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Detection of Heavy Metals Indifferent Soil Samples and Their Bioremediation with the Help of Fungus *Cunninghamella sp.*

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Abstract

Due to increased population, industrialization and urbanization are environmental contamination is growing day by day. Levels of some heavy metals (Zn, Pb and Cd) were investigated in various soil samples, nursery sample (NS), nursery sample containing herbicides (HS) and a sample from the area that has less human interference (LHI), after bioremediation with the help of fungus. There are various fungi (Aspergillus sp., Cunninghamella sp., Trichoderma sp. and Candida sp.) which can be used for the removal of different heavy metals. Fungus has very low nutritional value and high versatility and their biomass can be easily subjected to chemical and physical treatment. Toxicity of these heavy metals can lead to various diseases on the basis of their acute and chronic experience. Biosorption experiments were initially conducted with the isolation and identification of fungi Cunning lamellas on the SDA media. For the treatment of untreated soil wet fungal biomass is prepared, filtered and added in the soil samples. Quantity of soil samples (500 g, 100 g, 10 g) were distributed on the basis of temperature; room temperature and outside temperature and incubated for 15 days for bioremediation. Heavy metals were detected with the help of atomic absorption spectrophotometer and readings were taken at the interval of 7 days and 15 days after digestion of soil samples.

Keywords: *Bioremediation, metals, contamination, fungus*

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INTRODUCTION

The continuous release of heavy metals to the environment has been increasing continuously as a result of industrial activities and technological development and poses a significant threat to the environment, public and soil health. The heavy metals contamination of soil is a major problem on industrial and defense related sites all over the world. The industrial effluents are generated from hundreds of small and large lock manufacturing and plating industries and contain considerable amounts of heavy metals at elevated concentrations. Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake [1].

Fungi are recognized for their superior ability to produce a large variety of extra cellular proteins, organic acids, enzymes and their waste biomass may be used as effective bio-sorbent for removal, reduction and detoxification of industrial effluents ingredients. Use of waste water in agriculture has increased in recent years due to in herent treatment capacity of soil and high contents of major and micronutrients in it [2]. However, wastewater, particularly from industries contains high concentration of heavy metals which enter into human beings and animals through food chain. Therefore, it is desirable to remove these heavy metals from wastewater through low cost technology z methods such as reverse osmosis, solvent extraction, lime coagulation ion exchange and chemical precipitation. for removal of heavy metals from waste water are very expensive and these do not remove heavy metals from waste water upto desired limits [3]. The present study attempts to isolate and screen heavy metal tolerant fungi and find out their efficiency to remove heavy metals from liquid media under laboratory conditions [4].

REVIEW OF LITERATURE

Heavy metal analysis of agricultural field soil receiving long-term (> 20 years) application of municipal and industrial wastewater showed two- to five-fold accumulation of certain heavy metals as compared to untreated soil [5]. Metal-resistant fungi isolated from wastewater-treated soil belonged to genera *Aspergillus, Penicillium, Cunninghamella, Geotrichum, Fusarium, Rhizopus, Monilia* and *Trichoderma*. Minimum inhibitory concentrations (MIC) for Cd, Ni, Cr, Cu, and Co were determined [6]. The findings indicated promising bio-sorption of cadmium and chromium by the *Rhizopus* and *Aspergillus* spp. from aqueous solution. There is little, if any, correlation between metal tolerance and biosorption properties of the test fungi. The objectives of this study were to analyze the total contents of Cd, Cr, Cu, Ni, Pb, and Zn in the freshwater sediments and the arable and non-arable soils of Taiwan, and to compare the different digestion methods for their determination [7].

This study was focused on the heavy metal (Ni, Co, Mo, V, Mn, Fe, W and Zn) tolerance of fungi strains *Aspergillus niger, Aspergillus foetidus and Penicillium simplicissimum. Aspergillus Niger* was exposed to a mixture of Mo, V and Mn at a mass ratio of 1:2:6 as approximately present in the spent refinery processing catalyst [8]. This study highlights the adaptation of fungi strains to Tungsten and Vanadium to high concentration. The effect of different metals and metal concentration on different strains of fungi was evaluated. Results showed that Niwas one of the most toxic metals for strain so A*spergillus* and *Penicillium*. *Aspergillus foetidus* was the least tolerant, in particular for Ni, Co and Zn. In this work, *Aspergillus niger, Aspergillus foetidus and Penicillium simplicissimum* showed higher tolerance towards Mo and V, and the three fungi have a good potential for the bio-leaching of spent refinery processing catalysts that contain Mo and V. The main objective of this study is to identify the best strain, based on tolerance development, for the spent refinery processing catalyst [9].

Fungi, including *Aspergillus flavus, Aspergillus niger, Fusarium solani, and Penicillium chrysogenum*, resistant to heavy metals like Cr and Pb were isolated after screening soil samples from peri-urban agricultural areas. The objective of soil sample screening was to investigate the status of heavy metals and to identify the heavy metal-tolerant fungi. The results revealed that the majority of the isolates were resistant to Pb and Cr, and only few of them were able to grow. Among the isolated fungal strains, *Aspergillus Niger* was the most tolerant against Pb, with MIC of 600 mg/l, and *Aspergillus flavus* against Cr, with MIC of 400 mg/l, which makes them attractive potential candidates as bioremediation agents [10].

MATERIAL AND METHOD

Sampling

For the detection of heavy metals samples were collected from different areas of greater Noida (Table 1):

Table 1: *Samples.*

Sample 1	Nursery Sample (NS)
Sample 2	Nursery sample containing Herbicides (HS)
Sample 3	A sample from the area that has less human interference (LHI)

Isolation of Fungus

The soil samples (1 g) were suspended in 10 ml of distilled water, and subsequently 1 ml of this suspension was added to 9 ml distilled water to obtain desired dilutions up to 104 [11]. Four different dilutions was spread on SD A plates containing ampicillin to inhibit bacterial growth (Figure 1).

Saboruaud Dextrose Agar Media Composition

Sabouraud Dextrose Agar (SDA) is a selective medium primarily used for the isolation of dermatophytes, other fungi and yeasts but can also grow filamentous bacteria such as Nocardia shown in Table 2.

Table 2: *Composition of SDA.*

Ingredients	Quantity(/500ml)
Dextrose	20 g
Peptone	5 g
Agar	7.5 g

Final pH 5.6

The inoculated plates were incubated at 28 _°C for 72 h or more and fungal viable counts were determined. On the basis of frequency of occurrence of fungal growth on selection plates, common colonies of fungi were selected for purification and culture maintenance on SDA slants at 4 _°C.

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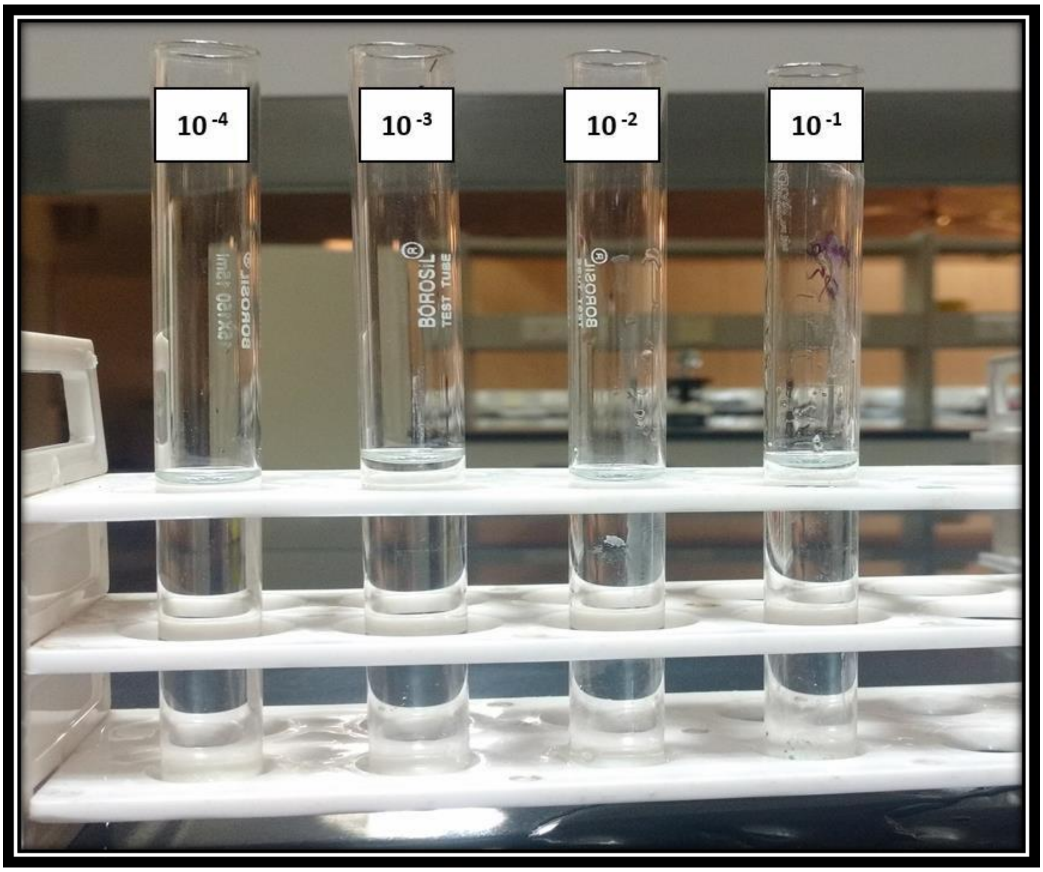

Fig. 1: *Serial Dilution.*

Identification of Fungus
Pure cultures of common fungi were tentatively identified by comparing the characteristic features of fungi [12]. The cultures were identified at the genus level on the basis of macroscopic characteristics (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape, and structure of conidia, and presence of sterile mycelium) (Figures 2 and 3).

Fig. 2: *Pure Culture of Cunninghamella sp.*

Filtration of Fungus
Biomass of the test fungi (*Cunninghamella*) was grown on SDB 500 ml (dextrose 20 g, peptone 5 g) medium with a pH adjusted to 5.6. The fungi were cultured in filamentous form under aerobic conditions for 3 days in shake flasks (215 rpm) [13]. The biomass was harvested by filteration through a filter paper.

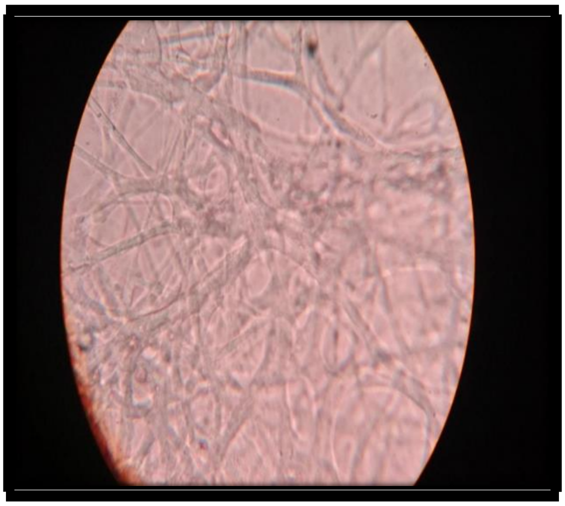

Fig. 3: *Microscopic View of Cunninghamella sp.*

The washed biomass (live biomass) was used immediately thereafter. The biomass was used immediately thereafter. Briefly, 1 g of biomass of the above fungi were added to each of the untreated soil samples and the samples were made in duplicates as 10 g, 100 g, 500 g. These samples were kept the nat room temperature and outside temperature. Proper moisture was maintained by sprinkling water timely.

Retained these samples till 15 days for bio-remediation process.

DIGESTION

One gram from each of the soil samples that is treated and untreated soil and analysed to determine different heavy metals.

The soil samples were oven dried at 80°C for 24 hours and passed through a 150micron sieve. 1 g of the soil samples in a conical flask was taken. 3 ml HNO_3 was added and conical flask was kept on the hot plate at 35°C. After 1 hour when white or yellow layer was formed then 4 ml of perchloric acid was added and again kept on the hot plate for the digestion [14]. After digestion 100 ml of distilled water was added in the conical flask and filtered it through the Whatman 42 filter paper. All the filtered soil sample were stored in the plastic bottles for analysis. Heavy metals were then detected with the help of atomic absorption Spectrophotometer (Figures 4 and 5).

ATOMIC ABSORPTION SPECTROPHOTOMETER

Atomic absorption spectrophotometry analyzes the concentration of elements in a liquid sample based on energy absorbed from certain wavelengths of light (usually 190–900 nm). Atomic absorption spectrophotometers are used by different industries for environmental testing, metal analysis, semiconductor manufacturing, petroleum and chemical production, and in pharmaceuticals.

RESULTS AND DISCUSSION

In the present investigation various filamentous fungi were isolated from agriculture field soil. The higher amount of heavy metals in the treated soil is likely due to long-term application of the wastewater containing these heavy metals. Soil fungi able to grow in the presence of heavy metals were isolated. The fungi belong to the genera *Aspergillus, Cunninghamella, Candida, Penicillium, and Trichoderma, Monilia* as well as the Mycelia sterile group. Some of the fungi could not be identified. The purpose of the investigation was to obtain filamentous fungi from polluted habitats for their possible exploitation in biosorption studies. The most frequently encountered isolates from the soil samples of all three sites were *Aspergillus spp.* and *Cunninghamella sp.* followed by *Penicillium sp.* and others (Table 3). In this study we carried *Cunninghamella sp.* and the whole digestion process is based on that particular fungi. The result shown that lead heavy metal has the highest concentration and cadmium has the lowest concentration, after treatment with the help of fungus *Cunninghamella sp.*

Fig. 4: *Digestion of Soil Samples with Nitric Acid and Perchloric Acid.*

Fig. 5: *Filtration of Samples with Whatman 42 Filter Paper.*

Table 3: *Total Samples.*

Samples	Total Samples
Untreated soil	3 samples
Treated soil (after 7 days)	18 samples
Treated soil (after 15 days)	18 samples

The study conducted the degrade at ion of heavy metals. The concentration of lead heavy metal decreased up to 0.043 (Figures 6–13). Detection of heavy metals from the soil samples with the help AAS:

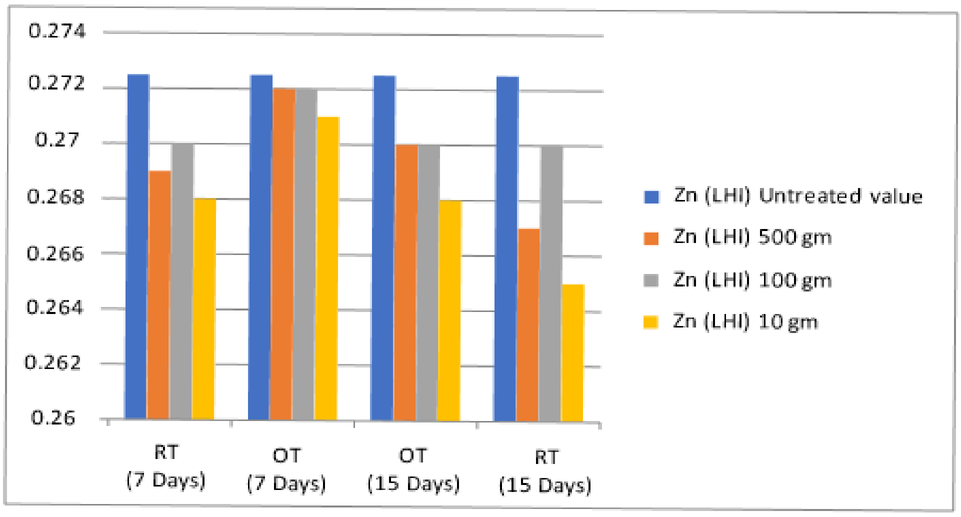

Fig. 6: *Zinc (Zn) in Less Human Interference Sample.*

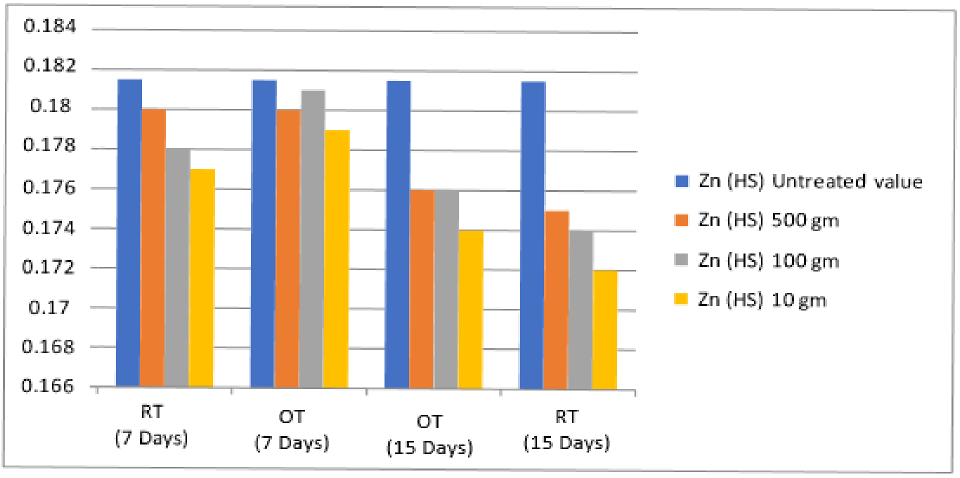

Fig. 7: *Zinc (Zn) in Nursery Sample Containing Herbicides.*

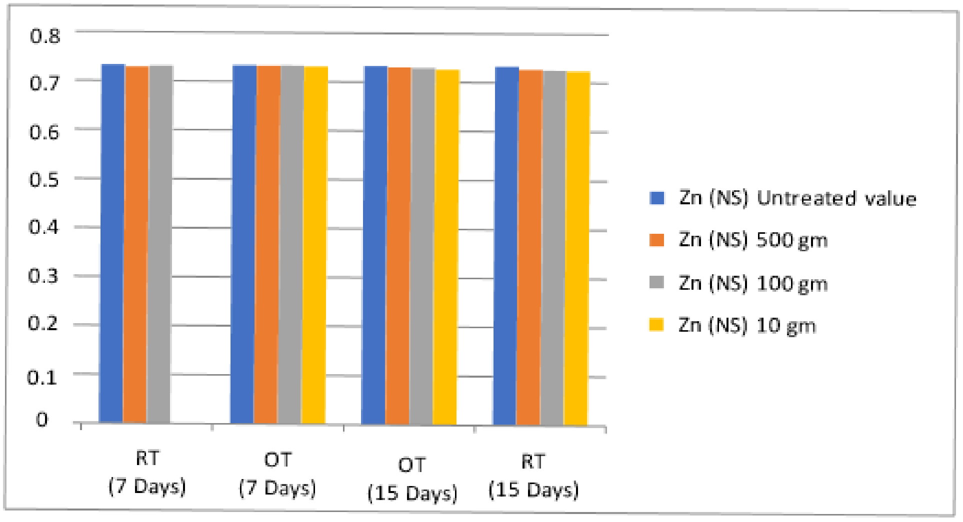

Fig. 8: *Zinc (Zn) in Nursery Samples.*

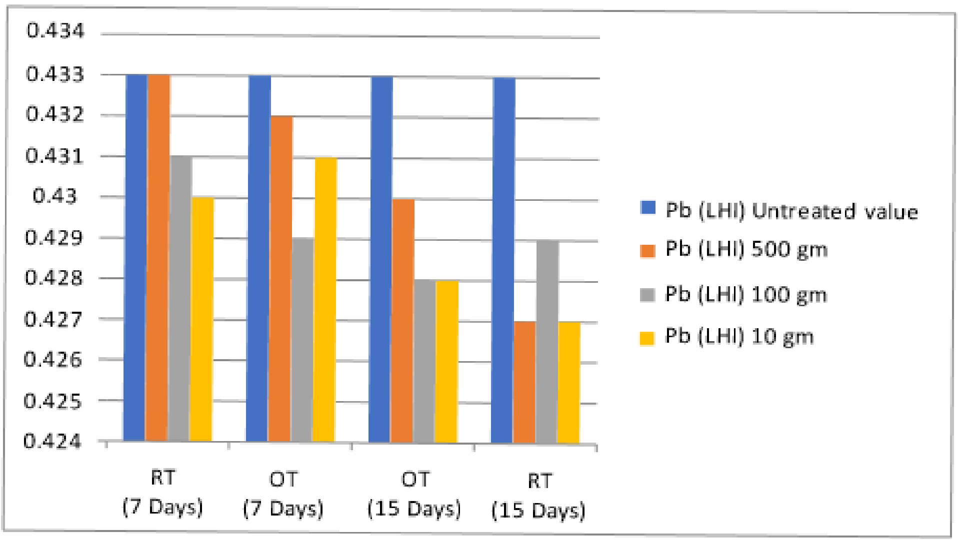

Fig. 9: *Lead (Pb) in Less Human Interference Samples.*

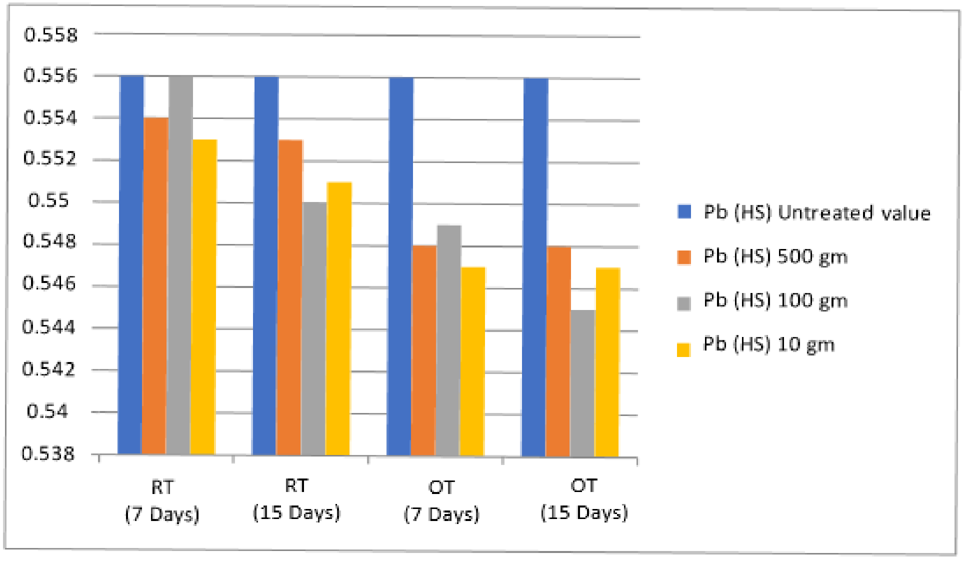

Fig. 10: *Lead (Pb) in Nursery Samples Containing Herbicides.*

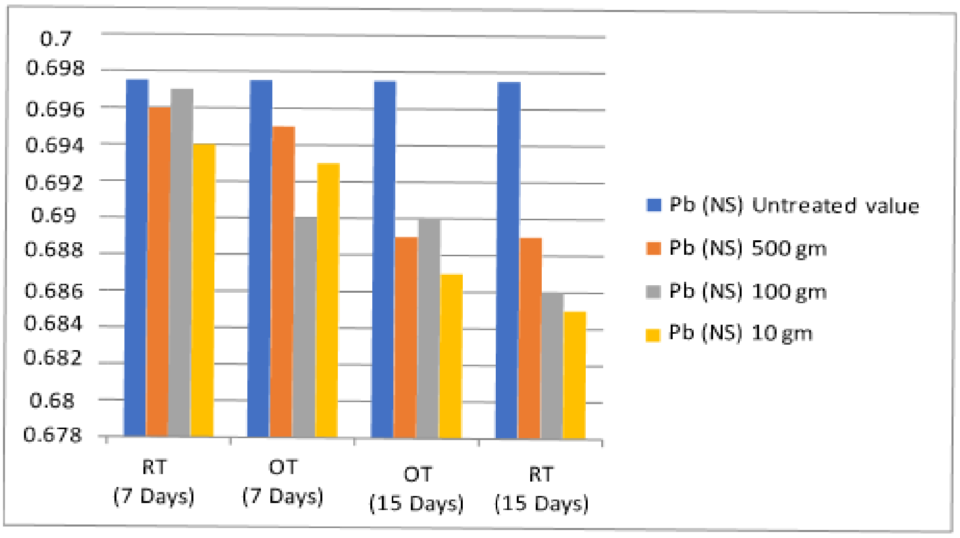

Fig. 11: *Lead (Pb) in Nursery Samples.*

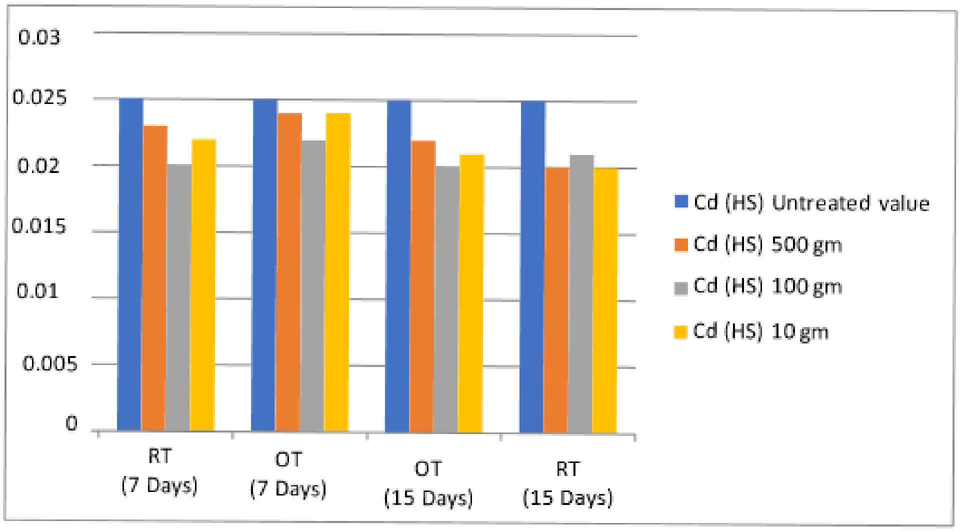

Fig. 12: *Cadmium (Cd) in Nursery samples containing Herbicides.*

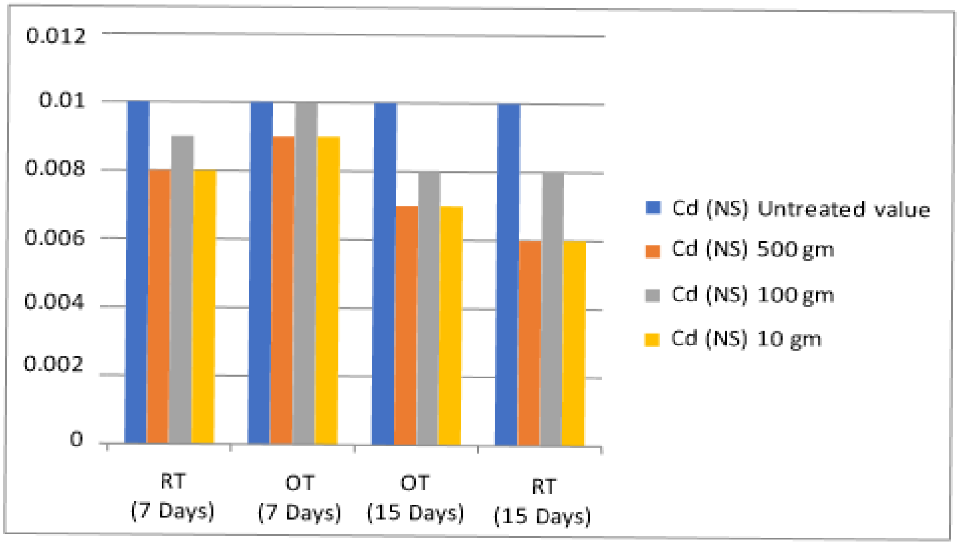

Fig. 13: *Cadmium (Cd) in Nursery Samples.*

CONCLUSION

The heavy metals release due to increased population, industrialization and urbanization are environmental contamination is growing day by day. However, in this time development of science and technology facilitates the use of the potential of biological diversity for pollution control which is termed as Bioremediation. Levels of some heavy metals (Zn, Pb and Cd) were investigated in various samples of soils, nursery sample, nursery sample containing herbicides and less human interference sample. And the result shown that Lead (Pb) heavy metal has the highest concentration and Cadmium (Cd) has the lowest concentration, after treatment with the help of fungus *Cunninghamella sp*. The study conducted the degradation of heavy metals. The concentration of Lead heavy metal decreased up to 0.043. Toxicity of these heavy metals can lead to various diseases on the basis of their acute and chronic experience.

So, it was observed that my core mediation is a biological process which is easy, convinent and beneficial process for the treatment of contaminated soil.

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