metal loaded on *B. amyloliquefaciens* MPA ' cells and it was observed metal ions distribution on the surface of the bacterial cells (Fig. 8 a&b). Along with the correspond metal ions, it was found that C and O are major elements present on both untreated and Pb-treated cells of *B. amyloliquefaciens* MPA. The EDX spectrum of lead-loaded *B. amyloliquefaciens* MPA appeared the peak of lead, 0.06%. Consequently, the existing of Pb(II) ion on the cell surface of *B. amyloliquefaciens* MPA was confirmed by EDX spectrum.

Analysis with SEM and EDX in previous studies confirmed the adsorption of metals on the bacterial biomass. For instances, SEM images of Gram-positive bacteria *Bacillus cereus* FIT10 (Dhanwal *et al*., 2018) and *Staphylococcus hominis* strain AMB-2 (Rahman *et al*., 2019) showed clear adsorption of Pb after heavy metals treatment. Moreover, EDX analysis also confirmed the presence of Pb signal on the bacterial surface. In addition, Shao *et al*., (2019) was investigated extracellular accumulation by SEM with EDX and found that extracellular accumulated Pb reached 61.7–95.9% of the total accumulation. Although when they examined the morphological differences of *Bacillus* sp. MPR-3 cells in the presence and absence of lead ions by SEM. They demonstrated that bacterial cells became bloated, smooth, and formed precipitates in the presence of Pb. The distinct morphological alterations, deformation, and severe membrane damage prove Pb toxicity to bacteria that result in different degrees of damage.

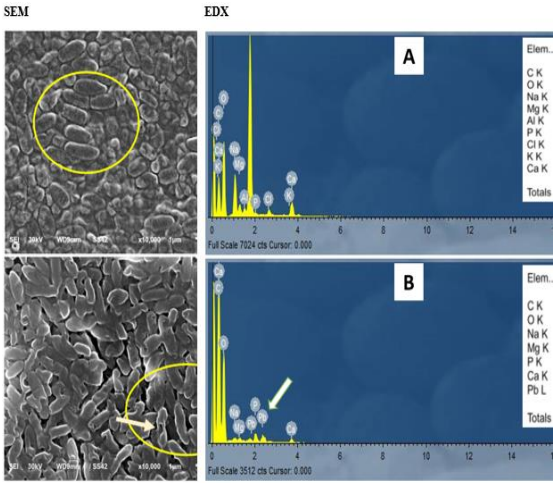

Fig. 8. Scanning electron micrograph (arrow points to changes) and EDX spectrum (arrow points to Pb peak) of Bacillus amyloliquefaciens MPA biomass cultured in optimized tryptone yeast extract broth medium. A) control – absence of metal; B) treated with lead solution (100 µg/ml).

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analyses were run to investigate the characteristic functional groups in surface of the microbial cell' samples and identify the change arises from the complex formation after metal exposure. The characteristic functional groups may have crossed a shift in their values than the control samples or disappearance of the frequency of a definite group. Here, the scale of the verified frequencies was expressed as the wavenumber in the ranges of 500 to 4000 cm^{-1}.

Cells of *Bacillus amyloliquefaciens* MPA that cultured in optimized tryptone yeast extract broth medium (both lead free and in the presence of lead solution, 100 µg/ml) were characterized by infrared spectroscopic analyses. The results stated to the representative frequencies and the deduced functional groups are shown in Table (4) and Fig. (9). The absorption band at ν= 1742 cm^{-1} for strong stretching C=O "esters" of the control sample was absence in the analysis of the cells treated with lead. On contrary, a new absorption band at 1232 is attributed to strong stretching C-O or medium stretching C-N "amine" groups appeared in the FTIR spectrum of cells treated with lead. Furthermore, stretching absorption band related to formation of new bond between lead and oxygen atom "Pb-O" at ν= 618 cm^{-1}.

Fourier-transform infrared (FTIR) spectroscopy is a powerful tool for monitoring and revealing microorganism composition and responses to the environment. So, it performed for confirming the interactions between metal ions

and functional groups on the surface of the microbial cells in comparison to untreated ones (Dhanwal *et al*., 2018; Rizvi and Saghir-Khan 2019; Pagnucco *et al*., 2023 and Tiquia-Arashiro 2023). Where, Dhanwal *et al*., (2018) identified the surface chemical functional groups of the metal tolerant strain, *Bacillus cereus* FIT10*,* by Fourier transform infrared (FTIR) spectroscopy as hydroxyl, carboxyl, amine, and halide, that might be involved in the biosorption of heavy metals, lead, chromium, copper, and nickel.

Table 4. The FTIR spectral analysis of *B. amyloliquefaciens* MPA' biomass cultured in optimized tryptone yeast extract broth medium both lead free (control) and in the presence of 100 µg lead /ml (Pb-treated).

Control		Pb-treated		
Wave No. (cm^{-1})	Annotations assigned functional groups	Wave No. (cm^{-1})	Annotations assigned functional groups	Shift
3422	–OH hydroxyl groups stretching	3528	–OH hydroxyl groups stretching "alcohol"	+106
2925	Strong broad –OH or N-H stretching	2924	Strong broad –OH or N-H stretching	-1
2856	Medium C–H stretching "alkane"	2872	Medium C–H stretching "alkane"	+16
1742	Strong C=O stretching "esters"	-	-	-
1653	C=O amidic carbonyl or strong C=C stretching	1657	C=O amidic carbonyl or strong C=C stretching	+4
1546	Symmetric bending of the amino group "N-H"	1545	Symmetric bending of the amino group "N-H"	-1
1459	C-H bending of CH_2 and CH_3 Proteins and lipids	1452	C-H bending of CH_2 and CH_3 Proteins and lipids	-7
1400	C-N stretching and N-H deformation or medium O-H bending "alcohol"	1406	C-N stretching and N-H deformation or medium O-H bending "alcohol"	+6
		1232	Strong C-O stretching or medium C-N stretching "amine"	-
1108, 1035	Strong C-O stretching	1073, 1031	Strong C-O stretching	-35 & -4
876	Strong C-H bending	826	Strong C-H bending	-50
777	Strong C-H or strong C=C bending	774	Strong C-H or strong C=C bending	-3
701	strong C=C bending	701	strong C=C bending	0
		618	Pb-O stretching	-
529	Polysulfides (S-S stretch)	541	Polysulfides (S-S stretch)	+12

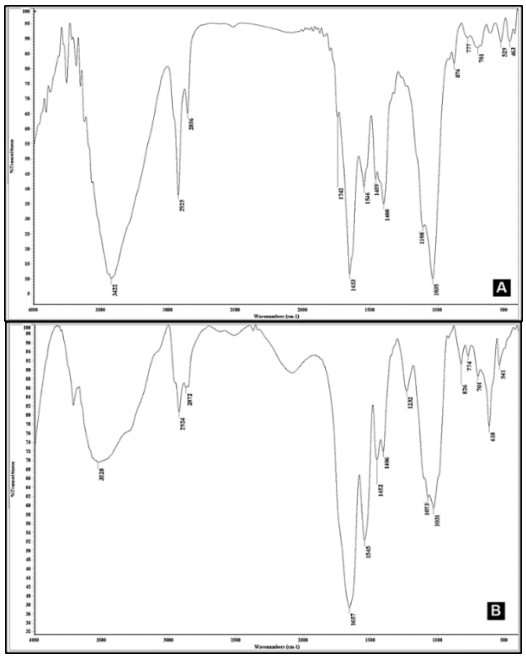

Fig. 9. FTIR spectra of *Bacillus amyloliquefaciens* MPA ' biomass cultured in optimized tryptone yeast extract broth medium A) Lead free (control) and B) in the presence of lead solution, (100 µg/ml).

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الإزالة البيولوجية للرصاص بواسطة *Bacillus amyloliquefaciens* MPA 1034 المعزولة من مياه الصرف الصحي في مصانع النسيج بالمحلة الكبرى، مصر.

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الملخص

تعتبر المعادن الثقيلة أخطر الملوثات البيئية حيث أدى النشاط الصناعي إلى إطلاق كميات كبيرة من النفايات السائلة الضارة والتي تحتوي على المعادن الثقيلة السامة في البيئة، ولذلك هناك حاجة ماسة وملحة لمعالجة مياه الصرف الصناعي. تهدف هذه الدراسة إلى الإزالة الحيوية لعنصر الرصاص بواسطة الخلايا البكتيرية النشطة والمقاومة للرصاص. تم الحصول على خمس عزلات بكتيرية فقط من عينات مياه الصرف الصناعي، وكانت العزلة NDSL2 مقاومة لتركيزات الرصاص وسجلتMIC عند 180 ميكروجرام/مل وتم اختيارها كأكثر عزلة مقاومة. وعلى اساس الخصائص المورفولوجية والجزيئية، تم تحديدها على أنها سلالة *Bacillus amyloliquefaciens* MPA 1034 . وقد تم الوصول الى الظروف المثلى لتحسين كفاءة الإزالة الحيوية للرصاص. وسجل الحد الأقصى من الإزالة الحيوية للرصاص بواسطة الخلايا البكتيرية النشطة بعد 3 أيام وعند درجة حموضة 7، وفي وجود اللاكتوز ونترات الصوديوم ومستخلص الخميرة. أكد تحليل SEM و EDXو FTIR إدمصاص أو امتصاص (أو كلاهما) معدن الرصاص بالخلايا البكتيرية. وبالتالي يمكن التوصية باستخدام هذه السلالة البكتيرية في الإزالة الحيوية للرصاص ومعالجة النفايات السائلة الصناعية الملوثة.