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Bioremediation potential of some heavy metals by bacterial strains isolated from municipal wastewater

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Abstract: Standard guidelines were used to assess the physicochemical characteristics of the effluent samples from municipal sewage treatment plant in Diwaniyah city. Physicochemical parameters assessed include temperature that was 29°C, PH value was 27.7, biochemical oxygen demand concentration was 449, chemical oxygen demand was 1470, BOD:COD ratio was 0.3, total suspended solids was 1990, ammonia nitrogen was 0.026, nitrate nitrogen was 8.515, phosphate was 5.579, total dissolved soilds was 1420.6, and lead concentration was 0.0210 and cadmium 0.0112, chromium 0.0410 is of great significance. From 20 samples, six isolates were able to grow in the wastewater agar media. These isolates were identified molecularly using partial sequences of the 16S ribosomal RNA gene as: Escherichia coli strain DAQSE1, Escherichia coli strain DAQSE2, Staphylococcus aureus strain DAQSE3, Pseudomonas aeruginosa strain DAQSE4, Enterobacter cloacae strain DAQSE5, Escherichia coli strain DAQSE6. A batch experiment was conducted to investigate bioremediation ability of some heavy metals by isolated bacterial strains. The results showed removal efficiency for lead ranged from 47% to 100%, and the most efficient strain was Staphylococcus aureus DAQSE3 with 100% removal efficiency. The cadmium removal efficiency ranged from 80% to 100%, with the most efficient Enterobacter cloacae DAQSE5 and Staphylococcus aureus DAQSE3 with 100% removal efficiency. The chromium removal efficiency ranged from 73% to 100%, with the most efficient Enterobacter cloacae DAQSE5 and Staphylococcus aureus DAQSE3 with 100% removal efficiency.

Keywords: Bioremediation Heavy metal Wastewater

1. Introduction

Municipal wastewater is a mixture of residential, agricultural, and industrial pollutants along with rainwater. Municipal wastewater treatment plants often release processed wastewater into surface water ecosystems. Municipal sewage discharges may include harmful compounds such heavy metals, bacteria, and chemicals that harm human health and the environment if not properly handled. Municipal treatment plants reduce organic waste to prevent dissolved oxygen drop in the receiving watershed, remove pollutants to prevent nutrient excess, and neutralize pathogenic microorganisms to protect

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human health [1,2]. Iraq's surface water quality has degraded owing to untreated wastewater in rivers. Over the years, mismanagement and insufficient wastewater treatment have worsened Iraq's water shortage. Government authorities regulate [3]. heavy metal usage and disposal because they are non-degradable and bioaccumulative, presenting serious risks[4]. Cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and lead (Pb) are heavy metals that may harm humans and the environment. Environmental issues like heavy metal water pollution affect people, animals, and plants. Due to their non-biodegradability, heavy metals may harm even in small doses. [5,6,7]. Among the most hazardous elements lead, cadmium, and chromium. are very toxic to living things and can damage cell membranes, disrupt DNA structure, and prevent enzyme activity. This toxicity is brought on by ligand interactions or the removal of essential metals from naturally occurring interacting sites [8]. Bioremediation is the process of converting poisonous heavy metals into a less dangerous by employing bacteria or enzymes to clean up a polluted environment. The strategy for revitalizing the environment is both ecologically benign and cost-effective [9]. Numerous adaptation mechanisms were established by microbial communities exposed to harmful heavy metals in the environment. These mechanisms can be used for environmental metal removal or detoxification. By altering their chemical characteristics through the processes of biosorption and bioaccumulation, bacteria can remove heavy metals or eradicate their toxicity. The ability of bacteria to remove metals varies by species [8]. This research aims to evaluate the efficacy of the wastewater treatment plant by studying the physicochemical parameters of the effluents from the primary wastewater treatment facility in Diwaniyah city, Al-Qadisiyah Province, Iraq. In addition to isolate and characterize bacterial strains from wastewater and tested its efficiency to remove lead, cadmium, and chromium.

2. Material and Methods

Study area

The main wastewater treatment plant is located in the southern part of the city of Diwaniyah, which is 11km away from the center, in Khairi village, behind the textile and rubber factory, about 4km away, near the Diwaniyah River. It was established in 1984. The designed capacity of the Khairi waste treatment plant was 12,000 cubic meters per day. However, this plant receives large quantities of wastewater exceeding its capacity, surpassing 35,000 cubic meters per day, which the plant cannot treat or control. The type of treatment in this plant is primary treatment, where large objects are screened using grates, followed by a grit removal unit. Secondary treatment is carried out using activated sludge in two basins containing aeration motors. Subsequently, the generated sludge is settled in final settling basins.

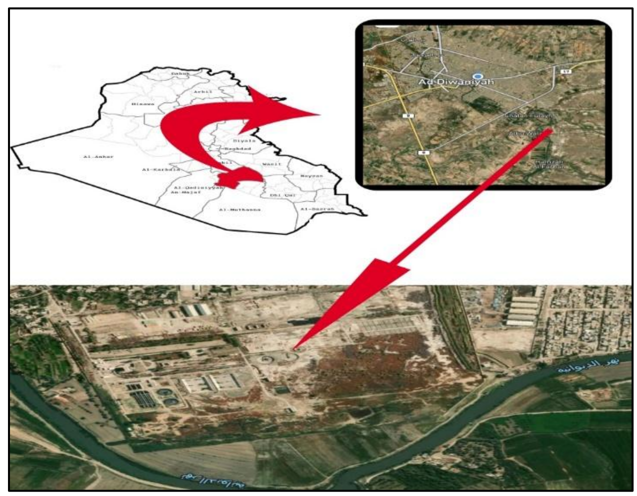

Fig 1. Location of the main wastewater treatment plant in the southern part of the city of Diwaniyah, Iraq.

Collection of samples

In this study, Twenty samples of wastewater effluents were collected from the Diwaniyah's wastewater treatment plant (Khairy) between September to January 2024. During sampling, PH and temperature were monitored on-site. Pre-sterilized bottles used for sampling following standard protocols [APHA 2005], and immediately transferred in in ice bags to the laboratory for analysis.

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Physochemical characteristics measurement of wastewater

The physiochemical properties of the wastewater treatment plant, Parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), nitrogen oxides (NO3), sulfur oxides (PO4), and ammonia nitrogen (NH3) were quantified utilizing the methodology outlined by [APHA 2005].

Heavy metal concentration estimation using flame atomic absorption

To prepare Five milliliters of concentrated nitric acid was introduced to 100 milliliters of wastewater and then evaporated on a hot plate at 80°C until desiccated. Subsequently, 5 ml of concentrated nitric acid (HNO3) was added and heated to dryness. Subsequently, 3 ml of concentrated hydrochloric acid (HCl) was included, and the total volume was adjusted to 10 ml with distilled deionized water. The concentrations of lead, cadmium, and chromium were quantified using a Flame Atomic Absorption Spectrometer[10].

Isolation and Primary Screening of Bacterial Isolates for Bioremediation Study

Twenty samples of Wastewater and sludge were serially diluted, and 0.1 ml of each diluted sample was inoculated onto nutrient agar plates, with duplicate samples for each dilution. After incubation at 37°C for 24–72 hours, distinct bacterial colonies were recovered and streaked onto sterilized wastewater agar (WWA), prepared by mixing 100 ml of wastewater with 2% agar. All WWA plates were incubated for 72 hours at 37 degrees Celsius. The selected bacterial isolates were chosen for further investigations.

Molecular Identification of Bacterial Isolates

Following the guidelines provided by the manufacturer of Geneaid Genomic DNA Purification Kit (Turkey), genomic DNA was extracted from bacterial isolates. The Macrogen company's universal primer (27F: AGAGTTTGATCCTGGCTCA, 1492R: GGTTACCTTGTTACGACTT) was utilized to amplify the 16s rRNA gene [11]. A PCR master mix reaction with a final volume of 50 µl was made using the Taq® G2 Green Master Mix kit 2X in the manner described below: Five microliters of DNA template, three microliters of each forward and reverse primer, twenty-five microliters of Taq® G2 Green Master Mix kit 2X, and fifty microliters of nuclease-free water. The PCR tubes underwent 30 cycles in a heat cycler under the following program conditions: Predenaturation takes 120 seconds at 95 degrees, denaturation takes 30 seconds, annealing takes 30 seconds at 53 degrees, extension takes 150 seconds at 72 degrees, and ultimate extension takes 300 seconds at 72 degrees. Using 1.5% agarose gel electrophoresis, 16s RNA gene ampilcons with 1498 pb were verified [12].The items were shipped to Korea to undergo Sanger sequencing at Macrogen. BioEdit 7.2 was used to examine 16s RNA gene sequences. To ascertain the level of genetic similarity, the sequences of the global strains' 16s RNA gene listed in the NCBI Gene bank were compared to the sequences of the local isolates' 16s RNA gene using BLAST.

A Study of bioremediation using A batch experiment

Inoculum preparation

Individual Loopful cultures of selected bacterial isolates were inoculated into presterilized 50 cc WWB with 0.5% peptone and sterile municipal wastewater. Flasks were 37 °C for 48 hours. The cell suspension's O.D. was adjusted to McFarland 0.5[13].

Bioremediation study

A batch experiment was conducted to investigate bioremediation ability of bacterial isolates. Ten ml (10%) A precise volume of each bacterial inoculum was introduced into a 250 ml flask containing 90 ml of non-sterile wastewater. The control flask was filled with non-sterilized effluent. The flasks were agitated at 37°C at 120 rpm for 72 hours. All samples underwent centrifugation at 10°C and 10,000 rpm for 20 minutes after incubation. The supernatants were used to measure the concentrations of lead, cadmium, and chromium using a Flame Atomic Absorption

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Spectrometer. The effectiveness of removal was established using the below equation.:

Removal Efficiency (RE%) = C0-CR\C0 × 100

C0 represents the initial concentration prior to treatment,

CR represents the final concentration after treatment.

Statistical analysis

In this study, The GraphPad Prism statistical software was used to analyze the data, which were presented as mean ± standard deviation using one-way ANOVA and two-way ANOVA.

3. Results and discussion

Physochemical characteristics

The concentrations and ratios of wastewater effluent are essential for evaluating wastewater treatment plant performance, recognizing risks to human health and the environment, and formulating efficient wastewater treatment and reclamation strategies. The examination of municipal wastewater treatment plant quality metrics, including BOD, COD, SS, NH3, TN, and P, is essential [14]. Disposing of municipal waste in drains and rivers adversely affects water quality and aquatic ecosystems. The release of untreated municipal wastewater into the river elevated BOD and TDS levels [15]. The physical and chemical properties affect the biodiversity of these aquatic systems. The release of untreated or inadequately treated waste pollutes the receiving water body, resulting in environmental and health detriment owing to the adverse effects on the physical and chemical properties of the watercourse. The temperature and pH levels were within permissible limits, since temperature significantly influences the aquatic environment; elevated temperatures in aquatic systems induce alterations in their physical, chemical, and biological characteristics, resulting in detrimental effects on the ecosystem [16]. Elevated colony counts and CFU/mL values indicate a higher concentration of microorganisms, which may threaten human health and the environment if wastewater is inadequately treated. The effective eradication or inactivation of germs during therapy is shown by a substantial reduction in colony counts and CFU/mL over time. This research, the CFU ratio was high (2.14×10^{7}). The temperature was within the permissible limits. The cause may be due to untreated wastewater or an increase in organic matter and this is similar to the study [36,37].

Table 1: Physicochemical parameters of wastewater from wastewater treatment plant

Parameters	**Mean**±S.D.
Temperature °C	**29^{a}**±1.908
PH	**7.27^{a}**±0.121
TDS mg/l	**1420.6^{a}**±37.197
TSS mg/l	**1990^{a}**±22.770
PO_4 mg/l	**5.579^{a}**±0.079
NO_3 mg/l	**8.515^{a}**±0.196
NH_3 mg/l	**0.026^{a}**±0.007
COD mg/l	**1470^{a}**±13.75
BOD mg/l	**449^{a}**±6.24
BOD:COD ratio	**0.3^{a}**±0.072
Pb mg/l	**0.0210^{a}**±0.0093
Cd mg/l	**0.0110^{a}**±0.0010
Cr mg/l	**0.0410^{a}**±0.0099
CFU $\times10^{7}$	**2.14^{a}**±0.04

The pH of wastewater varies based on its source and composition. Industrial processes can result in acidic or alkaline pH levels. pH fluctuations can alter pollutant solubility and toxicity, as well as the efficacy of treatment operations. Maintaining proper pH levels is crucial for effective wastewater treatment and minimizing environmental damage

[17]. TDS is a measure of salt in water systems [18]. TDS level was very high, above legal limits, indicating that the watershed could be contaminated by industrial effluent. High amounts of dissolved solids can be fatal to aquatic creatures, causing osmotic shock and disrupting the osmotic regulation of living organisms [19]. The total suspended solids (TSS) level was significantly higher than acceptable limits. High TSS levels in wastewater can be caused by insufficient TSS removal during primary treatment. TSS levels can degrade water quality because they absorb light [3]. Large amounts of phosphate contained in [20]. The phosphate concentration was also markedly over the permissible limits. As an increasing number of residences link to sewage systems and the delayed establishment of wastewater treatment facilities, this may result in significant eutrophication [21]. Except for NH4 and NO3, which were within legal ranges, all parameters exceeded the Iraqi Rivers Conservation Standards of 1967 and the US Environmental Protection Agency's allowable limits. BOD is an important measure in determining water quality. The BOD was higher than the permitted limit in this wastewater plant. High BOD levels deplete oxygen and generate anaerobic conditions, reducing aquatic biodiversity and dispersion. Organic debris promotes anaerobic activity, resulting in the accumulation of hazardous chemicals in water bodies [13]. Chemical oxygen demand (COD) is often used for the monitoring, planning, modeling, and operational analysis of wastewater treatment facilities[22].In this study, the COD value was significantly elevated and beyond the permissible limits. The rise in COD value correlates with the augmented influx of organic and inorganic constituents from the environment, alongside organic pollutants entering the aquatic system via domestic waste treatment facilities [16]. The BOD:COD ratio indicates wastewater biodegradability, with higher levels signifying less biodegradability. This analysis revealed a BOD:COD ratio of 0.3, indicating an inefficient plant[23].

Excessive use of lead, chromium, and cadmium in industrial processes can result in high concentrations in liquid waste, causing pollution and the emergence of metal-resistant microorganisms[8]. Lead (Pb) is one of the most hazardous persistent environmental contaminants, often known as a cumulative toxin, and has serious consequences for living creatures and ecosystems. Metal exposure can lead to the selection and adaptation of certain bacterial communities, which then thrive in contaminated environments. Human activity's increased lead emissions contaminated severe habitats, creating selection pressure for microbes to develop mechanism of lead toxicity resistance [38]. Prior to treatment, lead content was high owing to its usage in paint, pesticides, batteries, mining, and smelting, which may harm the environment[24]. Cadmium, a hazardous heavy metal, has no physiological purpose. Several human activities have released cadmium into the environment throughout the past century. As a caustic reagent, PVC stabilizer, color pigment, and nickel-cadmium battery stabilizer, cadmium pollution is largely industrial. House dust may include cadmium from contaminated soils. Copper and nickel smelting and refining, fossil fuel burning, and phosphate fertilizers release cadmium into the environment[25]. Cadmium levels were high regulatory limits. Chromium is a very toxic, nonessential metal for microorganisms and plants. The extensive industrial use of chromium has rendered it a significant pollutant in several ecosystems. The hexavalent form of chromium, Cr(VI), is more perilous than the less transportable trivalent version, Cr(III). The presence of chromium in the environment has resulted in the selection of microbial and plant species capable of tolerating elevated concentrations of chromium compounds [26]. The chromium concentration was within acceptable standards.

Screening and Identification of Bacterial Isolates

In the current study 54 bacterial isolates in total, showed growth on nutrient agar 26 of which came from wastewater samples and 28 of which came from sludge samples. Six of the 54 isolates were tested using WWA medium (Fig 2), which is free of any outside nutrients. These isolates grew well, which might be because they could use the organic matter in wastewater as nutrition [13]. Microbes thrive in environments contaminated by wastewater because they may easily exploit its harmful components as energy sources. By using wastewater as an electron source for respiration, heterotrophic microorganisms biodegrade contaminants in it. Here, among other nutrients in wastewater, nitrates and sulfates act as electron acceptors in anoxic, anaerobic, or aerobic environments. Some microbes have other energy sources, such as solar energy or chemicals, depending on whether they are phototrophic orchemotrophic. The development and reproduction of microorganisms utilized in bioremediation procedures are facilitated by hydrocarbons and organic matter as carbon sources [39]. After being selected, these isolates underwent molecular identification. Using 16S rRNA gene sequencing, bacterial isolates were identified and compared to the sequencing of global strains obtained in NCBI using BLAST [27]. The sequences of every isolate were deposited at Genebank NCBI with their accession numbers as following: Escherichia coli DAQSE1 (PV449165), Escherichia coli DAQSE2 (PV449166), Staphylococcus aureus strain DAQSE3 (PV449167), Pseudomonas aeruginosa strain DAQSE4(PV449168), Enterobacter cloacae strain DAQSE5(PV449169), and Escherichia coli strain DAQSE6 (PV449170).

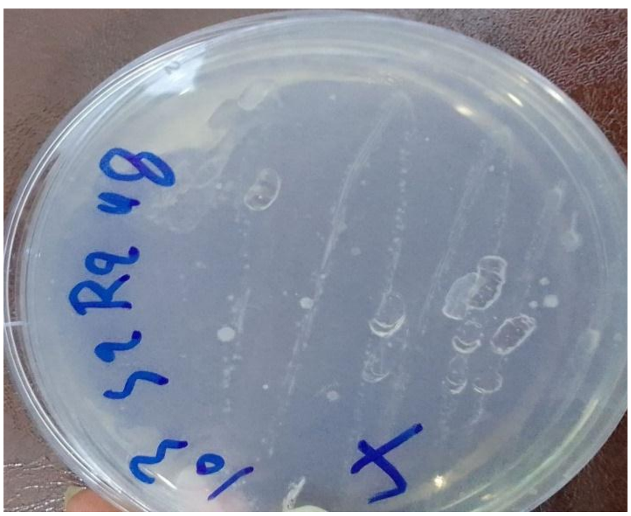

Fig 2. growth of bacterial isolates on wastewater media(WWA)

Removal Efficiency of bacterial strains

The current study demonstrated that bacterial strains isolated from municipal wastewater exhibit high efficiency in removal of heavy metals at varying rates. The variation in biological removal of heavy metals among bacterial isolates is related to their different affinities for metals. Microbes use a variety of strategies to tolerate the high levels of metal toxicity and to interact and thrive with organic metals in wastewater. Metallothionein and exopolysaccharide synthesis, enzyme synthesis, extrusion, and biotransformation are a few of the methods. In addition to producing metal chelators and biosurfactants, bacteria also mechanically oxidize, demethylate, and reduce metals and metal ligands in order to detoxify and resist metals.Microbial decontamination of metals can also occur by valence conversion, volatilization, and extracellular chemical precipitation. Because bacteria have an anionic structure that allows cationic metals to bind, their surfaces have a negative charge, making these methods viable. Both metabolism-based bioaccumulation and metabolism-free biosorption are methods by which bacteria can absorb heavy metals through biosorption[39].

The results revealed a difference in lead removal effectiveness among bacterial strains. Staphylococcus aureus DAQSE3 had a 100% removal efficiency, and Escherichia coli DAQSE1, isolates had lead removal efficiencies of 92%. Pseudomonas aeruginosa DAQSE4 removed lead with a 63% efficiency. Lead induces Pseudomonas aeruginosa to manufacture metallothionein proteins, which trap and accumulate lead within the bacterial cell, as demonstrated by the study [28,8]. Enterobacter cloacae DAQSE5 demonstrated lead removal efficacy of 60%.

Table 2 : Removal eficiency of heavy metals by bacterial isolates

Heavy metal	control	Enterobacter cloacae DAQSE5	Staphylococcus aureus DAQSE3	Pseudomonas aeruginosa DAQSE4	Escherichi coli DAQSE1
Pb	-4.7	60 %	100 %	63 %	92 %
Cd	-9.0	100 %	100 %	80 %	97 %
Cr	-2.4	100 %	100 %	73 %	96 %

Pseudomonas aeruginosa DAQSE4 was able to remove cadmium with efficiency of 80%. This result is consistent with the study [8], which indicated a removal efficiency of 81%. The study indicates that Pseudomonas aeruginosa may remove cadmium

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through many processes, including exopolysaccachride and dirhamnolipid biosurfactants. In reaction to cadmium stress, Pseudomonas aeruginosa produces metallothioneine proteins that bind intracellular cadmium. The removal mechanisms occur through adsorption on the outer polymer layer. The cadmium removal efficiency for Enterobacter cloacae DAQSE5 was 100%, which was similar to the results of the study [29], which reported a removal efficiency of 99%. The cadmium removal efficiency by Escherichi coli DAQSE1was 97%. This outcome was comparable study to that of [30].

For chromium, these bacterial strains were also efficient in chromium removal and reduction. The removal efficiency of Staphylococcus aureus DAQSE3 reached 100%, which is close to the results of study [31], in which the removal rate reached 91%. This indicates the possibility of using bacterial strains in the bioremediation of waste containing chromium and converting it to a non-toxic form. As for Pseudomonas aureus DAQSE4, the chromium removal efficiency was 73%, which is close to the results of study [32]. As for Enterobacter cloacae DAQSE5, the chromium removal efficiency was 100%, which was close to the results of study [33], where the removal rate reached 94.9%. Chromium removal efficiency of Escherichia coli DAQSE1reached 96%. This is similar to the study [34]. Several species of Staphylococcus, Escherichia coli, documented to convert harmful Cr(VI) to less toxic Cr(III). Bacterial bioreduction of Cr(VI) happens by using Cr(VI) as the ultimate electron acceptor, secreting specific extracellular enzymes, or generating H2S [35].

Conclusions

By measuring the physicochemical characteristics of wastewater treatment plants, the study conclude that municipal wastewater treatment plants pollute receiving watersheds and pose a threat to public health and the environment. The selected bacterial strains was capable effectively in reducing the metal concentration and Hence it can be recommended for municipal wastewater treatment.

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