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Research Article

Bacterial Removal of Lead and Mercury Elements from Water using *Pseudomonas aeruginosa in vitro*

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ABSTRACT

Background: Environmental pollution is the presence of a pollutant in the environment: air, water and soil, which may be lethal or toxic and will cause harmful to living things in the polluted environment. Thus removal of these toxic heavy metals from waste water is of crucial importance to protect the human population and the environment. The aim of the present study was to determine the capability of decreasing high concentrations of lead and mercury, in a laboratory experiment using *Pseudomonas aeruginosa* ATCC 27853 which was obtained from National Public Health Laboratory or The Stack Medical Laboratories.

Method and results: *P.aeruginosa* was cultivated in Peptone water and incubated for 48 hours. Different concentrations of heavy metal (Pb^{2+}, Hg^{2+}) were made in 30 ml of distill water, 5 ml of cultivated peptone water was added to each concentrations of heavy metals separately, incubated for 7 days at 37°C. Colorimetric method was done using ƛmax (520-540) nm to detect the absorbance of heavy metal (Pb^{2+}, Hg^{2+}) respectively. Absorbance was read before and after cultivation. The results of absorbance before cultivation were recorded. In the table for $Pb^{2+,}$ and for Hg^{2+}, the absorbance of Pb^{2+} after cultivation were revealed reduction which indicates removal of Pb^{2+} from solution and also absorbance of Hg^{2+} was decreased as result of removal Hg^{2+} from solution. The mean and Std. Deviation of concentration of (Pb^{2+}, Hg^{2+}) after cultivation were calculated by using SPSS. The results generally showed that Pb^{2+} and Hg levels were diminished in all the samples due to *P. aeruginosa* cultivation.

Conclusion and recommendation: It was concluded that *P. aeruginosa* assisted in removing harmful pollutants (lead and mercury) from water by uptake and accumulation of heavy metal from the water sample. It is recommended that further studies should be carried out to get rid of bacteria.

Keywords: Lead and mercury; *Pseudomonas aeruginosa; in vitro*

INTRODUCTION

Environmental pollution is the presence of a pollutant in the environment: air, water and soil, which may be lethal or toxic and will cause harmful to living things in the polluted environment [1]. Heavy metal pollution occurs directly by effuent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water systems and from the atmosphere *via* rainwater [2]. Heavy metals are defined as metals with a specific weight usually more than 5.0 g/cm^3, which is five times higher than the specific weight of water. The toxicity of heavy metals occurs even in low concentrations of about 1.0-10 mg/l while some strong toxic metals ions such as Mercury (Hg), Chromium (Cr), Lead (Pb), Zinc (Zn), Copper (Cu), Nickel (Ni), Cadmium (Cd), Arsenic (As), Cobalt (Co), Tin (Sn), etc., are very toxic even in lower concentration of 0.001-0.1 mg/l [3]. Thus removal of these toxic heavy metals from wastewater is crucial importance to protect the human population and the

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environment. Several heavy metals are particularly important in the treatment of industrial wastewaters i.e. zinc, copper, nickel, mercury, cadmium, lead and chromium [4]. Lead is a naturally occurring metal found deep in the ground. It occurs in small amounts in ore, along with other elements such as silver, zinc, or copper. Even though it is found in small amounts, there is an abundant supply of lead throughout the earth [5].

Lead released from manufacturing factories, recycling plants, automobile company and landfill leachate is abundantly found in wastewater. It can get into the body in two ways, through breathing it in or by eating it. For example, lead can enter the body through eating or inhaling paint dust or chips. The soil around the home can pick up lead from sources such as exterior paint. Lead can also enter in drinking water through plumbing [5].

Mercury is a naturally occurring heavy metal. It is unique because it is liquid at atmospheric temperatures and it uniformly expands and contracts in response to changes in temperature and pressure. Mercury can be taken into the body through the lungs, mouth, and skin or by eating mercury-contaminated fish. Mercury affects the human brain, spinal cord, kidneys, lungs and liver. Symptoms of short-term exposure to high levels of mercury include nausea, shortness of breath, fever, muscle aches, skin rash, sore gums, and an elevated white blood cell count [6].

REMOVAL METHODS OF HEAVY METALS

Many conventional methods have been applied in order to remove heavy metals from aqueous streams. Among the most commonly used techniques are chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, evaporative recovery and solvent extraction [7]. These conventional techniques offered several problems such as unpredictable metal ions removal and generation of toxic sludge [7]. Bioremediation is an alternative option to use of natural and recombinant microorganisms for the removal/reduction of toxic pollutants. It is considered as cost-effective and environment friendly approach [8]. The living and dead biomass of microbes have been used for the efficient removal of metal ions through biosorption and bioaccumulation process [9]. Bioaccumulation is a dependent, active and partially reversible process that needs energy and requires respiration. In contrast, biosorption is an independent, revisable process that does not require energy/ respiration [2]. The major advantage of biosorption is low operating cost, high ability, possibility of metal recovery and potent biosorbent revival [10].

Pseudomonas aeruginosa

Some studies have demonstrated microorganism`s ability to remove heavy metals from wastewater with better performance and lower cost. Many types of yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure [3]. *Pseudomonas species* is ubiquitous in soil, water ecosystems and are capable of metabolizing a wide range of organic and inorganic compounds. In addition, *Pseudomonas* was well studied and showed high resistance to antibiotics, heavy metals and detergents and organic solvents [11]. *P. aeruginosa* is a gram-negative, rod-shaped bacterium. It is found in desert, agricultural, grassland, and forest soils, water and humans, plants, sewage and hospitals [12] as well as in riverine ecosystems [13] and metal contaminated sites [14]. In the laboratory, the simplest medium for growth of *P. aeruginosa* consists of acetate as a source of carbon and ammonium sulfate as a source of nitrogen. Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees. It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics [15]. The objectives of this study were to remove Lead and Mercury elements from water using bacteria (*P. aeruginosa*).

MATERIALS AND METHODS

The present study is laboratory based study conducted in Gadarif state, Sudan, *P. aeruginosa* was cultivated in Peptone water and incubated at 37°C for 48 hours, different concentrations of both heavy metals (Pb^{2+}, Hg^{2+}) were made in 30 ml distill water (0, 2, 6, 8,10 ppm Pb^{2+}) and (0, 2,4,6, 8,10 ppm Hg^{2+}), the absorbance was read before inoculation of the *P. aeruginosa* and results were recorded, 5 ml of peptone water which was cultivated by the bacteria was added to each different concentrations of each heavy metals ($Pb^{2+,}$ Hg^{2+}) separately, incubated for 7 days at 37°C, absorbance was read for both heavy metals, the results were recorded [16,17]. Absorbance was read by Colorimetric method with different filters for (Pb^{2+}, Hg^{2+}) at λmax (520 and 540) respectively. The Mean and Std. Deviation of concentration of both heavy metals after cultivation were calculated by using SPSS and the results were recorded (Tables 1-3).

Table 1: Absorbance of different concentrations of Hg^{2+} before bacterial cultivation.

C (ppm)	Ab
0	0
2	0.13
4	0.35
6	0.37
8	0.48
10	0.55

RESULTS AND DISCUSSION

Our study results were showed that P. aeruginosa diminished the both heavy metals in water solution and that clearly in results. The absorbance of Pb^{2+} after cultivation was revealed reduction which indicates removal of Pb^{2+} from solution Table 4.

Table 2: Absorbance of different concentrations of Hg^{2+} after bacterial cultivation.

C (ppm) before cultivation	Ab after cultivation	C (ppm) after clutivation
0	0	0
2	0	0
4	0.01	0.0007
6	0.07	0.0053
8	0.09	0.0069
10	0.1	0.0076

Absorbance of Hg^{2+} was decreased as result of removal of Hg^{2+} from solution Table 5 and absorbance before cultivation.

Table 3: Mean and Std. Deviation of Concentration of Hg^{2+} after cultivation.

Mean of Concentration of Hg^{2+} before cultivation	Std. Deviation	Number	Mean of Concentration of Hg^{2+} after cultivation
0	.	1	0
2	.	1	0
4	.	1	0.0007
6	.	1	0.0053
8	.	1	0.0057
10	.	1	0.0076
5	0.003369	6	0.003217

Table 1 for Pb^{2+}, and Table 4 for Hg^{2+}, were showed normal absorbance compared with absorbance after cultivation, The mean and Std. Deviation of concentration of (Pb^{2+}, Hg^{2+}) after cultivation were calculated by using SPSS (Tables 3-6).

Generally the results showed that Lead and Mercury Elements were removed from water in all the samples due to inoculation of bacteria (P. aeruginosa). Another study was carried out by Karimpour et al. revealed that *P. aeruiginosa* has high ability to adsorption of Cd and Pb in aqueous solution [19]. Zeng et al. also concluded that *P. aeruginosa* has performed better biosorption of Cd than non-living cells [20].

A similar study curried out by Kalita and Joshi reported *Pseudomonas aeruginosa* isolated from extreme habitat of hot water spring of North – East India evaluated for its Lead biosorption property [21-24]. \

The present study described Lead and Mercury biosorption property of Pseudomonas aeruginosa ATCC 27853 which was obtained from National Public Health Laboratory or The Stack Medical Laboratories.

The bacterium revealed ability of removal of heavy metal (Pb and Hg) from water solution. Several studies have been reported that the ability of P. aeruginosa to remove heavy metal, similar study was conducted by Chang et al. showed that P. aeruiginosa has effective bioadsorbent for removal of Cd, Cu, Pb, from polluted water [18].

Table 4: Absorbance of different concentrations of Pb^{2+} before bacterial cultivation.

C (ppm)	Ab
0	0
2	0.47
4	0.9
6	1.3
8	1.69
10	1.9

Table 5: Absorbance of different concentrations of Pb^{2+} after bacterial cultivation.

C (ppm) before cultivation	Ab after cultivation	C (ppm) after cultivation
0	0	0
2	0	0
4	0.21	0.0044
6	0.23	0.0048
8	0.25	0.0053
10	0.28	0.0059

The current study showed that Pseudomonas aeruginosa removed (5-0.003217) 99% of Hg^{2+}, (5– 0.003333) 99% of Pb^{2+}. Another study conducted by Rehman et al. revealed that 93% of Hg^{2+} removed by *Pseudomonas aeruginosa*.

A similar study curried out by Jaysankar et al. showed that Pseudomonas aeruginosa removed more than 98% of Pb^{2+}.

Table 6: Mean and Std. Deviation of Concentration of Pb^{2+} after cultivation.

Mean of concentration of Pb2+ before cultivation	Std. deviation	Number	Mean of concentration of Pb2+ after cultivation
0	.	1	0
0	.	1	2
0.004	.	1	4
0.0048	.	1	6
0.0053	.	1	8
0.0059	.	1	10
0.003333	0.002656		5

CONCLUSION AND RECOMMENDATION

An increasing concentration of hazardous heavy metals like Pb^{2+} and Hg^{2+} in the environment especially in water has stimulated research to look for new possible ways for its removal/ neutralization. Study concluded that *Pseudomonas aeruginosa* is a very effective biosorbent for removal of lead and mercury in industrial wastewater as evidenced by its biosorptive capacity. This method offers a low-cost and environmentally friendly technology for treatment of, industrial or mixed wastewater. It is suggested as one of the choices to ensure treatment efficiency and performance for industrial effluents contaminated with heavy metals like lead and mercury. It is recommended that further studies should be carried out to get rid of bacteria.

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