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Bioremediation of Some Heavy Metals in Polluted Waters (A *Case Study* of Lake hoz-e Soltan Salt (Qom, Iran))

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Abstract

Bioremediation is considered as one of the safer, cleaner, cost effective and environmental friendly technology for removing the heavy metals ions from polluted area. Bioremediation is naturally living organisms to reduce the environmental pollutants into less toxic forms. It is followed by bacteria and fungi or plants to degrade or detoxify hazardous ingredients to human health /or the environment. Heavy metal contamination represents an major environmental problem due to the toxic effects of metals and their invasion in to the food chain leads to serious ecological and health problems. The aim of this study is to screen and characterize the resistance of the Halo tolerant bacteria of heavy metals from hoze Sultan lake in Qom. In this study, Halophilic and Halo tolerant microorganisms resistant to heavy metals nickel, cadmium, copper and cobalt were separated in Qom hoz-e Soltan Lake. Results shows that microorganisms which are respectively 13%, 5/19%, 75/43 and 7.3% of MGM, MH, SWN, LNSWN medium, 62/20% of samples from the environment without salt Nutrient agar was obtained. Resistance to heavy metals in the samples showed that Halo tolerant microorganisms most resistant to nickel and cobalt and cadmium and copper metals are the most susceptible.

Keywords: ***Bioremediation; Halo tolerant; Resistant; Microorganism***

Introduction

Contamination due to chemicals including heavy metals is a major problem that may have negative consequences on the biosphere. The levels of metals in all environments, including air, water and soil are increasing in some cases to toxic levels, with contributions from wide variety of industrial and domestic sources. The existing heavy metals in the environment and industrial wastewater increasingly contaminate ecosystems and threaten human health in-the developing countries. Heavy metal contaminants entering our water are a major concern due to both toxicity and persistence within the environment, necessitating physical extraction. Different concentrations of heavy metal elements commonly occur in all ecosystems. Several compounds have diverse properties such as Zn, Cu, Ni, Fe and Mn are essential trace elements for living organisms [1]. The soil and water pollution are often occurred by toxic heavy metals and organic pollutants as a result of human

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activities become a key concern in environmental and health problem. Several toxic metals (Pb, Mn, As, Ni, Zn, Cd, Cu, Hg, etc.) from industrial wastewater and other human activities are directly or indirectly released to the environment. Some metals including Fe, Mn, Cu, and Zn are micronutrients for most of the organisms; however, not all living organisms. It can play a biotic role in metalloenzymes. Cations usually play specific roles in nucleic acid structures, functions and metabolisms and increase membrane stability [2]. Heavy metals happen naturally in the environment from anthropogenic sources or pedogenetic processes of weathering of parent materials. Researchers have shown that bioremediation using microorganisms and plants have that potential to remove, degrade or inactivate heavy metals [3,4]. Bioremediation of metal pollutants from waste water using metal resistant bacteria is a very important aspect of environmental biotechnology. Today, due to industrialization and exploitation of natural resources, soil and water pollution is one of the major global concerns Bioremediation of microorganisms and essentially biological processes for reducing environmental pollutants into the uses little or no toxicity. Microorganisms doing actively absorb heavy metals. Among bioremediation methods seem more practical adsorption process of accumulation for use on a larger scale. Accumulation of heavy metals in contaminated soil is the main cause of the harmful effects of heavy metals on human health [2]. Heavy metals nerves, liver and bones, as well as the functional groups of vital enzymes harm. Some of these metals are essential to certain levels for microbial growth and higher levels of toxicity to the cells. The Cobalt, copper, nickel and zinc plays an important role in regulating gene expression and activity of biomolecules, enzymes or enzyme cofactors [3]. Bioremediation uses biological agents, mainly microorganisms i.e, bacteria, fungi and yeast to clean up contaminated water. Bioremediation with the help of microorganisms can effectively reduce heavy metals in the environment [5]. The ability of microorganisms to remove heavy metals from aqueous solutions, particularly in the range of less than 1 to 100 mg, bioremediation method is distinct from other cleaning methods [5]. In the last decade, a lot of research on the introduction of microorganisms capable with respect to the two properties was conducted bioremediation and biocompatible according to many studies on the nature of the interaction of microorganisms with toxic metals is in progress [3]. Bioremediation methods for heavy-metal-contaminated water include biosorption, microbial-driven *in situ* precipitation of heavy metals by dissimilatory reducing bacteria and the incorporation of HMHs in artificial wetlands. Microorganisms play a major role on nutritional chains that are an important part of the biological balance in the life in our planet. They are essential for the closing of nutrient and geochemical cycles such as carbon, nitrogen, sulfur, and phosphorous cycle. TABLE 1 shows the microorganisms that use of heavy metals. Overview of bioremediation potential for heavy metals contamination through microbe-heavy metals interactions are shown in TABLE 2. Ghom(Qom), is the capital of Qom Province and eighth largest city in Iran. It lies 125 kilometers or 78 miles by road southwest of Tehran. Hoz-e Sultan is the lonely salt lake in the heart of Iran. The aim of this article is the Bioremediation of some Heavy metals in polluted waters from hoz-e Soltan salt lake in Qom, Iran.

TABLE 1. **The Microorganisms that use of heavy metals.**

Microorganism	**Metals**	**References**
Bacillus spp. *Pseudomonas aeruginosa*	Cu, Zn	[4 ,9]
Zooglea spp. *Citrobacter spp.*	U, Cu, Ni Co, Ni, Cd	[8]
Citrobacter spp.	Cd, U, Pb	[9]

Chlorella vulgaris	Au, Cu, Ni, U, Pb, Hg,Zn	
Aspergilusniger	Cd, Zn Zn, Ag, Th, U	[9]
Pleurotusostreatus	Cd, Cu, Zn	[9]
Rhizopusarrhizus	Ag, Hg, P, Cd, Pb, Ca	[9,10]
Stereumhirsutum	Cd, Co, Cu, Ni	[11,12]
Phormidiumvalderium	Cd, Pb	[11,12]
Ganodermaapplantus	Cu, Hg, Pb	[11,12]

TABLE 2. **Overview of bioremediation potential for heavy metals contamination through microbe-heavy metals interactions [21].**

Heavy Metals	Types of contaminant alteration	Mechanisms of microbe contaminant interactions
Copper	Immobilized by sorption process.	Sorbs to extracellular polymer and biomass.
Nickel	Immobilized by sorption process.	Sorbs to extracellular polymer and biomass.
Zinc	Immobilized by sorption process.	Sorbs to extracellular polymer and biomass.
Mercury	Volatilized or immobilized by sorption, methylation, and precipitation.	Enzymatically oxidized, reduced or methylated to promote detoxification.
Chromium	Immobilized by precipitation.	Enzymatically oxidized or reduced to promote detoxification.
Manganese	Immobilized by sorption; methylation possible.	Sorbs to extracellular polymers and biomass.
Iron	Immobilized by sorption; methylation possible.	Sorbs to extracellular polymers and biomass.

Material and Methods

Bioremediation methods have drawn the attention of the researchers as chemical detoxification methods failed to handle the issue of water remediation economically. Specific microbial species are used to assimilate, precipitate, oxidize or reduce heavy metals to reduce their toxicity [6]. A wide variety of microorganisms are used as biosorbents for heavy metal remediation and some of them cause metal transition and reduce toxicity [7-9].

Sampling

Sampling from different parts of Qom hoz Sultan Salt Lake, including soil, water and salt was performed. Samples were collected in sterile conditions are coded and transferred to the laboratory, where they were prepared for planting (FIG 1).

FIG. 1. **Hoz-e soltan salt lake**

Isolation of microorganisms

Isolation of pure bacterial cultures: Isolated cultures were grown on the NA plates and were sub-cultured on fresh MHA plates from the master plates to get the pure cultures. 14 samples of 1 ml saline was added to 9 ml samples of soil, water and salt in different serial dilution were incubated at 37°C for two hours. Supernatants from them in liquid medium nutrient broth containing 3%, 7%, 10%, 15%, 20% sodium chloride and media (16) SWN, (13) MH, LNSWN, (14) MGM respectively 2 %, 10% and 23% sodium chloride and cultured at intervals of 24 hours, 72 hours and 14 days after the primary culture, were isolated and purified.

Identification of isolate based on morphological and biochemical characteristics

After purification of microorganisms, identification based on macroscopic characteristics, including size of colonies on solid culture medium, shape, color, surface, margins and sex colony were performed. In the next phase identification based on microscopic properties of microorganisms (rod, spherical, spiral), size, how the micro-organisms on the slides (or dispersed complex shape their community), the type of reaction to the Gram stain, the spore, position and spores done. With the motility, catalase and oxidase tests, some biochemical characteristics of the isolates were determined. Catalase activity in isolates from fresh 3% hydrogen peroxide and solid culture of microorganisms investigated.

Determination of Minimum Inhibitory Concentration (MIC)

The bacteria samples were determined by slowly increasing the concentration of heavy metals, each time on the nutrient agar plate until the bacterium failed to give colonies on the plate. When the samples failed to grow on the plates after incubation, minimum inhibitory concentration (MIC) was noted.

Screening based on the amount of sodium chloride salt tolerance

To determine Halophiles or Halotolerant of microorganisms, all of the isolates were cultured on medium with sodium chloride percent less or more. As a result of environmental isolates MGM (23% sodium chloride) on the SWN (2% sodium chloride) and inverse, and isolates of the MH (10% sodium chloride) were cultured on both the environment.

Screening based on resistance to the metal with blots methods with solid media and microplate

Resistant microorganisms to 10%-23% sodium chloride (resulting separation of the medium MGM and MH) to evaluate resistance to heavy metals, $NiSO_4.6H_2O$, $CdCl_2.H_2O$, $COSO_4.7H_2O$, $CuSO_4.5H_2O$ were selected. To measure metal resistance with blots methods in the solid medium, soluble metal salts of nickel, copper, cobalt and cadmium, with concentrations of 0, 0/5, 1, 2, 3, 5, 7 mM in the MH medium was prepared, then blotting was performed on solid medium and petri dishes were incubated for 36 h at 37°C [7]. In the microplate method concentrations mM 0/5, 1, 3/12, 6/25, 12/5, 25, 50 and each of the wells was prepared in the microplate MH medium. In this method, metal and non-inoculated culture medium microorganisms, as a negative control and metal-free culture medium containing the microorganisms as a positive control were considered. Each of the plates was incubated for 36 h at 37°C.

Results

Isolation of microorganisms

20 samples in MGM medium, 31 sample in MH medium, 70 sample in SWN medium, 6 samples in LNSWN medium and 33 samples in NA without sodium chloride medium were isolated. The growth of microorganisms were about 1-2 days in SWN, LNSWN, NA MH cultures, 2-3 days in MGM culture and the 2-10 days in MGM medium. Macroscopic screening isolates showed that most primary-colored colonies, some colonies terracotta work, some colorless, and a few also had a color between pink and red. Red pigments are often observed in isolates obtained from MGM medium and the colonies vary in size from very small (with a diameter of about 1 mm) to large (about 1 cm in diameter) varied.

Morphological characteristics of the isolates

About 90% of all isolates were gram-positive bacilli and other was gram negative bacilli, gram-positive and gram-negative and a few were actinomycetes. Microscopic observations showed that about 5% of all isolates containing spores (central, semi-final and final) were also larger than the cell size and cell size .20 % Strains isolated from the MGM culture medium were catalase-negative and 80%were positive catalase. All microorganisms isolated from the MH medium were positive catalase, 10% of strains in the MGM medium, with the addition of oxidase test solution showed no color change. 80% of strains, strong oxidase and the other 10%, were poor oxidase. 42% of microorganisms in MH culture, were oxidase negative, 6% of strains, strong oxidase and 52% were positive oxidase (TABLE 3).

TABLE 3. **Biochemical and morphological characterization and molecular identification of the metal –resistant bacteria isolates in The City of Qom, Iran.**

isolates	**J1**	**J2**	**J3**	**J4**	**J5**
Bacteria	Bacillus cereus	Bacillus amyloliquefaciens	Bacillus subtilis	Bacillus aerius	Pseudomonas aeuginosa
Morphological					
Gram stain	(+)	(+)	(+)	(+)	(–)
Color	White	White	Cream	White	Bluish green
Cell morphology	Rods	Rods	Rods	Rods	Rods
Motility	(+)	(+)	(+)	(+)	(+)
Endospore	(+)	(+)	(+)	(+)	(–)
Margin	Irregular	Irregular	Irregular	Irregular	Irregular
Biochemical	(+)	(+)	(+)	(+)	(+)
Catalase	(–)	(+)	(–)	(+)	(+)
Oxidase	(+)	(–)	(+)	(+)	(+)
Citrate					

Resistance of metal isolates and metal by microplate

To measure metal resistance for isolates of MGM 23% NaCl medium using microplate method, Isolates showed more resistance to nickel and the most sensitive to cadmium. The highest concentration of microorganisms growth were 6 mM for nickel metal and respectively 3, 0/5 and 0/5 mM for cobalt, cadmium and copper metals (FIG 2).

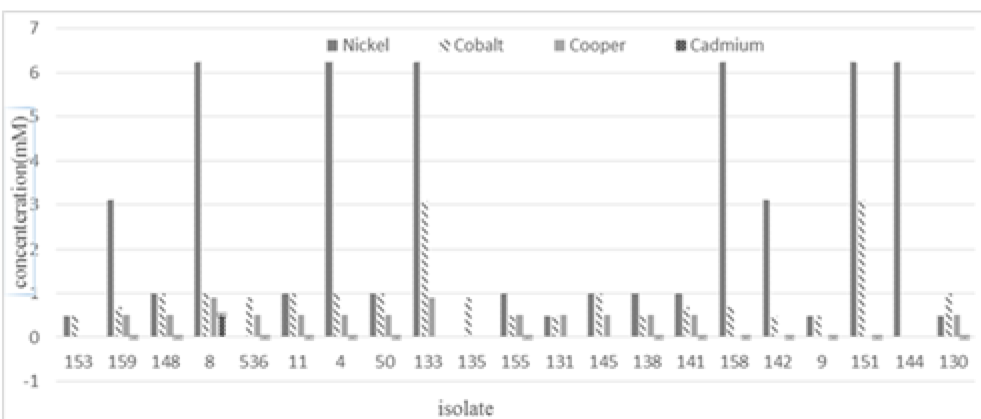

FIG. 2. **The results of resistance to the nickel, cobalt, copper and cadmium metals with microplate method: isolates have endured the maximum6 mM Nickel, 3Mm cobalt, 0/5mM copper and cadmium.**

Resistance of metal with the blotting method

Results In the case of nickel resistance showed that 10% of all isolates maximum of 3 mM, 30% of isolates 2 mM, 5% of all isolates 1 mM, 5% of all isolates was 5.0 mM nickel metal handle and 20% of all isolates not tolerated any concentration of metal. FIG. 3, shows the results of this method for a strain resistant to nickel, cobalt, copper, cadmium and a sensitive strain sensitive to the metals cobalt, copper, cadmium and nickel are relatively resistant.

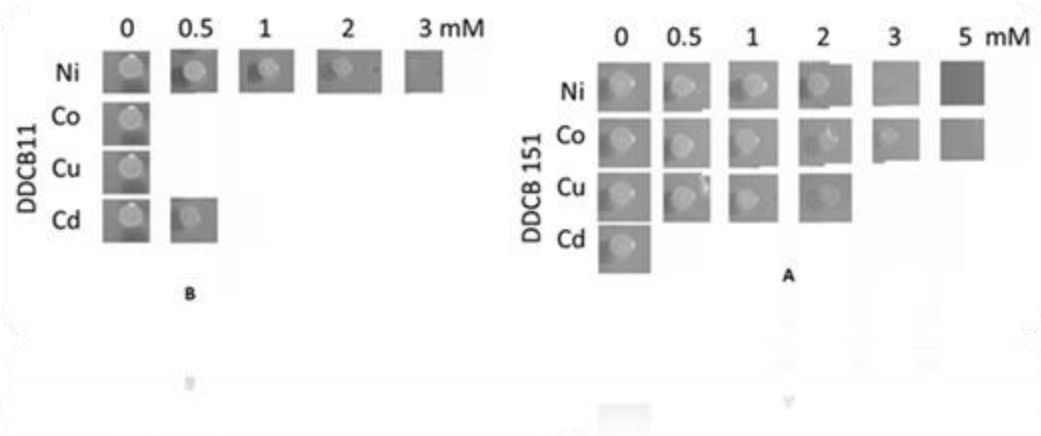

FIG. 3. **Resistance of metal with the blotting method**

Discussion and Conclusion

Bioremediation is a powerful tool available to clean up contaminated sites. The idea of bioremediation has a long history. However, other applications are relatively new and many other applications are emerging or being developed. Bioremediation occurs when the microorganisms can biodegrade the given contaminant and the necessary nutrients such as nitrogen, phosphorus, electron acceptors, and trace elements. Microorganisms had capacity to reduce the heavy metals depending on the factors like time and concentration of inoculum. As the time of incubation increases, more reduction was observed. Further identification of organism at genus and species level will help in heavy metal reduction in highly polluted effluent treatments. Zaki and Farag isolated *Entrobacter sp* ,*Chryseobacterium sp* and *Stenotrophomonas sp*, were the most resistant strains [10]. testosteroni, isolated *Ralstonia pickettii* and *Sphingomonas sp* to be resistant to high level of Zn,Ni , Pb and Cu from contaminated soil [11]. In examining the biodiversity of microorganisms Halophiles and halotolerant microorganisms by Amoozegar, of 217 isolates, 120 isolates of gram positive, gram negative bacilli and the rest of the 48 isolates were gram-positive cocci in this study, we found five bacterial isolates that were high resistant to the heavy metals cadmium, lead and nickel [12]. No microorganism showed multi resistance to two or more metals and obtained respectively 13%, 5/19%, 75/43 and 7.3% of isolates from MGM, MH, SWN, LNSWN% culture and 62/20% of the isolates obtained from nutrient agar medium without salt both bacterial and fungal isolates was observed microorganisms most frequent and the bacteria, gram-positive bacilli had the greatest number and Nickel metal shown greatest resistance, frequency, and cadmium, were the most susceptible to microorganisms also cobalt and copper metals were more resistant. It can be concluded that microorganisms isolated from the MH culture shown most resistant to nickel and cobalt. The greatest sensitivity obtained with cadmium and copper metals. So Halotolerant microorganisms isolated from the environment by 10% and 20% salt due to the ability to tolerate extreme environments can show better resistance to heavy metals and Microorganisms isolated from the environment with2% Salt or less do not shows this feature.

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