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# STUDIES ON HEAVY METAL TOLERANCE OF FUNGAL ISOLATES FROM SOIL SAMPLES OF JABALPUR REGION

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## ABSTRACT

*The presence of heavy metals in the environment has been a subject of great concern due to their toxicity and non-biodegradable nature. The fungal biomass can act as bio-sorbent for the removal of heavy metals and radio nuclides from polluted waste materials. In the present study, the heavy metal tolerant fungi were recovered from soil samples of iron workshop site and waste dumping sites of Jabalpur region. A total of 26 isolates were obtained in the present study and were then assessed for their growth in presence of heavy metals like Cr, Co, Hg, Zn and Mn. Among the fungal isolates, Aspergillus species Penicillium species and Cephalosporium species showed tolerance with the heavy metals used. The determination of heavy metal tolerance of Aspergillus sp, Penicillum sp and Cephalosporium sp was done at concentration range (100mM- 500mM) for Cr, Cu, Hg, Zn and Mn respectively. It was found that the tolerance limit of Aspergillus species to be 19mm in case of 120mM Cr, Penicillium species showed tolerance limit of 7mm with 160mM Cu and Cephalosporium species showed 21mm with 180mM Cr. The results suggest potential of soil mycotic flora of Jabalpur region for the removal of heavy metal from different sites.*

## KEY WORDS

*Heavy metal, heavy metal tolerance, soil mycotic flora, tolerance limit.*

## INTRODUCTION

Contamination through heavy metals is without doubt one of the most important environmental issues in lots of countries and these contaminants reach from various sources such as industrialization, agricultural activities and other activities. The associated anthropogenic have often resulted in environmental pollution. Heavy metals such as Cu, Ni, Zn, Pb, Cr, Hg Cd, Mn etc. are prominent components of industrial effluents which are discharged into the environment, consequently polluting the ecosystem [1].

Fungi are recognized for their superior ability to produce a wide variety of extracellular proteins, organic acids and enzymes etc. The biomass may be used as effective biosorbent for removal, reduction, and detoxification of industrial effluents. However, these effluents contain high concentration of heavy metals which may enter into human and animal's population through food chain, resulting in many metabolic disorders in the affected person [2]. Therefore, it is necessary to remove the heavy metals from soil.

Generally, the sites contaminated with heavy metals are the sources of metal resistant microorganisms [3]. Fortunately, microorganisms can affect the reactivity and mobility of metals and thus can be used to detoxify some metals and can be used to detoxify some metals preventing further metal contamination [4].

The chemical methods are used mostly for the removal of heavy metals but recently many micro-organisms have been reported as biological adsorbent at low cost. Bacteria, fungi, yeast and algae are able to remove heavy metals. Fungi and yeast accumulate micronutrients such as copper, zinc, manganese and non-nutrients metals like uranium, nickel, cadmium,

chromium and mercury in amounts higher than nutritional requirement. The potential of fungal biomass as biosorbent has been recognized for the removal of heavy metals and radio nuclides from polluted waste materials [5].

Fungal cell walls and their components have major role in the sequestering of metals because they contain the different functional groups like carboxyl, hydroxyl, sulfhydryl, amino and phosphate groups which help them in binding of the heavy metals [6]. Fungal biomass can also take up considerable quantities of heavy metals from aqueous solution by adsorption or related process, even in the absence of physiological pH, temperature and availability of nutrients [7].

Keeping in view the significant role of fungi in sequestration of heavy metals, the present study has been designed with the aim of isolating and identifying heavy metal tolerant fungi from the different site of Jabalpur region. The tolerance ranges of the selected fungal isolates *Aspergillus sp, Penicillum sp* and *Cephalosporium sp* was determined for heavy metals Cr, Co, Hg, Zn and Mn at concentration range (100mM-500mM). The concentration ranges at which ceased growth was found was considered as the minimum inhibitory concentration of particular heavy metal for each isolate.

## MATERIALS AND METHOD

**Collection of soil sample:**

The area for collecting soil sample was identified based on need, diversity and extent of pollutant produced by various industries and waste treatment plants. A survey was conducted near Gun Carriage Factory area in the district Jabalpur. The sites selected for the collection of soil sample were iron workshop site and waste dumping site. The soil sample from the different site was collected in sterilized polythene bags and was brought to the Department of Biotechnology, Mata Gujri Mahila Mahavidyalaya (Autonomous) Jabalpur (M.P.) for further studies.

**Isolation and screening of heavy metal tolerant fungi:**

The isolation of fungi from soil samples was done by serial dilution method [8]. The heavy metal used in the present study was chromium, copper, mercury, zinc, manganese. The fungal isolates obtained from different soil sample were screened individually for their growth in heavy metal. The spore suspension of each fungal isolate was grown on PDA plate containing individual heavy metal at concentration of 1mM. These were then incubated at $27^0$ C for 3 days and observed for the growth of colony. The survived colonies were transferred to freshly prepared potato dextrose agar (PDA) plates in order to obtain pure fungal culture. The colonies recovered for each heavy metal were further identified.

**Identification of fungal isolates:**

The fungal isolates from soil sample of Jabalpur region were identified on the basis of their macroscopic characteristics (on PDA plates) and microscopic examination (lactophenol cotton blue (LCB) staining) [9]. The morphological characteristics and appearance of the fungal isolates was done in accordance with the relevant data [10].

**Determination of heavy metal tolerance for the selected fungal isolate:**

The resistance of the selected isolates to heavy metal $Hg^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Mn^{2+}$and $Cr^{2+}$ was determined by dilution method [8]. The metal ions were added separately to PDA medium at concentration range 100mM to 500mM. The plates were inoculated with fungal colonies and incubated at 27°C for 7 days. The diameter of the colony was measured for each isolate in control (with no heavy metal) and plate containing the heavy metal. This was done to assess the effect of heavy metal on the growth of colony in PDA plate. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of metal that inhibited visible growth of the isolate [11].

## RESULTS AND DISCUSSION

**Isolation of fungi from soil sample**

A total of 26 isolates were obtained from the soil sample collected from iron workshop site and waste dumping site as depicted in Fig No.1. The isolates were referred as SB1-11 for iron workshop site and SD1-15 for waste dumping site. Table No. 4.1 shows that 11 fungal isolates were recovered from the iron workshop site and 15 fungal isolates.

Similarly, heavy metal tolerance of fungus isolated from soil contaminated with sewage and industrial waste-water was studied and reported *Aspergillus flavus, Aspergillus niger, Fusarium solani,* and *Penicillium chrysogenum* from peri-urban agricultural areas [12].

**Identification of fungal isolates**

The obtained isolates were identified on the basis of their macroscopic characteristics (on PDA plates) and

microscopic examination (lactophenol cotton blue (LCB) staining). Table No. 4.2 shows that 11 fungal isolates from soil sample B (iron workshop site) were identified as SB1 as *Aspergillus species,* SB2 as *Trichophyton species.,* SB3 as *Candida albicans,* SB4 as *Penicillum species,* SB5 as *Cladosporium species*, SB6 as *Aspergillus species,* SB7 as *Penicillium species,* SB8 as *Aspergillus flavus,* SB9 as *Aspergillus niger,* SB10 as *Aspergillus fumigates* and SB11 as *Fusarium species.* In a similar manner, 15 fungal isolates from soil sample D (waste dumping site) were identified as SD1 as *Aspergillus flavus*, SD2 as *Aspergillus species*, SD3 as *Cladosporium species*, SD5 as *Fusarium species*, SD6 as *Aspergillus species*, SD7 as *Aspergillus flavus*, SD8 as *Alternaria species,* SD10 as *Aspergillus species*, SD11 as *Aspergillus flavus*, SD12 as *Aspergillus niger*, SD13 as *Cephalosporium species* and SD4, SD9, SD14 & SD15 as unidentified species.

Similarly, isolation of fungi was studied from soil and phylloplane samples from sites in Sohag city, Egypt in which fungal isolates were identified according to morphological characterization as *Aspergillus niger, Aspergillus flavus, Aspergillus versicolor, Fusarium species* and *Penicillium species* [13].

**Screening of heavy metal tolerant fungi**

The fungal isolates obtained from different soil sample were screened individually for their growth in heavy metals: chromium, copper, mercury, zinc, manganese. The isolates were grown on PDA plate containing individual heavy metal at concentration of 1mM. These were then incubated at 27°C for 3 days and observed for the growth of colony. The isolates that survived in presence of heavy metal were selected and were used to determine tolerance limit for each heavy metal. Table 4.3 reveals that *Aspergillus sp, Penicillum sp* and *Cephalosporium sp* was able to survive in presence of minimum concentration (1mM) of heavy metal and was examined further for heavy metal tolerance at concentration range (100–500mM). The heavy metal tolerance was checked by assessing the ceasation in growth of fungal colonies on PDA plate containing heavy metal. The ceasation of growth was observed with increase in concentration of heavy metal.

The tolerance of heavy metal was determined by observing the survival of the fungal isolates at different concentration range (100mM-500mM). The tolerance limit was determined and it was found that decrease in zone diameter occurred with increase in concentration of heavy metal as depicted in Fig No.2, Fig No.3 and Fig No.4. Table 4.4 shows that the tolerance limit of *Aspergillus species* to be 19mm in case of 120mM Cr, *Penicillium species* showed tolerance limit of 7mm with 160mM Cu and *Cephalosporium species* showed 21mm with 180mM Cr.

In similar study, the adaptive tolerance behaviour of fungi in heavy metals was observed and it was found that *Aspergillus niger* and *Penicillium species* showed heavy metal tolerance of in presence of Ni, Co, Fe, Mg and Mn up to 2000ppm concentration [14]. Similarly, the effect of different metals and metal concentration on different strains of fungi was evaluated. Results showed that Ni was one of the most toxic metals for strains of *Aspergillus* and *Penicillium* [15].

**Table No. 4.1: Collection sites with fungal isolates**

| S. No. | Collection sites | Soil Sample code | Fungal isolate code | Name |
|---|---|---|---|---|
| 1 | Iron workshop site | B | SB1 | *Aspergillus species* |
| | | | SB2 | *Trichophyton species* |
| | | | SB3 | *Candida albicans* |
| | | | SB4 | *Penicillum species* |
| | | | SB5 | *Cladosporium species* |
| | | | SB6 | *Aspergillus species* |
| | | | SB7 | *Penicillium species* |
| | | | SB8 | *Aspergillus flavus* |
| | | | SB9 | *Aspergillus niger* |
| | | | SB10 | *Aspergillus fumigates* |
| | | | SB11 | *Fusarium species* |
| 2 | Waste dumping site | D | SD1 | *Aspergillus flavus* |
| | | | SD2 | *Aspergillus species* |

| S. No. | Collection sites | Soil Sample code | Fungal isolate code | Name |
|---|---|---|---|---|
| | | | SD3 | *Cladosporium species* |
| | | | SD4 | *Unidentified species* |
| | | | SD5 | *Fusarium species* |
| | | | SD6 | *Aspergillus species* |
| | | | SD7 | *Aspergillus flavus* |
| | | | SD8 | *Alternaria species* |
| | | | SD9 | *Unidentified species* |
| | | | SD10 | *Aspergillus species* |
| | | | SD11 | *Aspergillus flavus* |
| | | | SD12 | *Aspergillus niger* |
| | | | SD13 | *Cephalosporium species* |
| | | | SD14 | *Unidentified species* |
| | | | SD15 | *Unidentified species* |

**Table No. 4.2: Morphological Identification of the fungal isolates**

| S. No. | Source of soil sample | Isolate code | Macroscopic characterstics | Microscopic characterstics | Fungal species identified |
|---|---|---|---|---|---|
| 1. | Iron workshop site | SB1 | Peach –white colony becomes yellow with white edges, mature culture usually yellow colour and thread like edges, pigment- orange colour. | Single celled conidia. Connected with conidiophores. | *Aspergillus species* |
| | | SB2 | In centre of colony Greenish-white edges colony, dark brown pigment. | Spindle shape | *Trichophyton species* |
| | | SB3 | Leathery, compact, cream colour colony become in centre brown white line and cream edges, wrinkled – velvety colony. Pigment - brown colour. | Yeast like fungus produces pseudopomycelium. | *Candida albicans* |
| | | SB4 | Greenish-Grey with thin white edges, small velvat, wooly growing entire over the plate pigment –dark brown, | Single-celled spores in chains developat the end of the sterigma arising from the metula of the conidiophores. And chonidiophore arise from a septate mycelium. | *Penicillum species* |

| | | | | | |
|---|---|---|---|---|---|
| | | SB5 | small greyish – white colony, wooly colony, pigment –black colour. | Conidia develop at the end of complex conidiophores arising from a septate mycelium that is usually brownish. | *Cladosporium species* |
| | | SB6 | Wrinkled, leathery, in centre green with white edges, pigment orange brown. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus species* |
| | | SB7 | Wrinkled, small, green (blue-green) with white edges colony were grown over entire plate. Pigment- brown. | Single-celled spore (conodia), the srigma arising from the metula of the conidiophore, branching conidiophores arising from a septate mycelium. | *Penicillium species* |
| | | SB8 | Green powdery with yellow edges. Powdery growth as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus flavus* |
| | | SB9 | Small colony grown entire over the plate. Black powdery with white edges becomes black powdery as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from septate mycelium. | *Aspergillus niger* |
| | | SB10 | Wrinkled, leathery, in centre green with white edges, pigment orange brown. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus species* |
| | | SB11 | White cottony growth becomes pinkish- white colony with pink pigment. | Multi-celled spores or conidia are oval or crescent-shaped and attached to conidiophores arising from a septate mycelium. | *Fusarium species* |
| 2 | Waste dumping site | SD1 | Green powdery with yellow edges. Powdery growth as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus flavus* |

| | | | |
|---|---|---|---|
| SD2 | Bluish – green with white colony, cottony, fuzzy growth as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus species* |
| SD3 | Bluish- green smooth velvety colony growing entire over the plate. As mature culture usually greenish black and powdery. | Conidia develop at the end of complex conidiophores arising from a septate mycelium that is usually brownish. | *Cladosporium species* |
| SD4 | Small round, wrinkled, yellowish- green with white edges colony. Pigment - brown. | | *Unidentified species* |
| SD5 | White cottony, woolly, fuzzy, colony, becomes pinkish white growth, as mature culture, pigment- dirty colour. | Multi-celled spores or conidia are oval or crescent-shaped and attached to conidiophores arising from a septate mycelium. | *Fusarium species* |
| SD6 | Rapidly growing white becomes grey or bluish-grey colony as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus species* |
| SD7 | Green powdery with yellow edges. Powdery growth as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus flavus* |
| SD8 | Grey coloured colonies | Long branched chain conidia | *Alternaria species* |
| SD9 | Small round, wrinkled, yellowish- green with white edges colony. Pigment - brown. | . | *Unidentified species* |
| SD10 | Rapidly growing white becomes grey or bluish-grey colony as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long | *Aspergillus species* |

| | | | | |
|---|---|---|---|---|
| | | | conidiophores arise from mycelium. | |
| | SD11 | Green powdery with yellow edges. Powdery growth as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from mycelium. | *Aspergillus flavus* |
| | SD12 | Small colony grown entire over the plate. Black powdery with white edges becoming black powdery as culture mature. | Single –celled conidia in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from septate mycelium. | *Aspergillus niger* |
| | SD13 | Rapidly growing white cottony becoming grey colour coloured. | Single-celled conical or elliptical conidia held together in clusters at the tips of the conidiophores by a mucoid substance; erect, unbranched conidiophores arise from a saptate mycelium. | *Cephalosporium species* |
| | SD14 | Small, Yellow-white colony becomes dirty yellow | | *Unidentified species* |
| | SD15 | Black coloured colonies with white centre | | *Unidentified species* |

**Table No. 4.3: Growth of fungal colony in PDA with heavy metal (1mM)**

| S. No. | Soil Sample | Hg | Zn | Cu | Mn | Cr |
|---|---|---|---|---|---|---|
| 1 | Soil sample B | *Aspergillus sp.* *Cephalosporium sp* | *Aspergillus sp.* | *Penicillum sp.* *Cephalosporium sp* | *Penicillum sp.* | *Aspergillus sp.* |
| 3 | Soil sample D | *Aspergillus sp.* | *Aspergillus sp.* *Penicillum sp.* | *Aspergillus sp.* *Cephalosporium sp.* | *Aspergillus sp.* | *Aspergillus sp.* |

**Table No 4.4: Tolerance range (colony diameter in mm) of fungal isolate in presence of heavy metal**

| S.No. | Fungal isolate | Cr | | Cu | | Hg | | Zn | | Mn | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | T | C | T | C | T | C | T | C | T |
| 1 | *Aspergillus species* | 120mM 27 | 19 | 420mM 27 | - | 100mM 27 | 6 | 500mM 27 | 12 | 500mM 27 | 11 |
| 2 | *Penicillium species* | 100mM 12 | 7 | 160mM 12 | 16 | 100mM 12 | 7 | 100mM 12 | 12 | 100mM 12 | 12 |
| 3 | *Cephalosporium species* | 180mM 46 | 21 | 120mM 46 | 21 | 140mM 46 | 25 | 120mM 46 | 20 | 120mM 46 | 20 |

**a) C refers to control (without heavy metal); b) T refers to test (with heavy metal)**

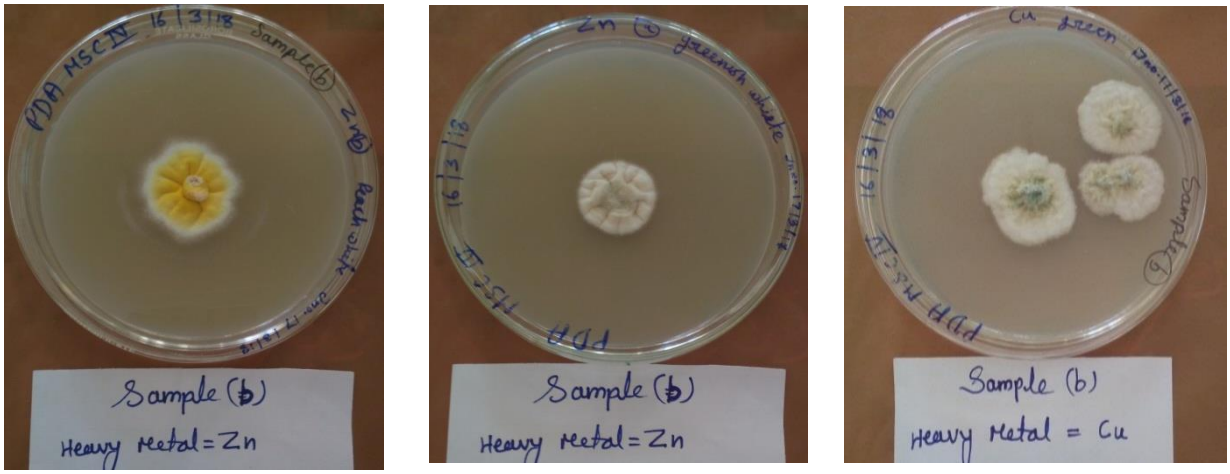
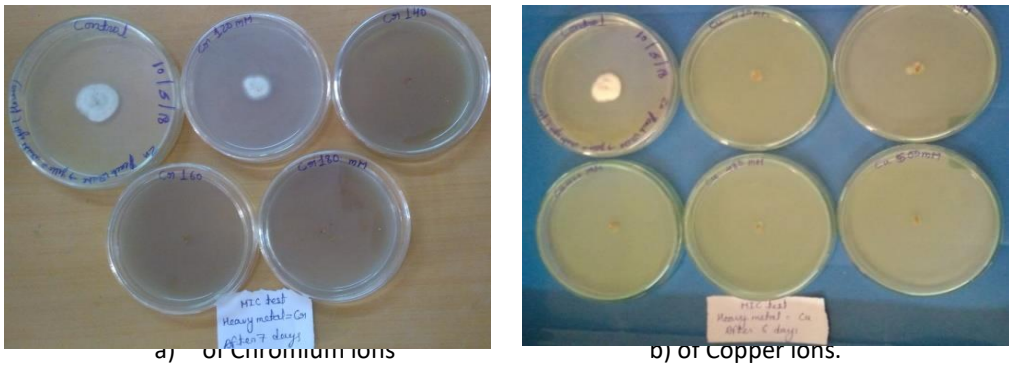
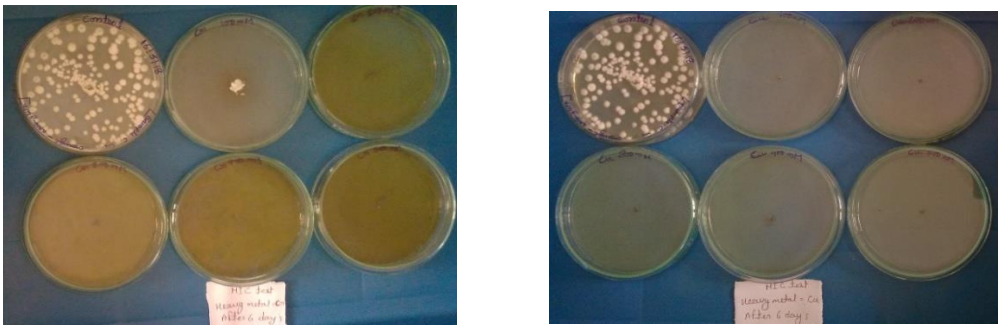



**Fig No.1: Macroscopic view of Fungal isolates from different soil samples**

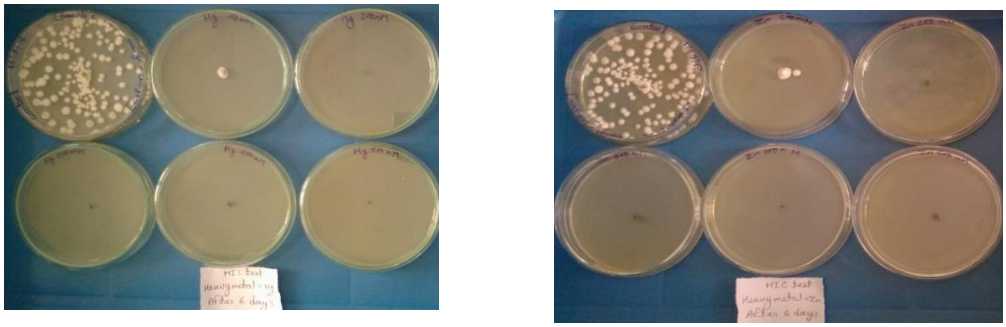


a) of Chromium ions b) of Copper ions.

**Fig No.2: Growth of *Aspergillus species* after exposure to different concentration**

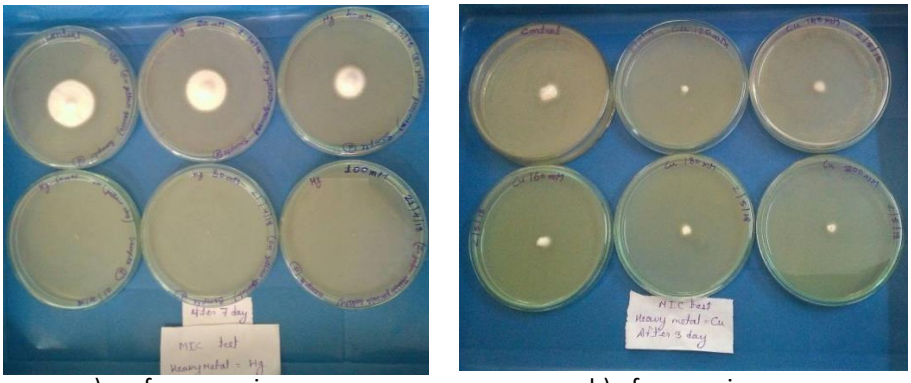


a) of chromium ions ions. b) of copper ions



c) of mercury ions. d) of zinc ions

**Fig No. 3: Growth of *Penicillium species* after exposure to different concentration**



a) of mercury ions. b) of copper ions.

**Fig No. 4: Growth of *Cephalosporium species* after exposure to different concentration**

## Conclusion

Heavy metals are considered hazardous waste metals that can accumulate in the human body with a relatively longer half-life. Several heavy metals such as Cd, As and Cr are considered as the pollutants that need to be removed either by chemical or biological methods. Introduction of heavy metal compounds into the environment generally induces morphological, cytological and physiological changes in the microbial communities. Thus there is urge for the search of biological candidates capable of removing heavy metals. The present study was done with the aim of isolating heavy metal tolerant fungi from soil samples. The isolates were identified on the basis of their morphological characteristics and microscopic examination of the isolates. They were identified as *Aspergillus niger*, *Aspergillus flavus, Aspergillus species Penicillum species Cladosporium* species, *Candida albicans*, *Fusarium species*, *Trichophyton species* etc. The fungal isolates which were found tolerant to all the heavy metals (chromium, copper, mercury, zinc, manganese) were *Aspergillus species, Penicillium species and Cephalosporium species.* The study showed that the tolerance limit of *Aspergillus species* to be 19mm with 120mM Cr, *Penicillium species* showed tolerance limit of 7mm with 160mM Cu and *Cephalosporium species* showed 21mm with 180mM Cr. From this study, it can be concluded that the selected fungi have high potential to remove the heavy metals from the toxic environment. These potential fungal isolates can be exploited in further studies for bioleaching and bioremediations under field trails. The use of biological method offers alternative strategy for the recovery of heavy metals.

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