Original article

Lead and Chromium Bioremediation Potential of *Rhizopus* sp isolated from a landfill in Sudan

Tasneem Abdelelah Mahmoud[1] and Seedahmed Ahmed Mohamed[2*]

[1]Department of Environmental Sciences, Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan; [2]Department of Biology and Biotechnologies, Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan;

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Abstract

The harmful effect of heavy metals on the environment has necessitated the need for their removal through efficient and cost-effective methods. The present study aimed to investigate the ability of *Rhizopus* sp. to remove lead and chromium from the polluted water. The fungus was isolated in potato dextrose medium supplemented with lead or chromium at 50 µg/ml concentrations. Tolerance to heavy metals was determined by exposing fungi to different concentrations of lead and chromium (0, 10, 20, 50, 100 µg/ml). The exposure time was one week under optimum conditions. The results showed that the isolated fungus was able to remove lead (50%) and chromium (37%), and to biosorb and accumulate lead and chromium inside its cells up to 4000% and 300% of that of the control, respectively. These results suggest that the isolated fungus can be used for removal of both Chromium and Lead from polluted water.

* Corresponding author E- mail: Seedahmed@neelain.edu.sd

Introduction

The rapid development of many industries, such as mining, fertilizers, energy and fuel production, pesticides, electric appliance manufacture and other activities, produce hazardous and toxic xenobiotics, which are directly or indirectly discharged into the biosphere. In addition to posing a serious threat to human health, these wastes also cause considerable environmental pollution (Khan et al., 2008; Zhang et al., 2010; Raymond and Felix, 2011; Iram et al., 2012). Heavy metals are known to be naturally occurring compounds. However, anthropogenic activities introduce them in enormous quantities in various environments. Because they are non-biodegradable in nature, they may bioaccumulate in the food (Vhahangwele and Khathutshelo, 2018).

The toxicity of heavy metals can last for a long time in nature. Some can even be transformed from low toxic species into more toxic forms within certain environments, where the bioaccumulation and bioaugmentation in the food chain can distort normal physiological activity and endanger human life eventually (Wang and Zhou, 2004).

Many conventional method are used for the removal of heavy metals. These methods include chemical precipitation and sludge separation, chemical oxidation or reduction, iron exchange, reverse osmosis, filtration, adsorption using activated charcoal, electrochemical treatment and evaporative recovery. (Rajasulochana, and Preethy, 2016). These techniques are too costly, intensive energy demanding and often related to production of toxic by-products (Khan and Mondal, 2021)

Bioremediation is today's most promising and cost-effective technology, widely used to clean up soil and wastewater containing organic and inorganic pollutants. According to reports, microorganisms can be used as biosorbents to remove heavy metals at low cost The potential use of microorganism in the treatment of heavy metal contaminated wastewater and improvement of metal in mining wastes or in metallurgical effluents is of especial importance (Ahirwar et al., 2016; Molalign et al., 2020)

Biosorption is essentially a non-directed physiochemical complexion reaction between dissolved metal species and charged cellular component, which involves sorption or complexion of metals to living or dead cell (White et al., 1995; Gadd, 2010). This process may simply be defined as utilization of inexpensive dead or alive microbial biomass to sequester metals from industrial effluents. It has gained importance during recent years due to their good performance, low cost, specificity, minimum sludge generation and amenability for repeated use (Iram et al., 2012; Shamim, 2018)

Pollution by lead and chromium is one of the most serious environmental problems facing developing countries now. Although studies on heavy metal pollution in Sudan are scarce. There has also been chromium leakage in the Nile River from the tannery waste from the factories in central Khartoum (UNICEF, 2017). In this research we sheds a light on the use of safe, environmentally friendly methods to solve the problem of Lead and Chromium pollution by using fungi.

The main aim of this study is to investigate the potentiality of soil fungi in removal of Chromium and Lead from polluted water. We Isolated and identified a soil fungus with capability of growing in chromium or lead containing medium, determined heavy metals tolerance using different concentrations of heavy metals and examined the heavy metals bioremediation ability of the isolated fungus.

Materials and Methods

Soil samples

The samples were collected in sterile Falcon tubes from Abu weleedat waste landfill at Karary locality, Khartoum state.

Culture media

YEPD medium

The YEPD medium was prepared by adding 20g of dextrose, 20g of peptone and 10g of yeast extract to 1000 ml of distilled water. The medium was autoclaved at 121°C and 15 psi for 15 min.

PDA medium

The PDA medium was prepared by dissolving 24.5 g of PDA medium powder in 100 ml D.W. The medium was autoclaved at 121°C and 15 psi for 15 min.

Isolation and identification of Fungi

The fungi were isolated from collected samples using serial dilution method. The dilution technique was made by placing one gram of the sample in a test tube containing 10 ml of sterile distilled water, and tenfold serial dilution was made by transferring 1ml of the suspension to another test tube which contains 9 ml of distilled water. This step was repeated thrice to obtain a dilution of 10^{-3}. An amount of 1 ml from the last dilution added on a plates containing potato dextrose agar (PDA) supplemented with $CrCl_3$ or $PbCl_2$ at concentration of 50 µg/ml. The cultures were incubated at room temperature for 3 days, distinct colonies were counted and identified under the microscope.

Screening of heavy metal tolerant fungi

The heavy metal-tolerance potential was examined by using PDA medium amended by various concentrations (e.g. 10, 20,

50, and 100 µg/ml) of $PbCl_2$ and $CrCl_3$. Media were autoclaved, and then the fungi were sub-cultured. After that they were incubated at room temperature for 3 days.

Bioremediation of Chromium and Lead

The YEPD medium was dispensed into 5 flasks. The first flask was kept as a negative control. In the second two flasks, 100 µg/ml of $CrCl_3$ or 100 µg/ml of $PbCl_2$ were added to the medium as positive controls. In the treatment flasks, another two 100 µg/ml of each heavy metal compound was added to the medium and the fungus was inoculated. Flasks were incubated at 28ºC in a shaking incubator at 100 rpm for 3 days. Then, the media were filtered by using filter paper and the fungi were dried in the oven, then they were ground by a mortar and a pestle inside a laminar air flow cabinet. The experiment was repeated thrice.

Digestion of Fungal Biomass

An amount of 1.0 g of the dried fungal biomass was dissolved in 3 ml solution consists of nitric acid and oxygen hydroxide at 3:1 ratio in sterile tubes. Then the solution was heated at a boiling temperature of 120ºC till complete dissolving. Six ml of distilled water was added to dilute the solution. The resulted solution was then filtered using Whatman filter paper and the concentrations of both lead and chromium were determined using Atomic Absorption instrument.

Heavy Metal Concentrations

The concentration of heavy metals in samples was investigated using Atomic Absorption Instrument (JPC/Model XplorAA, Australia) with deuterium correction of background in flame acetylene air. The accuracy of analytical procedure was checked out through the analysis of certified reference materials. The measurements were recorded at GRAS (Geological Research Authority of Sudan, Ministry of Minerals).

Statistical Analysis

Descriptive statistic were performed using Microsoft Office Excel 2008. One way Analysis of Variance (ANOVA) test was performed using StatPlus software along with Microsoft excel to determine the significant differences between samples and control at p= 0.05.

Results and Discussion

Isolation and Identification of Lead and Chromium Tolerant Fungi

Two Lead and Chromium tolerant isolates were recovered from the soil sample collected from Abu-weleedat waste landfill, and identified according to their morphological characteristics on potato dextrose agar (PDA), and the microscopic examinations (Fig. 1). Both isolates on PDA produced initially wooly white colonies, which were quickly became black with conidial production (Fig. 1). The microscopic examination for isolates showed black spores in round sporangia. The hyphae were undivided and the sporangiophore was unbranched (Fig. 1). These microscopic and morphological characteristics are apply to *Rhizopus* sp.

Determination of Heavy Metal Tolerance

The isolated fungus was further subjected to heavy metal tolerance test. The fungus was able to withstand all tested concentrations of Chromium and Lead. The growth patterns were variable and correlate negatively with the concentration used. However, the accumulated fungal biomass in flasks containing the control was not significantly different from those in flasks containing heavy metals (*P-value* > 0.05) as shown in (Fig. 2). This result is consistent with that of Ahmad et al., (2006) and Zafar et al., (2007) who reported that the fungus *Rhizopus* sp. has the ability to tolerance very high concentration of chromium up to 400 ug/mL and 7000 ug/mL, respectively. *Rhizopus* species have been reported to have notable tolerance to Lead and various heavy metals at varied concentrations (Rigoletto et al., 2020).

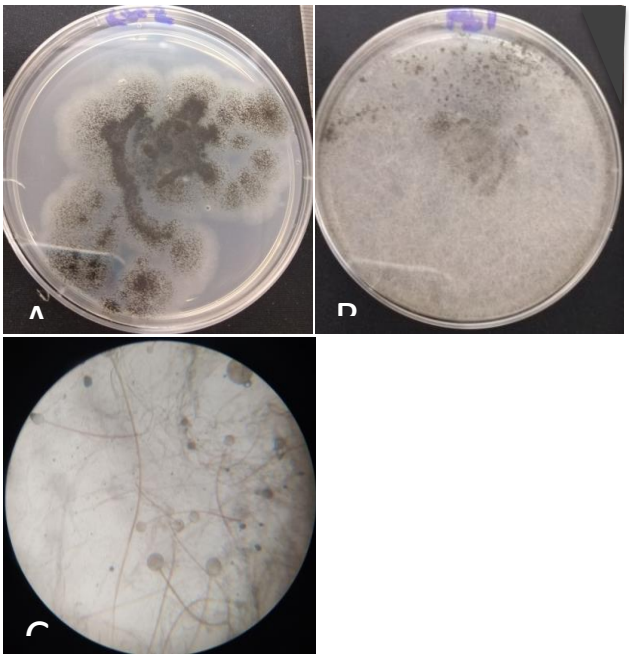

Fig. 1. Isolation of Heavy metal tolerant Fungi. (A) Colonial morphology of fungal isolates on potato dextrose agar (PDA) supplemented with Chromium at 100 µg/ml. (B) Colonial morphology of fungal isolates on potato dextrose agar (PDA) supplemented with Lead at 100 µg/ml. (C) Microscopic view of fungal isolate (*Rhizopus* spp.). The large globose conidial heads are visible

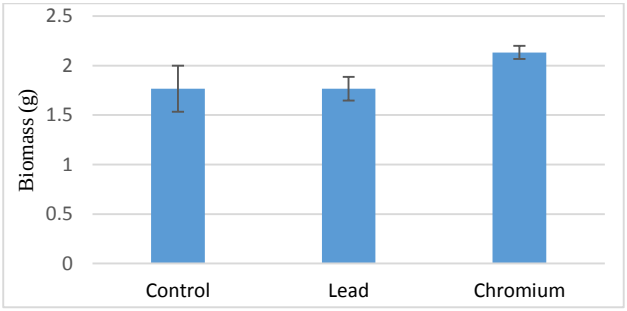

Fig. 2. Biomass accumulation of Fungi grown on Potato Dextrose Medium (Control), or PD medium supplemented with 100µg/mg Lead or100µg/mg Chromium. Results are represented as average ±SE. (n=3)

Bioremediation of Chromium

It is well known that tannery industries utilize Chrome salts particularly The trivalent Chromium as primary tanning agents during leather processing, but under suitable conditions, it can be readily oxidized to hexavalent Chromium which is more toxic (GracePavithra et al., 2019; Lofrano et al., 2013). Chromium (VI) can be reduced by microorganisms to chromium (Cr 3+), which then can precipitate to chromium oxides, sulfides, or phosphates (Bruce et al, 1993). Sukumar, 2010 used living biomass of *R. oryzae* to reduce 91.15% Chromium from hexavalent to trivalent. Thus we focused on the trivalent Chromium to test its bioremediation by *Rhizopus* sp.

Result showed that the isolated *Rhizopus* sp. absorbed Chromium in a large amount. The isolate accumulated Chromium inside cells on average of 4360% in concentration 100 mg/l when compared to the negative control (Fig. 3). It had also cleaned up to 37 % of the chromium added to the medium (Fig. 4).

Previous studies on bioremediation by *Rhizopus* sp. demonstrated high Chromium bioremediation capacity. Two independent studies performed by Martha et al., (2019) and Bai and Abraham (2001) observed 99% removal of chromium (VI) by using dead biomass of *Rhizopus* sp. and *Rhizopus nigricans*, respectively. Although there are many reports on biosorption potential for Chromium using *Rhizopus* sp. and other fungal dead biomass. Very few reports used alive biomass. Indeed, dead biomass offers certain advantages over living cells. Systems using living cells are likely to be more sensitive to high metal ion concentrations and adverse operating conditions such as pH and temperature. Constant nutrient supply is required for systems using living cells (Bishnoi and Nagpal, 2005). However, living biomass offers a renewed system for *in situ* bioremediation of heavy metals. For living cells, metal uptake is facilitated by the production of metal–binding proteins and the uptake occurs across the cell membrane depending on the cell metabolism (Volesky, 1990).

Our results broadly supports the work of Preetha and Viruthagir, (2007) who used living biomass of *R. arrhizus* to obtain a maximum percentage uptake yield of 93.84% using 25 mg/l of initial metal ion concentrations.

Result also showed that the tested *Rhizopus* sp. could absorb Lead in a large amount. The isolate accumulated Lead inside cells on average of 305% in concentration 100 mg/l in comparison to the untreated negative control (Fig. 5). The

isolate had absorbed 50 % of the Lead added to the medium (Fig. 6).

In previous studies, the fungus *Rhizopus*. sp. demonstrated varied capacities to remove Lead from polluted water. *Rhizopus arrhizus* removed 200 mg/g of Lead in 7.0 pH. (Eric et al., 1994), *R. arrhizus* bioabsorbed 45.54% of lead (Sauryya et al., 2002), whereas, *Rizopus* sp. g removed up to 51.5 mg/g dry weight fungus biomass (Mehrasbi et al., 2009). Ezzouhri et al., (2010) used both transmission electron microscope (TEM) and energy dispersive X-ray spectroscopy (EDX) to study the cellular location of accumulated lead in cells of *Penicillum* sp. Their results indicated the presence of lead deposits in the cytoplasm suggesting that the binding mechanism of lead to *Penicillium* sp. involves extracellular adsorption and intracellular uptake. Sun and Shao, (2007) have also demonstrated a similar mechanisms for Lead biosorption. Proteins and polysaccharides are potential binding sites for Lead in fungal cells (Ezzouhri et al., 2010). Several reports had confirmed a cell wall and periplasmic domain accumulation in fungal biomass (Bhaskar and Bhosle, 2006; Akhtar et al., 1996). It is likely that the biosorption of Lead in *Rhizopus* sp. follows a similar model. *Rhizopus* sp. biomass is interesting to be used for Lead removal due to its relatively high biosorption capacity and the natural form of the pellets, which facilitates the solid-liquid separation in biosorption reactors (Kogej et al., 2010).

Other fungi were also reported to be able to bioremediate Lead as well. These include *Aspergillus terreus* (Joshi et al., 2011), *A. niger* (Iram et al., 2015), *Penicillium chrysogenum* (Anupriya and Sharma, 2016), *Agaricus* sp (Corral-Bobadilla et al., 2019) and *Aspergillus fumigatus* (Iram et al., 2013).

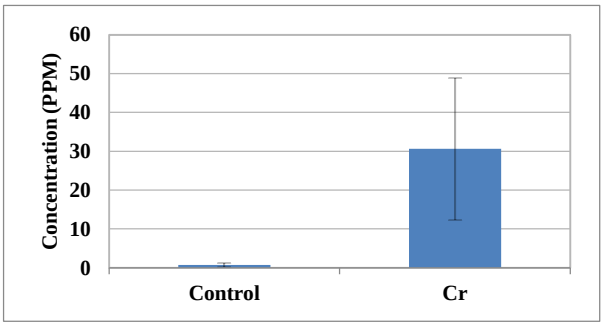

Fig. 3. Biosorption of Lead by the isolated fungus. The concentration of Chromium was measured inside the fungal cells grown on control medium or medium supplemented with 100µg/ml Chromium. The result is presented as average ± standard errors (n=3)

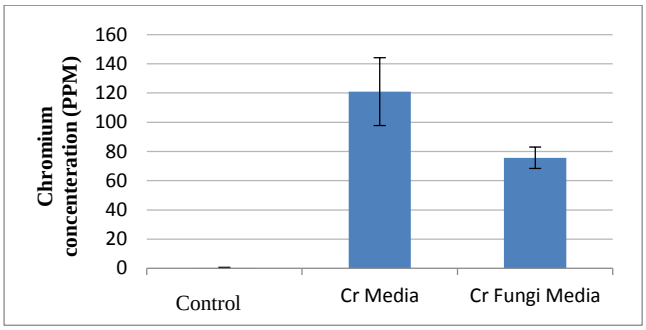

Fig 4. Bioremediation of Chromium by the isolated fungus. The concentration of Chromium was measured in the media of control and media supplemented with 100µg/ml Chromium. The result is presented as average ± standard errors (n=3)

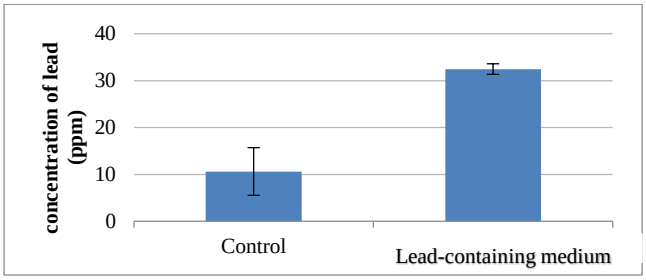

Fig. 5. Biosorption of Lead by the isolated fungus. The concentration of Lead was measured inside the fungal cells grown on control medium or medium supplemented with 100µg/ml Lead. The result is presented as average ± standard errors (n=3)

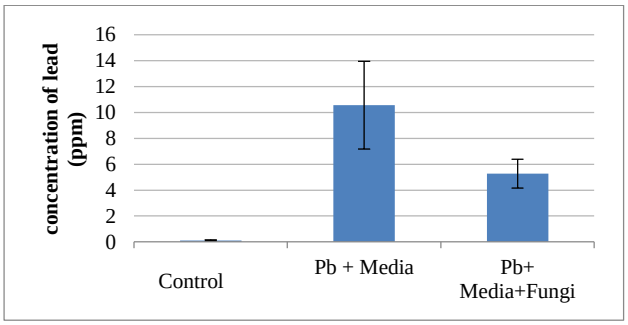

Fig 6. Bioremediation of lead by isolated fungus. The concentration of lead was measured inside the fungal cells of control and media supplemented with 100µg/ml lead. The result is presented as average ± standard errors (n=3)

Conclusion

In the present study, the fungal isolates were identified on the basis of morphological characteristics and microscopic examination as *Rhizopus* sp. The fungus demonstrated a remarkable tolerance to Chromium and Lead in high concentrations, and exhibited a great potential for Chromium and Lead removal from the media and accumulation in fugal cells by high percentages. These results indicate the potentiality of *Rhizopus* sp. as effective bioremediatice clean-up agent for Lead and Chromium. Further researches are recommended to optimize the bioremediation processes before the *in situ* implementation.

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