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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Isolation and Characterization of Chromium and Nickel degrading microbes from Industrial Effluent

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**ABSTRACT**

*Heavy metals are ubiquitous contaminants that have accompanied the man from the earliest ancient times, and unlike other environmental pollutants, heavy metals are chemical elements that man does not create or destroy. Heavy metals are ubiquitous contaminants that have accompanied the man from the earliest ancient times, and unlike other environmental pollutants, heavy metals are chemical elements that man does not create or destroy.In the present study heavy metal tolerant fungi were isolated from the compost soil sample contaminated by the industrial effluents. The isolation was done on PDA media supplemented with heavy metal that is Cr and Ni. The most dominating fungal species were found to be Penicillium spp. This fungus was screened for its ability to tolerate heavy metals by different methods and was found to be highly tolerating fungal species. The fungi were assessed for its ability to remove the heavy metal form the culture media and also the culture conditions for the fungus were experimentally optimized. The isolated Penicillium species was found to show maximum growth at 35ºC temperature with media pH 6 for an incubation period of 7 days. The isolate was able to tolerate 60-70ppm concentration of heavy metal under normal conditions. The ability of isolate for metal uptake was very effective as after 96 hrs of incubation it was capable of removing around 93.8% of Cr and 95.6% of Ni from culture media and the complete uptake was observed after 144hrs of the incubation period. The molecular characterization revealed the isolate to be Penicillium rubens. The Penicillium species have been reported under several studies to be one of the potential agents for the bioremediation process. The morphological characteristics of this fungus make it capable of biosorption of heavy metal imparting its bioremediation potential and economic importance.*

*Keywords: Bioremediation, Heavy metals, Penicillium, Biosorption.*

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## INTRODUCTION

Industrialization, in the present scenario, is growing increasingly in the world which is affecting the quality of water, food, feed, and weather [1]. The various types of industries like chemical, food, textile, and metallurgy release a very high amount of waste including toxic substances to the environment. The chemical fertilizers and pesticides used in the agricultural practices nowadays and the vehicle used for transportation discharge a large amount of pollutants containing heavy metals in the environment [2]. Most importantly the food safety is considerably threatened by the presence of heavy metal. Various studies carried out on this concern have proved the accumulation of heavy metals in water [3], rice [4], vegetables [5] and fish [6]. When consumed these food samples lead to the accumulation of heavy metals in human organs and tissues leading to some diseases such as kidney, cardiovascular system, and nervous system disorders [7]. Till now many strategies have been used for solving the problems of heavy metal pollution in the environment. Applying the bioremediation methods for decreasing the number of heavy metals from the environment is quite interesting. For this purpose plants, fungi, bacteria, yeast, cyanobacteria, and algae are used prominently. The microorganism becomes the most acceptable ones because they are easier to work with [8]. The most important advantage of microorganisms is their safety in human aspects. Due to excessive industrialization accompanied by rapid global population growth and increase in agricultural practices, heavy metals As, Cd, Cr, Cu, Hg, Pb, and Zn are the most common environmental pollutants [9].

The microorganism is capable of converting the toxic heavy metals to non-toxic heavy metals through the process of metabolism [10]. The methods involved in the bioremediation process by microorganisms are bioleaching, enzyme-catalyzed transformation, biomineralization, biosorption, and intracellular accumulation [11]. The maximum bioremediation of heavy metals occurs through the biosorption mechanism [12]. In order to obtain the efficient bioremediation process the ideal environmental conditions are necessary. Fungi are prominently used as biosorbents for the removal of toxic metals due to their excellent capabilities for metal uptake and recovery [13]. Several studies have proved that active and lifeless fungal cells are capable of inorganic chemical adhesion [14]. Penicillium spp. is known for its use to convert the toxic Cr (VI) to the non-toxic Cr (III) by biosorption [15].

In terms of the morphology, ecology, and metabolism the fungi are adapted so well according to the environmental conditions and are responsible for carrying out nutrient cycle and decomposition under the natural conditions [16]. Reports suggest that they can withstand and survive under the stress conditions of moisture, nutrients, pH, etc. Bioremediation carried out with the help of fungi is termed as the mycoremediation and it involves the use of live or dead fungus for the removal of contaminants [17][7]. The process does not leave any harmful product behind and is also cost-effective hence possessing the overall solution because of the full mineralization of pollutants in nature [18]. Mycoremediation cab is implicated as a successful method only when the correct identification and usage of the fungal species for the target heavy metal is done. Fungal species are capable of accumulating the heavy metals in their fruiting bodies in an efficient manner hence making them unavailable or decreasing their concentration form the substrate it's growing on [19]. The future availability of heavy metals and other contaminants in the media depends upon the life of the fungi, chemical behavior of the elements, and presence or absence of the fungi after sequestration. The process of biosorption involves fungal cell walls (having chitin, proteins, glucans, lipids, pigments, polysaccharides) and functional groups like hydroxyl, carboxyl, amino, sulfate, or phosphate and is mediated through interactions

## MATERIAL AND METHODS

### Sample collection and Study area

The sample collection was done from the electroplating efflux contaminated areas of Lucknow, Uttar Pradesh, and the local sites were randomly selected. From the selected sites surface soil samples (not exceeding 5cm depth) were collected in sterile bags. The composite soil samples were transported to the Soil Laboratory of Integrated Biotechnological Research Institute (IBRI) Lucknow for the physio-chemical analysis. A small portion of the soil was stored at 4ºC to ensure the minimal biological activity and the fungus isolation was carried out within 24 hours of sample collection.

### Isolation and Characterization of the fungi

For isolation of the fungus from the collected soil sample Potato Dextrose Media was used. Sterile Petri plates filled with sterile PDA media were inoculated with 0.1 ml of the sample soil suspension by spread plate technique and plates were placed in an incubator at 28±1 $^0$C temperature. For the morphological characterization, slides were prepared from the purified fungal isolates grown on PDA, stained with Aniline Blue stain and examined under 40X resolution. The identification of fungal isolate was done by comparing these morphological characteristics with those described by [20].

### Screening for selection of heavy metal tolerant Fungi

The fast-growing isolates with large biomass were selected for their screening of heavy metal (nickel and chromium) screening. For this PDA plates were prepared supplemented with 1mM of heavy metal. These heavy metal-containing plates were inoculated with a drop of spore suspension of isolates. The plates were incubated at 28±1 $^0$C temperature for 7 days. The effect of heavy metal on the growth of the fungus was estimated by measuring the radial colony extension against the control. The metal tolerance index was calculated as the ratio of the extended diameter of the treated colony to the untreated colony; *Tolerance Index = dt / dc*, where dt and dc is the radial extension (cm) of the treated colony and untreated colony respectively.

### Determination of heavy metal tolerance by fungal isolate

For determining the heavy metal tolerance capacity of isolate, two different methods were implicated that is plate diffusion method and broth method. For the first method PDA media was prepared and were separately amended with variable concentration of heavy metals *viz.*$NiSO_4$ and $K_2Cr_2O_7$. The media was sterilized and supplemented with 1 ml of streptomycin solution including a control medium. The solidified plates were inoculated with the fungal isolated being spotted in the center and incubated at 28±1 $^0$C temperature for 2-5 days, then colony morphology and radial growth diameter was measured [21]. Tolerance fungi were studied by the comparison among samples inoculated and control. for the later method fungal isolates were cultured in nutrient broth supplemented with variable concentration of the heavy metal that is 10, 20, 30, 40, 50, 60, 70, 80, 90 and, 100ppm. These sterile broths were inoculated

with 0.1 ml of the fungal culture suspension and incubated at 28±1 $^0$C temperature for 48 hours. After incubation period optical density at 405nm was measured against media blank using a spectrophotometer (Varian cary 50 UV-Vis double beam spectrophotometer), the process carried was described by Hassen [22].

**Optimization of culture conditions**

For the optimization experiment three parameters that are temperature, pH, and, incubation period were considered. The concentration of heavy metal was kept constant and this selection was done based on the result from the above experiment, the maximum concentration of heavy at which fungal growth was maximum was considered in this experiment. For this potato dextrose broth media was prepared and from it, three sets were prepared. From one set, different pH of media was set in different tubes that are from 5-7 at an interval of 0.5. These tubes were inoculated and kept at 28±1 $^0$C temperature for 48 hours. From the second set the pH was common but after inoculation the tubes were kept at a variable temperature that is from 25-45ºC at an interval of 5ºC. For the third set different tubes were incubated for different time intervals. After incubation period, the optical density of the samples collected from each tube of both sets was taken at 405nm to determine the optimum value.

**Determination of heavy metal removal**

The heavy metals removal from culture solution was determined by the estimation of the residual amount of heavy metal left in the culture medium after 96 hours of the culture period, followed by the method described by [23]. The reduction in chromium ion concentration in media was indicated by the loss of yellow to orange colorand by the quantitative decrease in Cr (VI) concentration in culture [24]. For this culture media supplemented with 50ppm concentration of metal, ion was inoculated separately by active and inactive mycelia of the fungus, inactivation of mycelia was done by autoclaving. Both were incubated under the same condition for 4-5 days and quantitative estimation small fraction of the media was filtered by membrane filters to remove the mycelia. The filtrate was used for quantitative measurement of heavy metal concentration, done at specific time intervals.

**Determination of total heavy metal uptake by fungal biomass**

For determining the heavy metal uptake by fungal biomass, the fungal biomass was acid digested under the method followed by [25]. The mycelia were separated from culture by filtration followed by washing with distilled water and air drying. The dried mycelia were weighed and suspended in a known volume of concentrated nitric acid and kept at RT for 30 minutes and then heated gently on a heating mantle at 60ºC for 30 minutes. The solution was then cooled at RT and to it hydrogen peroxide was added in a ratio of 7:3 v/v $HNO_3$ / $H_2O_2$ and it was again heated for 15 minutes. This mixture was centrifuged at 1500 rpm of 10 minutes and the supernatant so collected was used to determine the total chromium concentration using Atomic Absorption Spectrophotometer, AAS (Perkin Elmer, Japan Co, Ltd.) with Zeeman graphite furnace.

**Molecular characterization of the isolate**

The isolated Penicillium spp. was then taken forward for the molecular characterization by sequencing of its 18S rRNA gene sequence by commercial DNA sequencing service. Later on the nucleotide sequence so obtained was submitted in the Gen-Bank sequence database. The online available, program BLASTn was used to find out the related sequences with the taxonomic information in the databank at the NCBI website to accurately identify the species.

## RESULT AND DISCUSSION

In the current study heavy metal tolerant fungi were isolated from the compost soil sample obtained from the area contaminated by the effluent from electroplating industries. The isolation was carried out on potato dextrose media supplemented with the desired concentration of heavy metal (nickel sulphate and potassium dichromate). The effluent contaminating the soil sample was analyzed on the physio-chemical parameter and the result for the same is given in table no.1. The culture media plates when observed after incubation showed different kinds of fungal species but among them one species was prominent and dominant both in no. of colonies as well as in biomass. After the morphological characterization of this dominating colony is was found to be from the *Penicillium spp*. The morphological characteristics of the colony include elevated green color colonies with circular margins having a wolly texture and showed septate hyphae with globose spores under a microscope. This fungus of *Penicillium spp*. was transferred to pure culture and was undertaken for further analysis throughout the study.

Table no. 1:Physio-chemical characteristics of electroplating industrial effluent

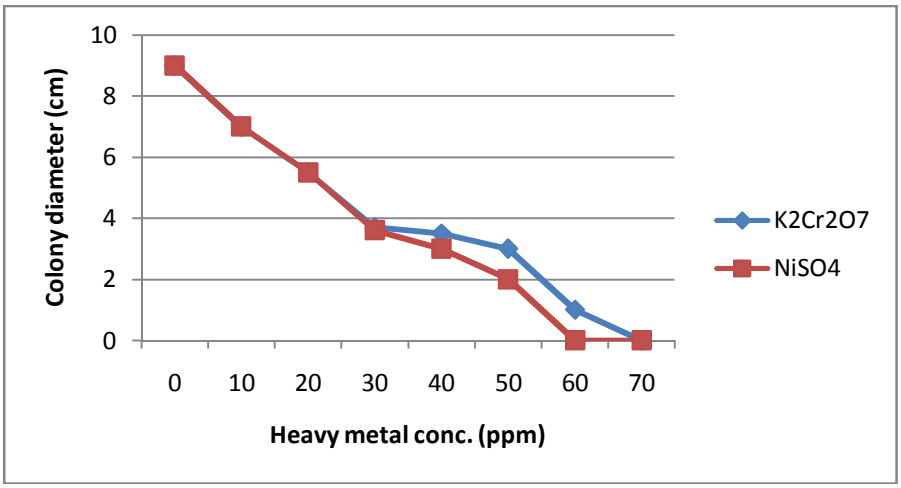
| Parameter | Value |
|---|---|
| **pH** | 5.89 |
| **Electrical conductivity** | 250 (mS/Cm) |
| **Total Dissolved Solids** | 153.6 ± 0.95 (mg/L) |
| **Organic carbon** | 3.8 ± 0.51 (%) |
| **Organic matter** | 6.11 ± 0.34 (%) |
| **Heavy metal** | Cr- 2.965 ± 0.55 (mg/L)<br>Ni- 2.541 ± 0.45 (mg/L) |

The selected fungal species was now screened for its metal tolerance fungus and the tolerance index of this fungus ranges from 4-4.5 cm (diameter of the colony) for 25ppm $NiSO_4$ and 4-4.9 cm (diameter of the colony) for 25ppm $K_2Cr_2O_7$. After this the isolate was used for determining its heavy metal tolerance capacity by growing it at a variable concentration of the selected heavy metal. The penicillium species showed maximum tolerance to chromium up to 60ppm concentration showing visible growth while visible growth was seen up to 50ppm nickel concentration. The result of the metal tolerance capacity of *Penicillium spp*. is shown in the graph no. 1 along with the data are given in table no.2.

Table no. 2: Table showing the result of the Radial growth of Penicillium spp. against 0-70ppm of heavy metal concentration

| S. No. | Conc. of heavy metal (ppm) | Colony Diameter (cm) and Tolerance Capacity | | | |
|---|---|---|---|---|---|
| | | $K_2Cr_2O_7$ | | $NiSO_4$ | |
| 1. | 0 | 9 | ++++ | 9 | ++++ |
| 2. | 10 | 7 | +++ | 7 | ++++ |
| 3. | 20 | 5.5 | +++ | 5.5 | ++ |
| 4. | 30 | 3.7 | ++ | 3.6 | ++ |
| 5. | 40 | 3.5 | ++ | 3 | ++ |
| 6. | 50 | 3 | ++ | 2 | + |
| 7. | 60 | 1 | + | 0 | - |
| 8. | 70 | 0 | - | 0 | - |

*Maximum growth = 8-9cm (++++), Moderate growth = (7-5cm) +++, Slightly growth = (4-3cm) ++, Very slightly growth = 2-1 (+), No growth = 0cm*

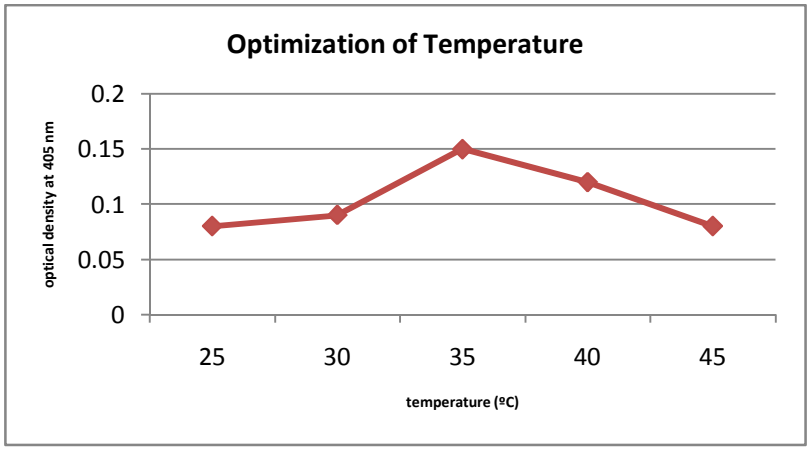



Graph no. 1: The Radial growth of *Penicillium* spp. against 0-70ppm of heavy metal concentration

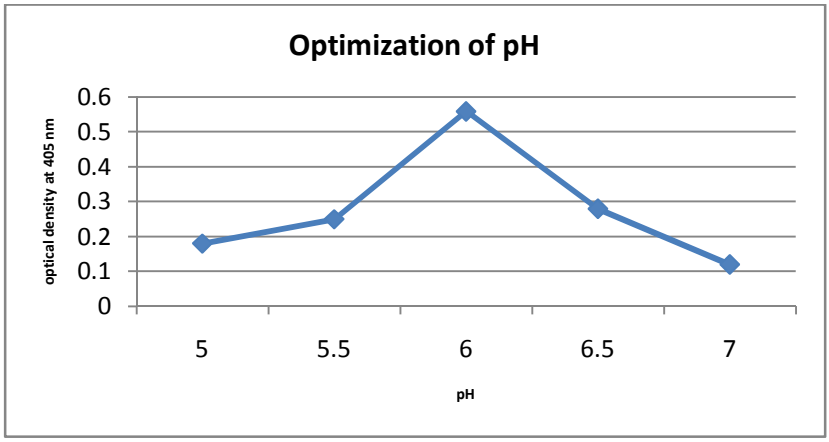
After determining the heavy metal tolerance capacity the culture was carried forward to determine its optimum culture concentration at a constant concentration of the heavy metal that is the maximum concentration at which visible growth was seen. These three parameters were undertaken and for all optical density was used for determining the fungal biomass concentration. The result of optimization showed that the selected *Penicillium spp*. shows maximum growth at 35ºC with media pH at pH 6 and incubation period of 7 days. The result for the same is summarized in table no. 3 along with graph no. 2, 3, and 4.

Table no. 3: Table Showing the result of the optimization of *Penicillium* spp under different growth parameters.

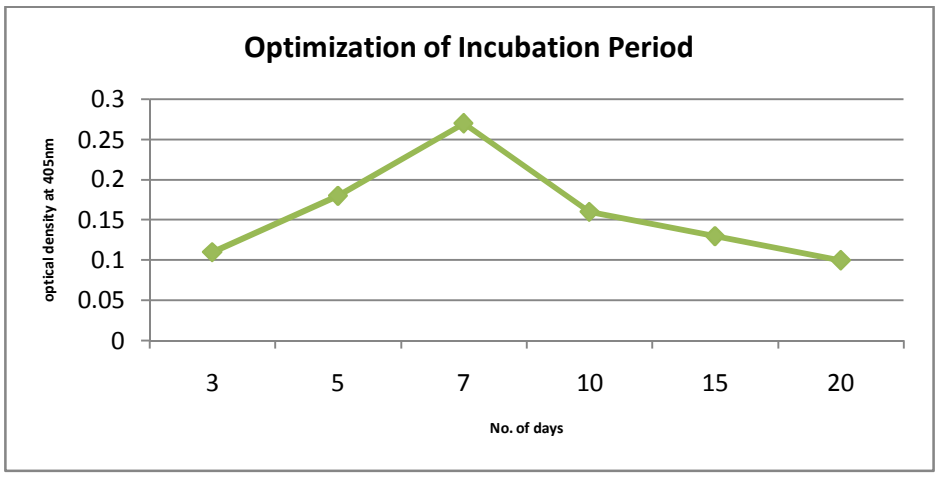
| Optimization of Temperature | | |
|---|---|---|
| **S. No.** | **Temperature** | **O.D. at 405nm** |
| **1** | **25** | 0.08±0.02 |
| **2** | **30** | 0.09±0.01 |
| **3** | **35** | **0.15±0.019** |
| **4** | **40** | 0.12±0.013 |
| **5** | **45** | 0.08±0.018 |
| **Optimization of pH** | | |
| **S. No.** | **pH** | **O.D. at 405nm** |
| **1** | **5** | 0.18±0.06 |
| **2** | **5.5** | 0.25±0.05 |
| **3** | **6** | **0.56±0.04** |
| **4** | **6.5** | 0.28±0.07 |
| **5** | **7** | 0.12±0.04 |
| **Optimization of the incubation period** | | |
| **S. No.** | **No. of Days** | **O.D. at 405nm** |
| **1** | **3** | 0.11±0.05 |
| **2** | **5** | 0.18±0.04 |
| **3** | **7** | **0.27±0.03** |
| **4** | **10** | 0.16±0.02 |
| **5** | **15** | 0.13±0.02 |
| **6** | **20** | 0.10±0.01 |




Graph no. 2: Optimization for growth of Penicillium spp. at different temperature

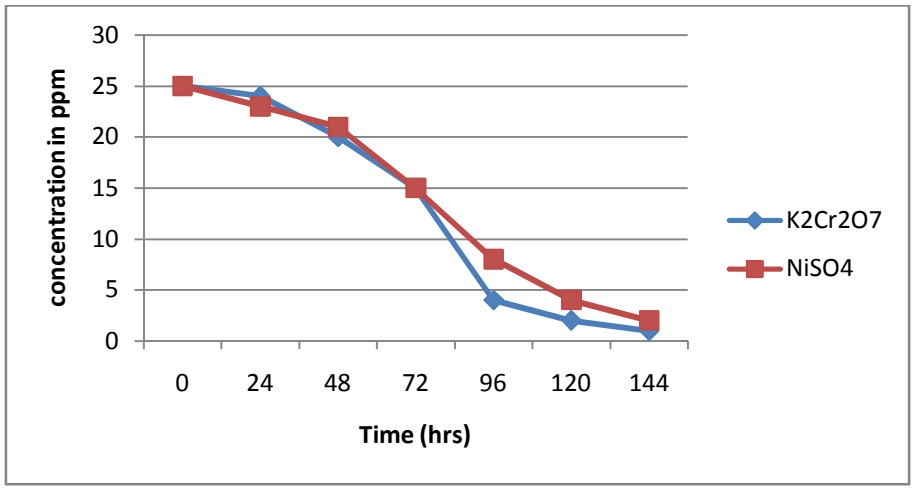



Graph no. 3: Optimization for growth of Penicillium spp. at different pH




*Graph no. 4: Optimization for growth of Penicillium spp. at different incubation period*

The fungal isolate that is the *Penicillium spp.* was assessed for its ability to remove the heavy metal from the culture media within a certain period. The changes in the concentration of both the heavy metals that is hexavalent chromium and nickel were significantly reduced in every 24 hours. At 96 hours, the media exhibited a Cr (VI) concentration of only 1.09 ± 0.12ppm and a nickel concentration of 1.53 ± 0.21 ppm. Thus, with the initial dichromate and nickel concentration of 25 ppm up to 93.8 % Cr (VI) and 95.6 % Ni removal was achieved within 96 hours by *Penicillium sp.*




Graph no. 5: Profile of Cr (VI) and Ni conc. during fungal growth in culture solutions treated with an initial concentration of 25 ppm

The isolated *Penicillium spp*. showed complete removal of both heavy metals from the culture media after 144 hours of the incubation period. This suggests the potential of the isolated fungal species in heavy metal removal, flashing the light on its environmental importance. The isolated fungal species was then taken forward for the Molecular characterization by 18s rRNA sequencing. The result of sequencing revealed that the isolated Penicillium spp. is *Penicillium rubens* and the sequence for the same is available with the accession number LC536286 in the nucleotide database.

The result of screening for the ability of biosorption of heavy metal by the isolated *Penicillium spp*. revealed its potential. Their ability to do so may come from their inherent physiological characteristics such as the cell wall. The Penicillium spp. have a rigid cell wall made up of the polysaccharides like chitins and glucans and also due to surface to volume ratio which helps these fungi to absorb the heavy metals like cadmium into their cell wall [26] P*enicillium spp*. are capable of releasing some extracellular enzymes like laccases and metal-binding proteins that act as the chelators that are capable of binding with the heavy metals and facilitates their absorption by the cell wall. The isolated *Penicillium spp.* is capable of surviving at high cadmium concentration and a similar finding was reported that the *Penicillium notatum* isolated from the polluted stream near the industrial area was able to grow and remove up to 100-folds higher cadmium levels after 13 days of incubation [27].

The uptake and selective binding of Ni(II), Zn(II), Cd(II) and Pb(II) by the mycelium of *P. digitatum* were demonstrated to be highly pH-sensitive and inhibited below pH 3, so as the study suggests that at pH above or equal to 7 is good for the fungal growth and so for its biosorption potential. The fungus *Penicillium canescence* was described to be able to remove the Cd, Pb, Hg, and As ions from aqueous solutions by biosorption [28]. The use of *Penicillium chrysogenum*to remove metal ions with high efficiency was first reported by Niu *et al.*. At pH 4.5, nonliving*P. chrysogenum*biomass not only removed Pb ions (116 mg/g dry biomass) from aqueous solutions but also exhibited selectivity for Pb(II) over the other metal ions studied [Cd(II), Cu(II), Zn(II) and As(III)]. Penicillium spp. are capable of biodegrading the polyaromatic hydrocarbons and the phenolic compounds. The PAHs (4-5 benzene rings), when provided in the medium as the sole carbon source in basal salt media, were degraded in a substantial amount by *P.janthinellum* [29]. The phenolic compounds are degraded by the ortho-pathway with catechol being the first intermediate [30]. *Penicillium simplicissimum* was capable of effectively degrading 8.5mM of phenol used as sole carbon source, after 22 days of the incubation period. The catabolism of phenol produced catechol, hydroquinone, and *cis, cis*-muconic acid [31]. All the above-given discussion indicates the environmental importance of *Penicillium spp.* in the bioremediation process and the versatility of the species against various kinds of pollutants.

## CONCLUSION

The present study depicts the importance of microorganisms in the bioremediation process. The isolated *Penicillium spp*. is found to be a very potential organism for the removal of heavy metals. The ability of these fungus increases their economical importance and their characteristics make them suitable for use in environmental cleanup. The identified *Penicillium spp.* that is *Penicillium rubens* can be considered as a good option for the bioremediation of heavy metals. Furthers studies can be carried out to assess the capabilities of this fungal species on other pollutants so the versatile nature of this fungus could be revealed. Other species of this genera are also reported to be potential agents for bioremediation. The increasing pollution rate alarms for the rapid research of such microorganisms that can act the novel agents for environmental cleanup.

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