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Research Article

Mycoremediation of *Trichoderma harzianum* and *Penicillium chrysogenum* to Pb Exposure: Effect on Metal Bioaccumulation, Oxidative Stress and Antioxidant System

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Keywords

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Penicillium chrysogenum,
Trichoderma harzianum,
Total oxidant.

Abstract

One of the biggest problems of the global world is environmental pollution. Much work is being done to prevent environmental pollution and expenditures are being made for this area. The use of biological molecules instead of physical and chemical methods for the removal of metals from industrial water is an alternative, economical and highly effective method. Metal tolerance and detoxification mechanisms in fungi, like many other microorganisms, with bioaccumulation ability to heavy metals, were quite a little knowledge. In this study, the relationship between lead (Pb) bioaccumulation and oxidative stress and antioxidant defenses of *T. harzianum* and *P. chrysogenum* was investigated. In order to determine the minimum heavy metal tolerance to lead in the first stage, PDA media containing lead at different concentrations were prepared and fungi growth. It has been observed that as the metal content in the medium increases, the number of fungi colonies developed decreases. Total antioxidant / oxidant levels were then measured, respectively, by TAS (Total Antioxidant Status) Assay Kit and TOS (Total Oxidant Status) Assay Kit and OSI (Oxidative Stress Index) values were calculated. Low OSI values (<0.4) suggest that *T. harzianum* and *P. chrysogenum* inhibits lead by tolerating lead.

1. Introduction

Heavy metals are one of the most important sources of pollution and are located in areas such as water waste streams of many industries, soil and air. Organisms in these dirty ecosystems are also affected by heavy metal exposure. This effect affects people both indirectly (diet foods) as and direct (water, soil, air) because of end user of food chain. The main heavy metals that threaten human health are lead, cadmium, mercury and arsenic. These metals can accumulate in the human body and cause toxicity [1].

Although the adverse effects of heavy metals on health have long been known, they have been used by humans for thousands of years and exposure still persists. Emissions in some parts of the world, especially in underdeveloped countries, are increasing [2]. During the past century, lead emissions to the ambient air caused significant pollution mainly due to the emissions of lead from petroleum. Lead toxicity increased due to increased environmental lead. Lead has neurotoxic [3], cytotoxic [4], genotoxic [5] and carcinogenic [6] effects. It is important to remove heavy metals because of their harm to the human body.

Microorganisms have the ability to bind metals to aqueous solutions. This phenomenon is known as biosorption and microorganisms responsible for the process are considered to be biosorbents. Many bacteria, mosses, fungi and plant species have the ability to sorption heavy metals. The adsorption ability of these species of fungi is described as mycosorption, and this is a topic of great interest to researchers all over the world [7]. Fungi are among the

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most powerful components of nature for the decomposition of waste matter and are an important component of the soil nutrient network that provides nutrients for other biota living in the soil [8].

Studies have shown that some fungal species, such as *Trichoderma* sp. and *Fusarium* sp., have the ability to tolerate the presence of different heavy metals. *Trichoderma* sp. are found everywhere in the soil, grow rapidly and have a high reproductive potential and highly competitive saprophytic ability. Also, some *Trichoderma* sp. have good antagonistic properties against plant pathogenic fungi and beneficial effects on plant growth. In addition, *Trichoderma* sp. are highly resistant to various toxins and xenobiotic compounds, including antibiotics, fungicides and heavy metals. *Trichoderma*'s resistance to heavy metal stress is an important point for bioremediation studies [9].

Another class of filamentous fungi, *Penicillium* sp., which are thought to play an important role in the naturally healing of metal-containing substances and degradation of different xenobiotics such as phenols, halogenated phenolic compounds, petroleum hydrocarbons, polycyclic aromatic compounds and polychlorinated biphenyls [10]. There are various *Penicillium* sp colonized in many different environments. In the land, food, drinks and the interior spread. *Penicillium*'s ability to remove heavy metals and xenobiotics is important for researchers and practitioners [11] and *P. chrysogenum* also has metal removal properties from wastes [12]. But there are very few reports available on using *P. chrysogenum* to bioleach of heavy metals in polluted soil [13].

Reactive oxygen species (ROS) can cause extensive damage to nucleotides, proteins, carbohydrates, fatty acids and lipids and eventually lead to cell death through cellular oxidative stress response. Exposure to heavy metals causes ROS formation in fungi, and then some antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are synthesized as a detoxification mechanism [14].

Accordingly the aim of our study were; to treat the lead in wastewater, which is an important problem for the environment, with fungi,

-To support the presence of antioxidant and total oxidant levels and bioaccumulation,

-Will be able to put forward ideas on how to use heavy metal pollution on the roads.

2. Materials and Methods

2.1. Fungal Strains and Culture Conditions

The fungi *T. harzianum and P. chrysogenum* were isolated in a metal-polluted soil and selected for its in vitro growth and high Pb accumulation capacity. The isolates were established on Potato Dextrose Agar (PDA, Merck) (25 ± 2 °C, 5 days) and sub-cultured periodically.

2.2. Accumulation of Lead in Fungal Mycelia

Firstly, to monitor the Pb-resistance of the fungal strain, conidiospores were cultivated in Petri dishes (d = 10 mm) with PDA supplemented with various concentrations (0, 50, 100, 200, 300, 500, 750 and 1000 mg/L) of $Pb(NO_3)_2$ for 10 days at a temperature of 30 °C.

Then, spore suspensions of fungi were prepared in the sterile distilled water at a concentration of 2.0×10^6 CFU mL^{-1}. 2 mL of as prepared spore suspensions were inoculated into 100 mL growth medium (Potato Dextrose broth) with desirable concentrations of Pb at 0, 50, 100, 200, 300, 500, 750 and 1000 mg/L (as $Pb(NO_3)_2$) and cultured at 30 °C with constant stirring at 125 rpm (three culture replicates for each group). Fungi cultured at 0 ppm Pb was defined as control sample in the whole experiment (controls). Biomass was collected at selected intervals and washed three times in distilled water and then centrifuged and filtered for monitoring the wet weight.

At the end of the incubation, fungi were removed from the medium and liquid medium was prepared with three levels of tolerance (100, 200 and 300 mg/L). Sowing mediums were liquefied by acid in the microwave combustion unit by following 2-4-6-9 and 14 days in a shaking incubator and lead levels were measured. For determination of Pb metal content the fungi material (100 mg) was digested in 10 ml mixture of $HNO_3/HClO_4$ (4:1, v/v) and heated at a temperature of 150 °C until the formation of white fumes. Distilled water was added to raise the total volume of the digested material to 50 ml. The content of Pb in fungi was determined by inductively coupled plasma - optical emission spectrometry (ICP-OES).

2.3. TOS Determination

Fungi samples (*T. harzianum, P. chrysogenum)* for the measurement of TOS and TAS were stored at -80 °C until needed. TOS levels of samples were measured using TOS kit (REL Assay Diagnostics), which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in the acidic medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the fungi samples. The test parameters were as follows: method, end-point measurement; fungi sample volume, 45 µL; R1 volume, 300 µL; R2 volume, 15 µL; reaction time, 10 min; temperature, 37 °C; wave length, 530 nm. The results were expressed in µmoL H_2O_2 equivalent/L (µmol H_2O_2 eq/L).

2.4. TAS Determination

TAS was measured colorimetrically using the Total Antioxidant Status kit (REL Assay Diagnostics). The color change was measured by spectrophotometrically (Thermo Scientific UV/VIS Multiskan GO). The reaction was calibrated with Trolox (a water-soluble analogue of vitamin E, 6-hydroxy-2.5.7.8-tetramethylchroman- 2-carboxylic acid), and the TAS value of the samples tested was expressed as mmol Trolox equivalent/L (mmol Trolox eq/L).

2.5. Oxidative Stress Index

OSI was defined as TOS to TAS ratio and was calculated as follows: OSI (arbitrary unit) = (TOS, µmol H_2O_2 eq/L)/(TAS, mmol Trolox eq/L).

3. Result and Discussion

To determine the relationship between oxidative stress and lead toxicity, we first evaluated the lead resistance of the

model strains and then measured several oxidative stress biomarkers and the antioxidant status of the fungi.

3.1. Effect of Pb Ions on Growth and Cell Differentiation

The growth of fungal spores on agar medium supplemented with Pb^{2+} from 50 to 1000 mg/ml is demonstrated in Figure 1 and 2; exposure to $Pb(NO_3)_2$ affected the growth of fungi spores at all concentrations used in a concentration-dependent manner. Moreover, heavy metal content inhibited the formation of conidiospores.

Figure 1. Growth of *T. harzianum* 50-1000 mg/L Pb^{+2}

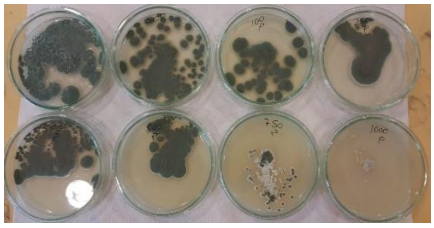

Figure 2. Growth of *P. chrysogenum* 50-1000 mg/L Pb^{+2}

3.2. Pb Bioaccumulation in T. harzianum and P.chrysogenum

Pb amount curves shown in Figure 3. plotted out that Pb bioaccumulation in *T. harzianum* occurred in all tested concentrations. After 6 d of exposure, a significant accumulation of Pb was observed in *T. harzianum*. As the Pb concentration increased, there was a concomitant promotion in Pb bioaccumulation content. The highest Pb accumulation (8.832 g/kg at 6 d) was observed in *T. harzianum* exposed to 100 ppm Pb. However, Pb bioaccumulation in *P. chrysogenum* occurred in all tested concentrations (Figure 4.). After 14 d of exposure, a significant accumulation of Pb was observed in *T. harzianum*. The highest Pb accumulation (9.928 g/kg at 14 d) was observed in *T. harzianum* exposed to 200 ppm Pb.

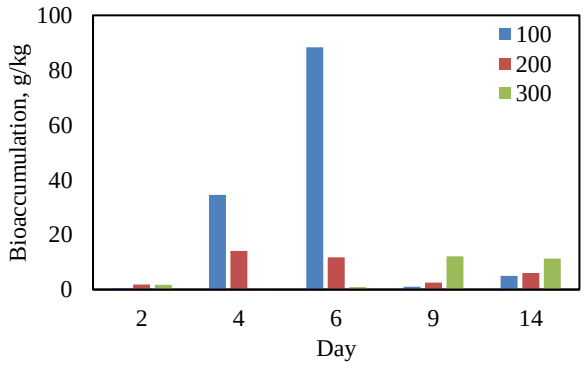

Figure 3. Bioaccumulation of Pb in *T. harzianum* exposed to 100, 200 and 300 ppm Pb.

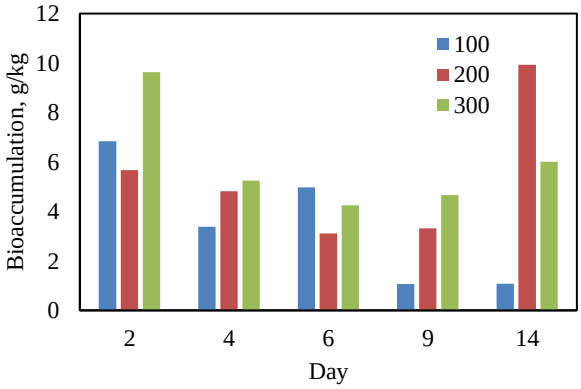

Figure 4. Bioaccumulation of Pb in *P. chrysogenum* exposed to 100, 200 and 300 ppm Pb.

3.3. Pb-Induced Oxidative Stress in T. Harzianum and P. Chrysogenum TAS-TOS and OSI Values

The TAS and TOS levels of *T. harzianum* and *P. chrysogenum* are shown in the following graphs (Figure 5 and 6). OSI (Oxidative Stress Index) values were calculated using TAS and TOS values and presented in Figure 5 and 6. A low OSI value is indicating that *T. harzianum* and *P. chrysogenum* fungi used inhibit lead poisoning.

Heavy metals are one of the most serious problems of today because they are poisonous due to their ionic properties. Heavy metals can bind to many cellular ligands and displace native binding sites from normal binding sites. Metals can also interfere structure and function of protein and nucleic acid by binding to sulfhydryl, phosphate or hydroxyl groups [7].

Fungi have also proved useful in the remediation of heavy metals such as lead (Pb) and cadmium (Cd). Because these metals are the simplest and will not deteriorate any more, the fungi pick them up from the soil or water and accumulate them in tissues such as mycelia or fruit. For this reason some fungi species are thought to be effective as mycoremediators [15].

Raspanti et al. studied the ability of four different fungal species, *Trichoderma harzianum*, *Fusarium antophyllum*, *Fusarium compactum* and *Fusarium phyllophilum*, to grow in the presence of heavy metals and they reported that Pb did not substantially affect the growth rate of *T. harzianum* at either 5 ppm or 10 ppm [9].

In this study we explored Pb tolerance and accumulation in *T. harzianum* and *P. chrysogenum* as well as the oxidative stress and antioxidant response under different concentrations Pb. The results indicated that the exposure to Pb would affect the growth of fungi cells and induce the oxidative stress as well as the defense response against toxicity.

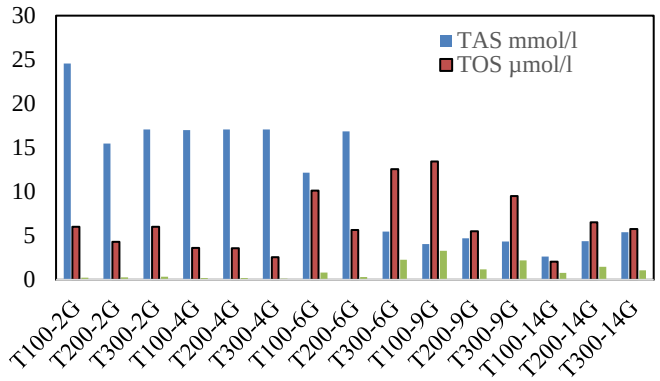

Figure 5. The TAS, TOS and OSI levels of *T. harzianum*

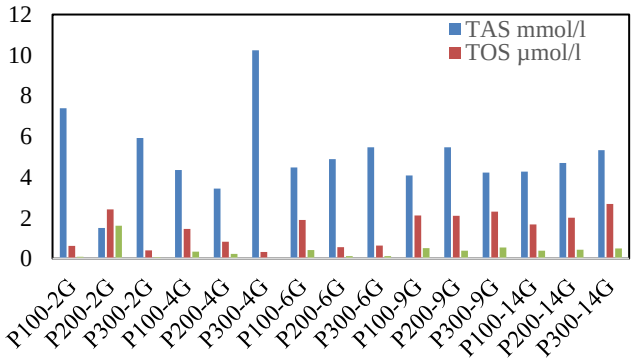

Figure 6. The TAS, TOS and OSI levels of *P. chrysogenum*

4. Conclusions

A lot of work is being done to prevent environmental pollution and to make a livable world, and expenditures are made for this area. In this study, the demonstration of the presence of fungi capable of refining with heavy metals bioaccumulation will bring innovation and reduce costs. From this point of view, it seems economically advantageous against other methods of bioremediation.

Another effect of environmental pollution is that it starts from the lowest level of the food chain and damages all units, that is, all living. Therefore, prevention of environmental pollution is important not only for providing social benefits but also for the whole vitality world. Moreover, in terms of being an innovative, cheap and simple method of preventing environmental pollution from biological materials, it is also a vision to bring innovation.

In this regard, comprehensive treatment studies can be carried out by considering the effect of different fungi species on different heavy metals. Therefore, in fact; to prevent pollution in nature, nature can be verified thesis that can still use a living creature, which is its product.

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Conflict of Interest

The authors declares that there is no conflict of interest regarding the publication of this article.

Ethical approval

This article does not contain any studies with animals performed by any of the authors. This article does not contain any studies with human participants or animals performed by any of the authors.

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